BIOACTIVE COMPOUNDS AND BIOLOGICAL ACTIVITIES OF SOME MEDICINAL PLANTS FROM CENTRAL NEPAL

M.Sc. Thesis

2016

Submitted to

CENTRAL DEPARTMENT OF BIOTECHNOLOGY

Tribhuvan University

Kirtipur, Kathmandu, Nepal

By

Gyandra Prasad Ghimire

Exam Roll No.: BT105/069

Registration No.: 5-2-37-540-2007

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Supervisors

Prof. Dr. Krishna Das Manandhar

Prof. Dr. Krishna Kumar Shrestha

Acknowledgements

I am deeply indebted to my research supervisor, Prof. Dr. Krishna Das Manandhar, for his kind encouragement, patient supervision, insightful advice, enduring support, and creative suggestions. He has taught me to think critically, select problems, solve them, and present their solutions. I would also like to express my gratitude to my another supervisor, Prof. Dr. Krishna Kumar Shrestha, Former Head, Central Department of Botany, for offering me an opportunity to work in a research project entitled "Bioprospecting of underutilised plants of Dhading district" which was conducted in the Central Department of Biotechnology, in collaboration with Ethnobotanical Society of Nepal (ESON), Kathmandu, and Korea Research Institute of Bioscience and Biotechnology (KRIBB), South Korea. My sincere gratitude goes to Prof. Dr. Rajani Malla, Head, Central Department of Biotechnology for her persistent love and guidance. She rendered her valuable time and effort and helped arrange this work for me.

I would like to thank Tribhuvan University Teaching Hospital (TUTH), Maharajgunj, Kathmandu for providing bacterial cultures of *Staphylococcus aureus,* methicillin resistant *Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella* Typhi. I would like to appreciate Central Department of Chemistry, Tribhuvan University for providing *Artemia salina* eggs. Special recognition must be given to National Centre for Cell Science (NCCS), Pune for providing different cell lines including HeLa and MDCK. The author's gratitude goes to Dr. Rabindra M. Sapkota, Mr. Bikash Pandey, Ms. Priyam Shah, and Mr. Rajendra Ghimire from Everest Biotech Pvt. Ltd., Khumaltar, Lalitpur for their technical assistance and first-class facilities on cell culture and MTT assay. I cannot go without mentioning National Forensic Science Laboratory (NAFOL), Khumaltar, Lalitpur, and Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi for offering the training, and the technical support on GC-MS analysis.

I am grateful to my respected teachers, Prof. Dr. Tribikram Bhattrai, Prof. Dr. Mohan Kharel, Prof. Dr. Ganga Prasad Kharel, Prof. Dr. Tilak R. Shrestha, Dr. Giri Raj Tripathi, Ms. Jarina Joshi, Mr. Bal Hari Poudel, and Dr. Smita Shrestha, for their endless encouragement and support throughout the whole period. I would like to acknowledge Mohan Bahadur Shrestha and Ujjwal Pradhan for their assistance in the lab and in the library, respectively. Appreciation is given to Shrawan Kumar Mishra, Birendra Prasad Gupta, Bimlesh Kumar Jha and Anurag Adhikari for their support and scholarly interactions at various points of my research. My sincere thanks to Sujindra Subedi, Namita Bhattarai, Suyog Lamichhane, Yadu Nath Poudel, and Sneha Joshi. Thanks are due to my friends Rajindra Napit, Nirmal Aryal, Pradip Paudel, Ishwar katawal, Mitesh Shrestha, Roshan Nepal, Baidhyanath Kumar Jha, Santosh Dahal,

Uddhav Karki, Mukesh Thapa, Bimala Dhakal, Sandipty Kayastha, Aarati Poudel, and Suvash Chandra Awal. Here I would like to express my infinite gratitude to my mother for her unconditional love and support.

Gyandra Ghimire

Abstract

Considerable attempts have been made globally to validate the traditional use of plants in phytomedicine. Phytomedicine can counter the challenge posed by drug resistance and toxicity by continuous search for novel drugs. This study focuses on the estimation of total phenolic content, total flavonoid content, examination of antioxidant activities, GC-MS identification of bioactive compounds, observation of antimicrobial, cytotoxic, and anticancer effects. Twelve medicinal plants with some history of use as traditional medicine have been selected for the purpose. They include *Loxogramme involuta, Hydrocotyle javanica, Phyllanthus emblica, Chrysopogan aciculatus, Rhododendron lepidotum, Geranium wallichianum, Tsuga dumosa, Anemone rivularis, Scurrula elata, Terminalia bellerica, Ranunculus scleratus,* and *Symplocos lucida.*

Total phenolic content was determined by the Folin-Ciocalteu method, total flavonoid content by aluminium chloride colorimetric method. Antioxidant activity was tested by their capacity to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals. GC-MS analysis was performed to identify the bioactive compounds. The antimicrobial efficacy was tested against *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella* Typhi*, Saccharomyces cerevisiae* and *Pichia sp.* by agar well diffusion method. Cytotoxicity was determined by brine shrimp bioassay. Anticancer activity was determined by MTT assay on HeLa and MDCK cell lines.

P. emblica (55.66 ± 0.84 mgGAE/g) possessed the highest amount of total phenolic content. Higher amount of TPC was also observed in *G. wallichianum* (54.99 ± 0.74 mgGAE/g), *S. elata* (54.71 ± 0.68 mgGAE/g), *T. bellerica* (54.69 ± 0.77 mgGAE/g), and *T. dumosa* (54.19 ± 0.93 mgGAE/g). The lowest amount of TPC was observed in *R. scleratus* (6.74 ± 0.62 mgGAE/g). The highest amount of total flavonoid content was observed in *P. emblica* (25.65 ± 0.77 mgQE/g). The lowest amount of TFC was observed in *T. bellerica* (7.51 ± 0.54 mgQE/g). TFC for other plant extracts locate within the short range between these two values. *T. dumosa* (4.54 ± 0.18 µg/ml) showed the highest antioxidant activity with the lowest IC_{50} value – lower than that observed in ascorbic acid (4.57 ± 00 µg/ml). Higher antioxidant activity was also observed in *P. emblica* (6.64 ± 0.71 µg/ml), *G. wallichianum* (7.24 ± 0.48 µg/ml), *T. bellerica* (12.08 ± 0.76 µg/ml), and *S. elata* (14.20 ± 0.94 µg/ml). *R. scleratus* (195.55 ± 2.45 µg/ml) showed the lowest antioxidant activity. A significant Pearson's correlation between TPC and IC_{50} of DPPH RSA (r = -0.9684) attributed phenolic compounds to be the main contributor of antioxidant activity in plants. GC-MS analysis showed the presence of a wide range of bioactive compounds such as fatty acids, esters, sugars, steroidal, phenolic, and heterocyclic

compounds. Maximum inhibition of *S. aureus* and MRSA both were shown by *T. bellerica*. Highest inhibition of *S.* Typhi was caused by *T. dumosa*. Minimum number of plants inhibited *K. pneumoniae* on which *L. involuta* exerted the highest effect. Ten of the twelve plants inhibited at least three of the six organisms. Eight of them inhibited at least four organisms. *G. wallichianum* inhibited five of the six organisms. *S. elata* and *T. bellerica* both inhibited all of them. Plants showed higher effects on gram positive bacteria. Nine of the twelve plants showed significant toxicity to brine shrimps (LC_{50} <500µg/ml) of which three were found to be highly toxic (LC_{50} <100 µg/ml) which includes *A. rivularis, L. involuta* and *T. bellerica.* Maximum number of plants showed higher inhibition of HeLa cell line than of MDCK. The highest inhibition of HeLa cell was shown by *S. lucida* (52.22 µg/ml) which precedes other ten with EC_{50} value less than 1000 µg/ml. *T. bellerica* (1048 µg/ml) showed the lowest inhibition. Highest inhibition of MDCK cell was shown by *L. involuta* (240 µg/ml) followed by *T. dumosa* (305 µg/ml), *A. rivularis* (407.7 µg/ml), *S. elata* (751.1 µg/ml), and *P. emblica* (925.3 µg/ml). Other showed less inhibition with EC_{50} value more than 1000 µg/ml.

The results provide justification for traditional use of medicinal plants in treatment of various diseases. The biological activities of medicinal plants may have been attributed due to the presence of bioactive compounds identified. These plants can be the candidates as the potential source of high valued phytopharmaceuticals. Further meticulous analysis of plants might lead to isolation, identification and characterization of biologically active substances.

Keywords: medicinal plants, phenolic, flavonoid, antioxidant, bioactive compounds, antimicrobial, cytotoxicity, anticancer

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List of Abbreviations

µg	Microgram
µl	Microliter
amu	atomic mass unit
ATCC	American Type Culture Collection
EC_{50}	Effective concentration 50
CDBT	Central Department of Biotechnology
CDB	Central Department of Botany
CDC	Central Department of Chemistry
DPPH	2,2-diphenyl-1-picrylhydrazyl
EB	Everest Biotech
ESON	Ethnobotanical Society of Nepal
EMEM	Eagle's Minimum Essential Medium
eV	electron volt
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography - Mass Spectroscopy
HeLa	Human Cervical Carcinoma Cell Line
IC_{50}	Inhibitory Concentration 50
IUCN	International Union for Conservation of Nature and Natural Resources
KRIBB	Korea Research Institute of Bioscience and Biotechnology
MDCK	Madin-Darby Canine Kidney Epithelial Cells
MDR	Multi-Drug Resistance
MHA	Mueller Hinton Agar
MRSA	methicillin resistant *Staphylococcus aureus*
MTT	3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide

NA	Nutrient Agar
NCCS	National Centre for Cell Science
NPHL	National Public Health Laboratory
NAFOL	National Forensic Science Laboratory
QE	Quercitin Equivalent
RSA	Radical Scavenging Activity
RT	Room Temperature
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV	Ultral Violet
WHO	World Health Organization

1 Introduction

1.1 Background

Recorded history of the use of medicinal plants is much shorter than the actual one which in fact began with the beginning of the human civilization. The vast majority of modern medicines have been derived originally from ancient herbal traditions. Plants are a great source of therapeutic molecules. It has been more than three billion years since nature started its own combinatorial chemistry (Holland, 1997). The total number of natural products produced by plants has been estimated at over 500,000 (Mendelson & Balick, 1995). Medicinal plants rich in phytochemicals have been used for centuries in the treatment and prevention of diseases. Phytochemicals may affect health, but are not essential nutrients. Most of the phytochemicals used in the modern pharmaceutical industry are secondary metabolites. Emerging factors like drug-resistance, cost-effectiveness and side-effects of synthetic drugs have led to the resurgence of phytomedicine.

It is estimated that the worldwide market potential for herbal drugs is around US$40 billion (Patwardhan et al., 2004). About one-fourth of the drugs approved by FDA during the period 1981–2002 was either natural products or based on natural products (Newmann et al., 2003). About two dozen of such drugs introduced to the market during the period 2000–2005 include drugs for cancer, neurological, cardiovascular, metabolic and immunological diseases, and genetic disorders (Chin et al., 2006).

Total number of higher plant species on earth has been estimated to be more than 250,000 (Ayensu & DeFilipps, 1978). Of that only around 6% are screened for biological activity, and 15% are phytochemically characterized (Verpoorte, 2000). Fifty million screening tests have been conducted so far making a variation of molecules, concentrations and/or bioassays (Drews, 2000).

1.2 Medicinal Plants

WHO has estimated that almost 80% of the world population rely on traditional medicine for their primary health protection (Diallo et al., 1999; Kim et al., 2005). Some countries have incorporated traditional medicine extensively in their public health system (WHO, 2000). Plants continue to be the major source of medicine in rural regions of developing countries

(Chitme et al., 2004). More than 3.3 billion people have been reported to utilize medicinal plants on a regular basis (Davidson-Hunt, 2000).

The number of species of plants estimated to exist on earth has been on the order of 250,000 to 500,000 of which only 1 to 10% are used as food by humans and other animals (Borris, 1996; Cowan, 1999). Nearly 3000 species of plants have been documented to be used as food during the course of human civilization, of which only about 150 species have been cultivated (Council, 1982), and less than 10 plant species are meeting more than 90% of the world food demand (Wilkes, 1981). About 20,000 to 70,000 species of plants are used medicinally (Alves & Rosa, 2007; Farnsworth & Soejarto, 2009; Schippmann et al., 2006).

Medicinal plants have been used in treatment of different diseases for thousands of years (Palombo, 2011). Plants have been used in the treatment of cancer for a long time (Richardson, 2001). More than one thousand plants have been found to possess significant anticancer properties (Mukherjee et al., 2001). About 50% of breast cancer patients and 37% of prostate cancer patients are estimated to use herbal products (Sini et al., 2012). Phytochemicals have also been suggested to prevent colorectal cancer and other cancers (Birt et al., 2001; Yuvaraj et al., 2010).

1.2.1 Medicinal Plants in Nepal

Nepal contains about 1,950 species of medicinal plants (about 28% of the local flora) (Baral & Kurmi, 2006; Ghimire, 2008; Manandhar, 2002; Shrestha & Shrestha, 1999; Shrestha et al., 2002; Tiwari, 1999), including 1,614 native medicinal species (Ghimire et al., 2008; Shrestha et al., 2002). The contribution of medicinal plants in Nepal is important (Kunwar, 2006). More than 80% of the rural population of Nepal are reported to use herbal remedies (Dani, 1986). Herbal medicine has been used since ancient time in many parts of the country where access to formal and modern healthcare is limited (Kunwar et al., 2010).

1.3 Bioactive Compounds

The medicinal values of plants have been claimed to lie in their phytochemical components including alkaloids, tannins, flavonoids and other phenolic compounds (Anyasor et al., 2011). With the discovery of profound physiological effect exerted by active principles on mammalian systems, efforts are being made to know the exact chemical nature of compounds and to obtain by chemical synthesis (Ramawat, 2007). Natural products are a major source of inspiration for drug development (Newmann & Cragg, 2007), with only 30% of all novel molecules introduced into the market in the period 1981–2006 being pure synthetic and all others being natural products or natural product related.

1.3.1 Phenolic

Phenolic compounds and polyphenols are the most abundant structures in plants. Polyphenols are a structural class of natural and synthetic or semisynthetic, organic chemicals characterized by the presence of large multiples of phenol structural units. The number and characteristics of these phenol structures underlie the unique physical, chemical and biological (metabolic, toxic, therapeutic, etc.) properties of particular members of the class. Examples include tannic acid and ellagitannin (Chuang et al., 2013; Quideau et al., 2011). More than 8000 different polyphenols of diverse structures have been reported (Strack & Wray, 1992).

Polyphenols are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. Some show metal chelation properties (Kähkönen et al., 1999; Pietta, 2000). Some have antimicrobial activity (Rauha et al., 2000). A great number of aromatic plants have been reported as having anti-inflammatory, antiallergic, antimutagenic, antiviral, antithrombotic, and vasodilatory actions (Hollman et al., 1996). Polyphenols can have favorable effects on the incidence of cancers and chronic diseases, including cardiovascular disease, type II diabetes, and impaired cognitive function (Rio et al., 2013).

Polyphenols historically have been considered as anti-nutrients by nutritionists, because some, eg tannins, have such adverse effects as decreasing the activities of digestive enzymes, energy, protein and amino acid availabilities, mineral uptake and having other toxic effects (Salunkhe et al., 1982). Recognition of the antioxidant activities of many polyphenols has realigned thinking toward the health benefits provided by many of these compounds (Bravo, 1998). The most important dietary phenolics are the phenolic acids (including hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins) and flavonoids (King & Young, 1999).

1.3.2 Flavonoid

Flavonoids are secondary metabolites widely distributed in the plant kingdom. They are the most abundant polyphenols in the human diet. There are six subclasses of flavonoids including anthocyanins, flavonols, flavanols, flavanones, flavones and isoflavones. More than 6000 varities of flavonoids have been identified in plants (Khatiwora et al., 2010). The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. Among the biological activities of flavonoids are actions against free radicals, free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, microbes, ulcers,

viruses and tumors and hepatotoxins (Kinsella et al., 1993). Flavonoid intake has been associated with reduced risk from death from coronary heart disease (Hertog et al., 1993).

1.4 Biological Activities

1.4.1 Antioxidant Activities

Free radicals are atoms or group of atoms with an unpaired electron. These highly reactive free radicals can start a chain reaction. Free radicals such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated through endogenous processes such as metabolism, respiration and phagocytosis. They are also generated by exogenous systems such as chemical agents, pesticides, pollutants, toxins, organic solvents, radiation, cigarette smoke, deep fried foods and herbicides (Davies, 1995; Thatoi et al., 2014). Chemical compounds that can generate free radicals are called Prooxidants. In a normal cell there is a proper balance between pro-oxidants and antioxidants but when the level of pro-oxidants increases as compared to antioxidants, they lead to oxidative stress (Collins, 2005). Free radicals react with important cellular components such as DNA, and the cell membrane, and can lead to lipid peroxidation, tissue injury and protein degradation (Gilbert, 1981; Phang et al., 2011). Oxidative stress has been implicated in the etiology of a number of diseases diseases including cancer (Kinnula & Crapo, 2004), cardiovascular disease (Singh & Jialal, 2006), neural disorders (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000), mild cognitive impairment (Guidi et al., 2006), Parkinson's disease (Bolton et al., 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna et al., 1997), aging (Hyun et al., 2006), arthritis (Ramamoorthy & Awang, 2007), atherosclerosis (Upston et al., 2003), nephritis, diabetes mellitus, rheumatism, and ischemia (Amel et al., 2013).

Antioxidants are compounds that interact with free radicals and terminate the chain reaction before the vital molecules such as lipids, proteins and DNA are damaged (Becker et al., 2004; Halliwell & Guteridge, 1989; Kumar & Chattopadhyay, 2007; Ozsoy et al., 2008; Ratnam et al., 2006; Temitope et al., 2010). Generation of these free radicals is normally balanced by an equivalent production of antioxidants through our natural antioxidant defense mechanism, which are the enzymatic antioxidants (superoxide dismutase, glutathione peroxidase, quinone reductase and catalases) and the non-enzymatic antioxidant (ascorbic acid, α-tocopherol, melatonin, β-carotene) obtained from the diet (Halliwell, 1996; Davies, 2000; Phang et al., 2011). All aerobic organisms have antioxidant defence systems to protect from harmful effects caused by free radicals. In the case of failure of the antioxidant defence system, antioxidants need to be supplemented from outside sources which can be either

synthetic and natural. Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate have toxic effects due to which restrictions have been imposed on their use. Natural antioxidants are safe with nutritional and therapeutical effects (Lopez-Velez et al., 2003). Therefore, researchers have focused their studies on plant-derived natural antioxidants (Kulisic et al., 2004). Carotenoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, polyphenols such as phenolics acids, flavonoids, proanthocyanidins, among others, are some of the antioxidants produced by plants for their survival (Jimoh et al., 2008; Ramamoorthy & Awang, 2007). Higher level of natural antioxidants (vitamins, carotenoids, phenolics) in human everyday diet can protect against cardiovascular diseases, cataract, cancer and aging- related disorders (Steffen et al., 2003).

1.4.2 Antimicrobial Effects

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries. Drug resistance to pathogenic bacteria has been commonly reported from all over the world (Mulligan et al., 1993; Piddock & Wise, 1989; Singh et al., 1992). Antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganism, immunosuppression and allergic reactions (Lopez et al., 2001). This has created immense clinical problem in the treatment of infectious diseases (Davies, 1994). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties. Plant materials remain an important resource to combat serious diseases in the world.

Major groups of antimicrobial compounds from plants include simple phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and essential oils, lectins and polypeptides (Cowan, 1999).

The action mechanisms of natural compounds are related to disintegration of cytoplasmic membrane, destabilization of the proton motive force, electron flow, active transport and coagulation of the cell content. Not all action mechanisms work on specific targets, and some sites may be affected due to other mechanisms (Burt, 2004). Important characteristics responsible for the antimicrobial action of essential oils include hydrophobic components that allow the participation of lipids from the bacterial cell membrane, which disturbs cell structures and make them more permeable (Sikkema et al., 1994). Chemical compounds from essential oils also act on cytoplasmic membrane proteins (Knobloch et al., 1989). Cyclic hydrocarbons act on ATPases, enzymes known to be located at the cytoplasmic membrane

and surrounded by lipid molecules. In addition, lipid hydrocarbons may distort the lipid-protein interaction, and the direct interaction of lipophilic compounds with hydrophobic parts of the protein is also possible (Sikkema et al., 1995). Some essential oils stimulate the growth of pseudo-mycelia, evidencing that they may act on enzymes involved in the synthesis of bacterium structural components (Conner & Beuchat, 1984).

1.4.3 Anticancer Properties

Cancer is considered to be the third leading cause of death worldwide (12.4%), the first being cardiovascular disease (30%) and the second being infectious diseases, including HIV/AIDS (18.8%) (Mathers et al., 2001). An estimated 12.7 million new cases are registered each year with 7.6 million deaths and 24.6 million persons living with cancer worldwide (Jemal et al., 2011). It is estimated that the number of deaths due to cancer may double in next 50 years and new cases of cancer may rise to 15 million by 2020 (Ferlay et al., 2015).

Cancer cells occur as a result of unique multiple genetic disorders that may arise from exposure to environmental and occupational carcinogenic agents or dietary habits and infectious agents (Sugimura, 1992). Drug resistance, particularly multidrug resistance (MDR), can make many of the clinically established anticancer drugs ineffective (Borowski et al., 2005).

Chemotherapy remains the treatment of choice in many malignant diseases (Carter & Livingston, 1982; Lage et al., 2010). Natural product could serve as chemopreventive and chemotherapeutic agent with minor side effect on normal cells and high tumor selectivity (Mehta et al., 2010). More than 60% of the anticancer drugs are of natural origin, such as plants (i.e., vincristine, irinotecan, camptothecines) and microorganisms (i.e., doxorubicin, dactinomicines, mitomycin and bleomycin) (Grever, 2001; Newman & Cragg, 2012; Newmann & Cragg, 2007; Stevigny et al., 2005). Over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them (Newmann & Cragg, 2007). The plant-derived drugs such as taxol from *Taxus* species, vinblastine and vincristine from *Catharanthus roseus*, topotecan and irinotecan from *Camptotheca accuminata*, and etoposide and teniposide from *Podophyllum peltatum* are currently used in treatment of various types of cancers (Patwardhan et al., 2004).

1.4.4 Brine Shrimp Cytotoxicity

Although many plants have valuable properties, some of them are known to carry toxicological properties as well. Recent studies indicate that although numerous plants are used as food sources, some of them may have mutagenic or genotoxic potential (Çelik &

Aslantürk, 2007). Study on pharmacology and toxicity of medicinal plants is of high importance in order to achieve a safe treatment with plant products (Parra et al., 2001).

Various assays are used for the research of potential toxicity of herbal extracts based on different biological models, such as *in vivo* assays on laboratory animals. However, recent studies employed efforts for alternative biological assays that include species of *Artemia salina, Artemia franciscana, Artemia urmiana* and *Thamnocephalus platyurus*. These toxicity tests are considered a useful tool for preliminary assessment of toxicity (Carballo et al., 2002; Mayorga et al., 2010; Veni & Pushpanathan, 2014).

During the past 30 years, the Brine Shrimp Assay has been widely used to test the toxicity of a great variety of plant products. Brine shrimp (*Artemia salina*) is most extensively studied of the *Artemia* species, estimated to represent over 90% of the studies in which *Artemia* is used as an experimental test organism (Campbell et al., 1994). The Brine Shrimp Toxicity Assay was proposed and developed by Michael et al. (1956) and later adapted by Vanhaecke et al. (1981), Meyer et al. (1982), and Sleet & Brendel (1983).

Brine Shrimp Lethality Assay has been applied as an alternative bioassay technique to screen the toxicity of plant extracts (Gadir, 2012; McLaughlin et al., 1998; Meyer et al., 1982; Moshi et al., 2010; Ogugu et al., 2012; Sharma et al., 2013; Solanki & Selvanayagam, 2013).

Since its introduction, this *in vivo* test has been successively employed for bioassay-guided fractionation of active cytotoxic and antitumor agents (Ahmed et al., 2010; Ramachandran et al., 2011). Additionally, several studies demonstrated that there is a good correlation between the results for the lethal concentration that kills 50% of the exposed population (LC_{50}) obtained with the Brine Shrimp Lethality Assay using *A. salina* and the results of the acute oral toxicity assay in mice (Arslanyolu & Erdemgil, 2006; Parra et al., 2001).

Brine Shrimp Lethality Assay is a convenient system for monitoring biological activities of various plant species. Although this method does not provide any adequate information regarding the mechanism of toxic action, it is a very useful method for the assessment of the toxic potential of various plant extracts (Gadir, 2012; Naidu et al., 2014). This method provides preliminary screening data that can be backed up by more specific bioassays once the active compounds have been isolated (Pisutthanan et al., 2004).

Due to the ethical issues in toxicological tests, substituting animals with alternative models is very important. The effectiveness of the *Artemia salina* bioassay for predicting the toxicity of plant extracts was evaluated by comparing the LC_{50} results for the brine shrimps with the LD_{50}

results for acute toxicity in rats and mice (Naidu et al., 2014; Parra et al., 2001; Sharma et al., 2013).

1.5 Hypothesis

People have been using plants in medicine since ancient past. They contain bioactive compounds such as phenolics and flavonoids. They possess antioxidant, antimicrobial, anticancer properties due to which the medicinal plants are being used for a number of ailments.

1.6 Objectives

1.6.1 General Objective

Study of twelve medicinal plants for the presence of bioactive compounds and biological activities.

1.6.2 Specific Objectives

1. Methanol extraction of twelve medicinal plants
2. Estimation of total phenolic and flavonoid content
3. Determination of antioxidant activity by DPPH free radical scavenging assay
4. Antibacterial property evaluation of the extracts on *S. aureus* ATCC – 25923, MRSA, *K. pneumoniae*, and *S.* Typhi
5. Antifungal property evaluation on *S. cerevisiae,* and *Pichia sp.*
6. Analysis of cytotoxicity of the extracts by brine shrimp bioassay
7. Observation of anticancer effects on HeLa and MDCK cell lines
8. Identification of bioactive compounds by GC-MS analysis

1.7 Rationale

Medicinal plants are known to contain bioactive compounds responsible for antioxidant, antimicrobial and anticancer activities. Antioxidants such as flavonoids and phenolics are associated with reduced risk of chronic diseases like heart disease, cancer, diabetes, neurodegeneration and other age related degenerative diseases. Growing problem of resistance and toxicity of presently available commercial drugs has necessitated the search for novel phytopharmaceuticals. Phytomedicine can counter the challenge posed by drug resistance and toxicity by continuous search for novel drugs. Nepal preserves highly valued medicinal plants which find use in varieties of ailments such as in cough, fever, cuts, wounds,

malaria, nephritis, hepatitis, cancer, diabetes, tooth aches, and joint pains, and so on. People are intimately connected to phytomedicine when faced with frequent diseases. Research inspired from tribal medicine contributes to drug discovery and socioeconomic development.

2 Literature Review

2.1 *Anemone rivularis* Buchanan-Hamilton ex De Candolle

Anemone rivularis (Ranunculaceae) (Plate 2) is a perennial herbaceous plant distributed in Nepal, northern India, Bhutan, Sri Lanka, southern Tibet, southwestern China and Myanmar (Manandhar, 2002). The genus *Anemone* (Ranunculaceae) consists of about 150 species with a near global distribution, of which about 50 species were found in the northwest and southwest of China (Wang, 1980). More than 10 species of this genus have been used as Chinese folk medicines for a long time. The rhizome of *A. raddeana* is recorded in the Chinese Pharmacopeia for the treatment of rheumatism and neuralgia (National Pharmacopoeia Committee, 2010). The entire plant and rhizome of *Anemone rivularis* have been used as folk medicines for treatment of hepatitis, muscle and joint pain, stranguria, emission, edema, etc. (Wang, 1980) (Flora of China Editorial Committee of Chinese Academy of Sciences, 1980). In Nepal, paste of the plant is given to treat cough and fever, a decoction of the root is applied to cuts and wounds and juice of the leaf is inhaled to relieve sinusitis (Manandhar, 2002).

A new saponin, rivularinin, shown to be [*α*-L-arabinofuranosyl(1→2)-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→4)-*β*-D-glucuronopyranosyl(1→3)]-3*β*-hydroxy-olean-12-en-28-oic acid was isolated from the ethanolic extract of *Anemone rivularis* (Tiwari & Singh, 1978). Six saponins were first reported from *Anemone rivularis* var. *flore-minore* one of which was a new compound with gypsogenin as aglycone first reported in genus *Anemone* (Ding et al., 2011). A new triterpene ester olean-9(11),12-dien-3-O-palmitate and six known triterpenoids lupeol, betulin, betulic acid, oleanolic acid, ursolic acid and *β*-amyrin were isolated from *Anemone rivularis* (Zhao et al., 2012). Phytochemical investigation on the whole plant of *Anemone rivularis* var*. flore-minore* led to the isolation of a new labdane-type diterpene glycoside, *β*-D-glucopyranosyl (13S)-13-hydroxy-7-oxo-labda-8,14-diene-18-oate and a new trihydroxyfuranoid lignanoid glycoside (7S,7'R,8R,8'S)-7'-butoxy-7,9'-epoxy-4,4',9-trihydroxy-3,3'-dimethoxylignane 9-O-*β*-D-glucopyranoside, together with three known triterpene and triterpenoid glycosides 3-O-*α*-L-arabinopyranosyl-hederagenin, 3-O-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-oleanolic acid, hederagenin (Ding et al., 2012). A new cerebroside, rivulacerebroside, was isolated from *Anemone rivularis* (Shao et al., 2013). Phytochemical investigation of the *n*-BuOH extract of the rhizomes of *Anemone rivularis* var. *flore-minore* led to the isolation of of five new oleanane-type triterpenoid

saponins 3-O-*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl gypsogenin 28-O-*β*-D-glucopyranosyl ester, 3-O-*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabino-pyranosyl gypsogenin 28-O-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester, 3-O-*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)-*β*-D-gluco-pyranosyl-(1→4)]-*α*-L-arabinopyranosyl oleanolic acid 28-O-*β*-D-glucopyranosyl ester, 3-O-*α*-L-rhamnopyranosyl-(1→2)-[*β*-Dglucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl 21*α*-hydroxyoleanolic acid 28-O-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester, 3-O-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-[*α*-L-rhamno-pyranosyl-(1→2)]-*α*-L-arabinopyranosyl 21*α*-hydroxyoleanolic acid 28-O-*α*-L-rhamnopyranosyl-(1→4)-*β*-Dglucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester, together with five known saponins sapindoside B, pulsatilla saponin D, 3-O-*β*-Dxylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl oleanolic acid, sieboldianoside B, 3-O-*α*-L-arabinopyranosyl gypsogenin 28-O-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (Wang et al., 2014).

The genus *Anemone* has been found to be the rich source of glycosides, especially triterpenoid glycosides with a range of biological and pharmacological activities including antitumor, antiperoxidation, antibacterial, insect deterrence (Lu et al., 2009; Mimaki et al., 2009; Sun et al., 2011; Wang et al., 2012; Wang et al., 1998; Wang et al., 1997; Ye et al., 2001; Zhang et al., 2008). The triterpene ester, olean-9(11),12-dien-3-O-palmitate, from *Anemone rivularis* was found to be effective against *B. subtilis, S. aureus, E. coli, S. typhimurium,* and *P. aeruginosa* (Zhao et al., 2012). The monodesmosidic saponins from *Anemone rivularis* var. *flore-minore* exhibited cytotoxic activity against HL-60 (promyelocytic leukemia), HepG2 (hepatocellular liver carcinoma), A549 (lung carcinoma) and HeLa (cervical carcinoma) human cancer cell lines (Wang et al., 2014).

2.2 *Chrysopogon aciculatus* (Retzius) Trinius

C. aciculatus (Retz.) Trin. (Plate 1) is a perennial grass with a creeping rhizome (Paria & Chattopadhyay, 2005) widely distributed in tropics of Asia in hills and plains common along the banks of rivers, streams and canals usually forming dense carpets (Dileep & Nair, 2015). Its culm is divided into creeping base and erect portion. The creeping base is covered with imbricate scale like old sheaths. Sheaths are long, striate, sometimes purple-tinged and imbricate (Singh et al., 2001). Leaf-blades are flat. Panicles are reddish purple, narrowly elliptic and long. Chorant is usually found in sunny, dry, exposed areas such as roadsides,

lawns, pasture, bank of rivers, water courses, etc. (Noltie, 2000). Cattle eat this species in default of anything else. It can tolerate grazing, mowing and trampling by animals (Kabir & Nair, 2009). It is very difficult to eradicate if it becomes established. It is a very good soil binder which prevents soil erosion (Dileep & Nair, 2015). The spikelets are easily disarticulating, acicular and the callus is extremely sharp, often gets attached to the skin and hairs of animals and clothes of human beings. Hence it is popularly called 'Love Grass' (Sreekumar & Nair, 1991).

Whole plants of *Chrysopogon aciculatus* is reported to be applied topically as decoction in treating swollen bodies, legs, arms in Sepik Province, Papua New Guinea (Koch et al., 2015). Leaves of *Saraca asoca*, *Chrysopogon aciculatus* and *Vitex negundo* are warmed and applied topically to affected areas 2-3 times daily in cases of pain and paralysis (Shahnaj et al., 2015). Rhizome of *Chrysopogon aciculatus* powdered with *Piper nigrum* and made into a paste is taken early in the morning on empty stomach for stomach ache and gastric disorders by tribal people in India (Saha et al., 2014; Dileep & Nair, 2015). The plant is used in Ayurveda as a diuretic (Johnson T, 1998). In Malaysia, the plant is burnt with lalang and the ashes swallowed as a remedy for rheumatism. The plant has been used in traditional Chinese medicine to treat common cold and fever. In China and Taiwan, a decoction of the roots is used to treat poisonous snake bites. In Indochina, the "seeds" are used to expel intestinal roundworms. In Nepal, a paste of the root is applied to boils (Manandhar, 2002) and smoke of the plant is used for healing the wound of toes caused due to mud (Rajbhandari, 2001).

Four new apigenin derivatives, 7-de-O-methylaciculatin, 8-C-*β*-D-boivinopyranosylapigenin, aciculatinone, and 4'O-glucosylaciculatin, along with eight known compounds, apigenin-8-carbaldehyde, kaempferol, tricin, taxifolin, 6,7,4'-trihydroxyflavone, *trans*-oxyresveratrol, aciculatin, and luteolin-7-sulfate, were isolated from an ethanolic extract of *Chrysopogon aciculatis* (Shen et al., 2012). Aciculatin (8-((2R,4S,5S,6R)-tetrahydro-4,5-dihydroxy-6-methyl-2H-pyran-2-yl)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one), a major bioactive component of *Chrysopogon aciculatus*, was first reported in 1991 showing DNA-binding activity and cytotoxicity *in vitro* (Carte' et al., 1991; Krause & Eggleston, 1991).

Methanol extract of *Chrysopogon aciculatus* exhibited *α*-glucosidase inhibitory activity (Nguyen et al., 2012). Compounds isolated from *Chrysopogon aciculatis* have been evaluated for cytotoxic activities on human cancer cell lines including MCF-7, H460, HT-29, and CEM (Shen et al., 2012). It has been found that aciculatin exerts dual inhibitory effects on inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) due to regulation of the NF-kappaB and c-Jun N-terminal kinase (JNK)/p38 pathways (Hsieh et al., 2011). Aciculatin shows

a potent anti-inflammatory effect by inhibiting the expression of lipopolysaccharide-mediated inducible nitric oxide synthase and cyclooxygenase-2 (Shen et al., 2012). Aciculatin inhibits IL-1*β*-stimulated G-CSF expression and the subsequent neutrophil differentiation, suggesting that it might have therapeutic potential for inflammatory arthritis (Shih et al., 2012). Aciculatin induces cell cycle arrest and apoptosis *via* inhibition of MDM2 expression, thereby inducing p53 accumulation without significant DNA damage and genome toxicity (Lai et al., 2012). Water extracts of *Chrysopogon aciculatus* exhibited allelopathic effects on *Borreria latifolia* (Li et al., 2012).

2.3 *Geranium wallichianum* D. Don ex Sweet

Geranium wallichianum D. Don ex Sweet (Plate 2) belongs to family Geraniaceae and is commonly known as "Shephred's needles" (Nadkarni, 1976). It is a tall much branched procumbent or erect perennial herb found in the Himalayas, from Kashmir to Nepal at the altitude of 7000-11000 feet (Vakil, 1956; CSIR, 1968). The herb possesses the astringent properties of the genus to a marked degree. The root stock is used in eye troubles as a substitute for that of Cotis teeta Wall. The herb is also in the treatment of toothache (Watt, 1972). The rhizomes of the plant are used in the treatment of mouth ulceration, dysentery, diarrhea, passive hemorrhage, and leucorrhoea (Shinwari et al., 2003). Juice of the plant is applied to stop bleeding from fresh cuts and a paste is applied to relieve joint pains while root of *Geranium wallichianum* mixed with bark of *Prunnus cerasoides* is used for peptic ulcer (Manandhar, 2002). *G. thunbergii* has been used in intestinal disorders in Japan (Ito et al., 1999).

The GC and GC-MS analysis of essential oil of *G. wallichianum* showed the presence of bioactive compounds such as germacrene D, *β*-caryophyllene, selinene < 7-*epi*-*α* >, δ-cadinene, *α*-cadinol, linalool, *β*-bourbonene, *γ*-amorphene, *α*-humulene, caryophyllene oxide, isophytol, *β*-(*E*)-farnesene, (*E,E*) geranyl linalool, terpineol-4-ol, sabinene and cubenol (Tewari et al., 2015). Tannins, Geranin, Geraninic acid B and C, Phyllanthusine F, Dehydraallagitallin, Dehydrogernin, Furosinin and Furosine were isolated form *G. wallichianum* (Okuda et al., 1982). Six compounds ursolic acid, *β*-sitosterol, stigmasterol, *β*-sitosterol galactoside, herniarin, and 2,4,6-trihydroxyethylbenzoate were isolated and characterized for the first time from *Geranium wallichianum* (Ismail Mohammad et al., 2009).

The antioxidant capacity of *Geranium wallichianum* has been investigated by DPPH radical scavenging assay and EtOAc extract was found to be most potent with IC_{50} 19.05 ug/mL (Ismail et al., 2009). Antibacterial, antifungal (Atta-ur-Rahman et al., 1991), phytotoxic

(McLaughlin et al., 1991), cytotoxic (Meyer et al., 1982) and insecticidal (Naqvi & Parveen, 1991) activities of *G. wallichianum* has been reported. The crude extracts and pure compounds isolated from rhizomes and leaves of *Geranium wallichianum* showed varied degree of antimicrobial activities (Ismail et al., 2012). The crude extract of *Geranium wallichianum* when tested for antifungal, antibacterial, insecticidal, phytotoxic and cytotoxic activities, displayed moderate activity against human, animal and plant pathogens in antifungal tests and similar activity against *Staphylococcus aureus* in antibacterial tests but not any remarkable activity in rest of the bioassays (Ahmad et al., 2003). Ursolic acid and benzoic ester from *Geranium wallichianum* were found to be capable of inhibiting urease enzyme but *β*-sitosterol, stigmasterol and *β*-sitosterol galactoside were not (Ismail et al., 2012). Lipoxygenase enzyme was more significantly inhibited as compared to urease (Ismail et al., 2012).

2.4 *Hydrocotyle javanica* Thunb

Hydrocotyle javanica Thunb (Umbelliferae) (Plate 1) is a prostrate herb, occurring from tropical to temperate zones, distributed in Nepal, northern India, Bhutan, Tibet, and Myanmar (Manandhar, 2002). Juice of the plant is used to treat fever, cough and boils (Manandhar, 2002). The genus *Hydrocotyle* contains some 130 species worldwide (Du and Ren, 2010). Most of them inhabit marshy, moist shady and understory environments in tropical and subtropical forests. The whole plants of the *Hydrocotyle* species are often used in Taiwan folk medicine for treating common cold, tonsillitis, cephalitis, enteritis, dysentery, zoster, eczema, period pain, hepatitis and jaundice (Chang et al., 2003). Traditionally, the fresh plant parts of *H. javanica* are ingested orally to cure sores of throats and lungs. Leaf juice is often used as eye drops to cure eye infection and leaf paste was used in dressing of wounds to reduce swelling and juice of shoots can treat gastritis and constipation. The leaves of *H. javanica* Thunb. are used in traditional medicine in China and Thailand as liniment on rashes (Inta et al., 2008). The poultices of *H. bonariensis* Lam. are applied externally to heal wounds, including freckles. Internally, its decoction is diuretic, antiphlogistic, hepatic, against dropsy and pectoral. The juice is emetic, diuretic, and useful against illnesses of lungs, liver, spleen and bladder (Eduardo et al., 2006). The leaf juice of *Hydrocotyle mannii* Kook.f. along with *Cynoglossum amplifolium* is used locally in cataract (Giday et al., 2009). *Hydrocotyle rotundifolia* Roxb. leaf juice is taken to cure fever and sometimes is used as brain tonic (Barukial & Sarmah, 2011). *H. sibthorpioides* is used to treat fevers, wounds, boils, abscesses, boils, cirrhosis, cold, cough, hepatitis, itching, jaundice, sinusitis and sore throat in Madhya Pradesh, India (Quamar & Bera, 2014).

Methanol extract of *Hydrocotyle javanica* showed the presence of alkaloids, flavonoids, phenols, tannins, leucoanthocyanidins and cardiac glycosides while water extract showed the presence of alkaloids, saponins and triterpenoids (Mandal et al., 2016). Five new oleanane-type triterpene saponins including steganogenin 3-O-*β*-D-glucopyranoside, steganogenin 3-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, steganogenin 3-O-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, chichipegenin 3-O-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside and chichipegenin 3-O-*β*-D-glucopyranosyl-(1→2)*β*-D-glucopyranoside, along with four known oleanane-type triterpenes and triterpene saponins were isolated from ethanolic extract of the whole plant of *Hydrocotyle nepalensis* (Ma et al., 2016). Seven new oleanane-type triterpenoid saponins, hydrocotylosides I−VII, and one known saponin, udosaponin B, were isolated from the methanol extract of the whole plants of *Hydrocotyle sibthorpioides* (Matsushita et al., 2004).

Several studies have confirmed the pharmacological activity and therapeutic potential of the genus *Hydrocotyle* (Srinivasan et al., 2001). Water extracts of four *Hydrocotyle* species *H. nepalensis*, *H. setulosa, H. batrachium* and *H. sibthorpioides* showed antioxidant activities in descending order consistent with the antiproliferative activities against human hepatoma Hep3B cells suggesting *Hydrocotyle nepalensis* to be a good source of natural antioxidant among the *Hydrocotyle* genus (Huang et al., 2008). The compounds isolated from *H. nepalensis* exhibited cytotoxic activities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) (Ma et al., 2016). *H. javanica* exhibited larvicidal activity against *Culex quinquefasciatus* (Tennyson et al., 2012), venom neutralization effect and antihaemolytic activity against *Naja nigricollis* venom (Kumarapppan et al., 2011), bactericidal activity against food poisoning, human pathogenic gastrointestinal and topical bacteria (Mandal et al., 2016) and a high antioxidant and genotoxic properties (Wan-Ibrahim et al., 2010). *H. sibthorpioides* inhibited the growth of transplanted tumors in mice, such as Hep (hepatic carcinoma), S180 (sarcoma) and U14 (uterine cervical carcinoma) (Yu et al., 2007), demonstrated *in vitro* and *in vivo* antiviral activities against the replication of hepatitis B virus and dengue virus (Huang et al., 2013b; Husin et al., 2015) and showed effectiveness in curing chronic alcohol-induced hepatic injury and fibrosis in rats (Huang et al., 2013a). *H. leucocephala* and *H. sibthorpioides* exhibited immunomodulatory effects (Ramos et al., 2006; Yu et al., 2007). *Hydrocotyle umbellata* exhibited analgesic, anti-inflammatory, anxiolytic and sedative effects in mice (Florentino et al., 2013; Rocha et al., 2011). *H. bonariensis* showed antioxidant activity and chemopreventive effect against oxidative stress and galactose-induced cataract (Ajani et al., 2009).

2.5 *Loxogramme involuta* (D. Don) Persl

Loxogramme involuta (Polypodiaceae) (Plate 1) is an epiphytic fern found in Nepal, India, Sri Lanka and Indo-China (Manandhar, 2002). Juice of the rhizome is used to treat cuts and wounds (Manandhar, 2002).

2.6 *Phyllanthus emblica* Linnaeus

Phyllanthus emblica Linnaeus (Euphorbiaceae) (Plate 1) is a deciduous tree indigenous to tropical South-East Asia and occurs mainly in Nepal, India, Sri Lanka, Malaysia, Mayanmar (Khan, 2009; Zhang et al., 2003). Traditionally, the fruit of *P. emblica* is used to treat various ailments including diarrhoea, jaundice, inflammation, cerebral and intestinal disorders, diabetes mellitus, coronary heart disease, cancer, rheumatic pain, diseases of the eye and genitalia, gonorrhoea, constipation, asthma (Aslokar et al., 1992; Baliga & Dsouza, 2011; Perry, 1980; Rao & Siddiqui, 1964; Scartezzini & Speroni, 2000).

P. emblica contains hydrolyzable tannins Emblicanin A, Emblicanin B, punigluconin and pedunculagin (Ghosal et al., 1996). The seed contains various fatty acids like linolenic acid, linoleic acid, oleic acid, palmitic acid, stearic acid and myristic acid (Thakur et al., 1989), flavonoids such as Kaempferol-3-O-*α*-L-(6”-methyl) rhamnopyranoside, Kaempferol-3-O-*α*-L-(6”-ethyl) rhamnopyranoside (Rahman, 2007), alkaloids such as Phyllantidine and phyllantine (Khanna & Bansal, 1975). The fruit contains quercetin, Gallic acid, ellagic acid, 1-O-galloyl-*β*-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, chebulagic acid, corilagin and isostrictinnin (Zhang et al., 2003). A new acylated glucoside, apigenin7-O-(6”-butyryl-beta)-glucopyranoside was isolated from the methanolic extract of the leaves of *P. emblica* (El-Desouky et al., 2008). Phyllaemblic acid, a novel highly oxygenated norbisabolane were isolated from the roots of *P. emblica* (Zhang et al., 2000). Roots of *P.emblica* are also a rich source of Ellagic acid and lupeol (Kapoor, 1990; Rastogi & Mehrotra, 1993). Four new compounds 3,4,8,9,10-pentahydroxydibenzo[b,d]pyran-6-one, 3,4,3'-tri-O-methylellagic acid, lup-20,29-en-3*β*,30-diol and betulin were isolated first time from the *P. emblica* (Zhang et al., 2013).

Methanol extract of *P. emblica* exhibited the significant scavenging activity against DPPH, O2•–, OH• and NO radicals and also significantly inhibited the oxidation of low density lipoprotein (Nampoothiri et al., 2011). The ethyl acetate fraction of a methanolic extract of *P. emblica* fruits showed strong NO scavenging activity *in vitro* (Kumaran & Karunakaran, 2006). Extracts of *P. emblica* also exhibited significant protection to DNA against oxidative damage (Kumar et al., 2006). *P. emblica* inhibited the growth of Chinese hamster ovary cells

(CHO) (Sumantran et al., 2007), human hepatocellular carcinoma (HepG2) and lung cancer cells (A549) (Pinmai et al., 2008). *P. emblica* fruits inhibited hepatocarcinogenesis (Jeena et al., 1999), protected from sublethal gamma radiation (Singh et al., 2005), and reduced the effects of radiation (Jagetia et al., 2002). *P. emblica* inhibited cyclophosphamide-induced suppression of humoral immunity (Haque et al., 2001), chromium induced free radical production, apoptosis and DNA fragmentation (Sai Ram et al., 2003) and restored the antioxidant status. *P. emblica* showed potent antibacterial activity against *E. coli*, *K. pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *P. aeruginosa*, *S.* Typhi, *S. paratyphi* A & B, and *Serratia marcescens* (Saeed & Tariq, 2007) and also inhibited *Bacillus subtilis, S.* Typhi, *Bacillus cereus*, *P. aeruginosa,*, *Shigella boydii*, *Shigella dysenteriai*, *S. aureus*, *Sternbergia lutea*, *E. coli*, *S. paratyphi*, *Vibrio parahaemolyticus* and *V. mimicus* (Rahman et al., 2009). *P. emblica* reduced bacterial colonisation in the lung of *Klebsiella pneumoniae*-induced mice (Saini et al., 2008). *P. emblica* inhibited the mRNA expressions of tyrosinase and related proteins (TRP-1 and TRP-2) in B16 murine melanoma cells and also suppressed the LPS-induced pro-inflammatory genes (COX-2, iNOS, TNF-α, IL-16 and IL-6) expression in RAW 264.7 murine macrophage cell (Sripanidkulchai & Junlatat, 2014).

2.7 *Ranunculus scleratus* Linnaeus

Ranunculus sceleratus Linn. (marsh-crowfoot) (Plate 3) belonging to the family Ranunculaceae is an erect annual herb which is often found on damp terrain, riverbanks and small water bodies (Kanjilal, 1997). This species originated in the northern hemisphere and is widely distributed in Nepal, northern India, Bhutan, central Asia including Pakistan, China, Mongolia, Siberia, Japan, Europe, and North America (Manandhar, 2002). It is widely used in traditional Chinese medicine (Wu et al., 1999) due to its excellent therapeutic effects (Mei et al., 2012). The fresh or dry plant can be used to treat cancer of the esophagus and the breast (Li, 1999). *Ranunculus sceleratus* has unique toxicological and pharmacological activities. The leaves of this plant cause dermatitis, raise blisters on the skin and were formerly used by professional beggars to produce or maintain open sores and blisters to gain sympathy (Chopra et al., 1958). The plant is very acrid and poisonous, producing violent irritant effects if taken internally (Manandhar, 2002). However, the toxic properties of the plant get destroyed when consumed after boiling. The plant is considered stimulant and diuretic. Leaf juice is used in sciatica, rheumatism, dysuria, asthma and pneumonia. It is use in cutaneous disorders. Seeds are prescribed to cure kidney troubles (Ambasta et al., 1992; Gangwar & Joshi, 2008).

Ranunculuss sceleratus was investigated for pharmacologically active substances, namely histamine, acetylcholine, and 5-hydroxytryptamine, which are found in other plants that cause irritation (Bhargava et al., 1965). The various tryptamine derivatives were detected in *Ranunculus sceleratus* which are involved in the synthesis and metabolism of 5-hydroxytryptamine (Bhargava et al., 1965). Six compounds stigmasta-4-ene-3,6-dione, isoscopoletin, scoparone, protocatechuic aldehyde, protocatechuic acid and stigmasterol were isolated from *Ranunculus sceleratus* (Gao et al., 2005).

Leaf extract of *Ranunculus sceleratus* was thermostable up to 100°C and retained its antifungal activity even after autoclaving. It possessed a quick fungicidal action and even its volatile vapours were fungitoxic (Misra & Dixit, 1978). *Ranunculus sceleratus* exhibited anti-dermatophytic activity against five strains of dermatophytes *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporum gypseum* and *Microsporum fulvum* (Sharma et al., 2012). Chloroform, ethyl acetate, *n*-butanol and aqueous fractions of *R. sceleratus* have exhibited enzyme inhibition, antimicrobial and antioxidant effects, which identifies it as a potential drug candidate for the treatment of Alzheimer's and other diseases (Shahid et al., 2015). In addition to its medicinal value, *R. sceleratus* has other potential applications. Recent studies suggest that it is capable of purifying organic sewage and the industrial wastewater containing abundance of heavy metals. *R. sceleratus* has also been considered as a potential bio-indicator of eutrophication in aquatic habitats (Xu et al., 2004).

2.8 *Rhododendron lepidotum* Wallich ex G. Don

The genus *Rhododendron* comprises more than 900 species, the majority of which are indigenous to Asia (Tiwari & Chauhan, 2005; Zhao et al., 2006). *Rhododendron lepidotum* (Ericaceae) (Plate 2) is distributed in Nepal, northern India, southeastern Tibet, southwestern China and northern Myanmar (Manandhar, 2002). It is mainly found in the Western Himalayas along with *R. campanulatum*, *R. arboretum*, and *R. anthopogon*. The genus *Rhododendron* has a long history of use in inflammation, symptoms of cold, gastrointestinal disorders, skin diseases and as pain killers (Popescu & Kopp, 2013). *Rhododendron lepidotum* has been used in headache (Khan et al., 2008; Li et al., 2000), fever, cough, cold, tonsillitis (Rajbhandari et al., 2009). *Rhododendron adamsii* Rehder is used as a stimulant and tonic (Aleksandrova, 1975; Blinova et al., 1961). Dried leaves of *R. dauricum* are used as an expectorant and in the treatment of acute and chronic bronchitis (Tang & Eisenbrand, 1992).

Rhododendron lepidotum showed the presence of flavonoids, terpenoids, steroids, glycosides, coumarins and saponins (Bhattarai et al., 2010). *Rhododendron lepidotum* afforded the isolation of 3*β*-*O*-acetyllup-20(29)-ene, lupeol, *β*-sitosterol and 3*β*-hydroxy-12-ursen-28-oic acid, 3*β*-olean-12-en-28-olic acid and *β*-*D*-fructofuranosyl-*α*-*D*-glucopyranoside (Lamichhane et al., 2014), rhodonin and rhodonetin (Khan et al., 2008), 7-*O*-*β*-*D*-glucopyranosyl-8-methoxybenzopyranone and 7-hydroxy-8-*O*-*β*-glycosylbenzopyranone, 7,8-dihydroxy coumarin (daphnetin) (Ahmad et al., 2010), daphnin, daphnetin, daphnetin glucoside, rhodonetin, rhodonin, and umbelliferone (Shakeel-U-Rehman et al., 2010), 8-[2',6'-dimethoxy-4'-(1",2",3"-trihydroxy-propyl)-phenyl]-7-hydroxy benzopyranone, 3-O-*β*-d-glycopyranosyl betulinic amide and 8-hydroxy-7,7'-oxydicoumarin (Shakeel-U-Rehman et al., 2013), 2,4,6-trihydroxacetophenone-3,5-di-*C*-*β*-*D*-glucoside, hopenol B, lupeol, ursolic acid, avicularin, quercetin, myricetin, hyperoside, myricetin-3'-*O*-*β*-*D*-xyloside, (+)-taxifolin-3-*O*-*α*-*L*-arabinopyranoside, (+)-taxifolin-3-*O*-*β*-*D*-glucopyranoside, lyoniside, confluentin, 2-(4-hydroxyphenyl)-ethyltriacontanoate, 2,6-dimethoxy-4-hydroxyphenyl-1-*O*-*β*-*D*-glucopyranoside, (–)-2-hydroxy-5-(2-hydroxyethyl)phenyl-*O*- *β*-*D*-glucopyranoside, (–)-isolariciresinol, and isofraxoside (Zhou et al., 2012). Chemical constituents such as triterpene and flavanone glycosides (Takahashi et al., 2001), anti-HIV principles rhodoaurichromanic acid A and rhodoaurichromanic acid B (Kashiwada et al., 2001), diterpenoids rhodomolleins XV-XVIII, kalmanol and rhodojaponins III and VI (Li et al., 2000), dihydroflavonol glycoside(Chosson et al., 1998a), phloroacetophenone glycoside (Chosson et al., 1998b), terpenoids (Kloocke et al., 1986) and iridiod glycosides (Fan et al., 2001) were isolated from different species of *Rhododendron* genus.

Six coumarin compounds isolated form *Rhododendron lepidotum* showed better antibacterial activity than their acyl derivatives against *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* in which Daphnetin displayed the best antibacterial activity followed by rhodonetin against *S. aureus* and MRSA (Shakeel-U-Rehman et al., 2010). *Rhododendron lepidotum* showed high anti-proliferative activity against HeLa cells (Bhattarai et al., 2010), very mild to no toxicity against Vero and MDCK cells (Rajbhandari et al., 2009) and a weak activity against Influenza virus A (Rajbhandari et al., 2009). *Rhododendron lepidotum* exhibited cytotoxicity against brine shrimp larva and antimicrobial activity against *Staphylococcus aureus* (Bhattarai et al., 2010). The leaves and twigs of *R. dauricum* display significant anti-HIV activity (Kashiwada et al., 2001).

2.9 *Scurrula elata* (Edgeworth) Danser

Scurrula elata (Edgeworth) Danser (Plate 3) is a hemiparasitic plant of family Loranthaceae distributed in Nepal, India, Bhutan, Tibet, and western China. The family Loranthaceae contains about 75 genera most of which are known as mistletoes (Barlow, 1991; Calvin & Wilson, 2006). The leaves of *S. elata* are ground into a paste to relieve joint pain (Acharya & Acharya, 2009) and entire plants to hasten fracture recovery (O'Neill & Rana, 2016). The fruits of S. elata are edible and used for trapping birds (Acharya & Acharya, 2009; Joshi & Joshi, 2007; Kunwar & Adhikari, 2005; Limbu & Rai, 2013). Leaves are also used as fodder (Duwadee & Kunwar, 2001; Manandhar, 2002; Nepal, 1999; Shrestha, 1988, 1989).

Chemical and pharmacological studies of plants from the genus *Scurulla* led to the isolation of several bioactive compounds. Quercetin, quercitrin and glycoside 4"-O-acetylquercitrin were isolated from *Scurrula ferruginea* (Lohézic-Le et al., 2002). (*Z*)-9-octadecenoic acid, (*Z,Z)*-octadeca-9,12-dienoic acid, (*Z,Z,Z)*-octadeca-9,12,15-trienoic acid, octadeca-8,10-diynoic acid, (*Z*)-octadec-12-ene-8,10-diynoic acid, octadeca-8,10,12-triynoic acid, theobromine, caffeine, quercitrin, rutin, icariside B, aviculin, (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, (-)-epigallocatechin-3-*O*-gallate, (+)-gallocatechin, and (-)-epigallocatechin were isolated from *Scurrula atropurpurea* (Ohashi et al., 2003).

Fatty acids, monoterpene glycoside, lignan glycoside and flavanes from *Scurrula atropurpurea* inhibited cancer cell invasion (Ohashi et al., 2003). *Scurrula atropurpurea* also modulated the total plasma nitrate/nitrite levels and reduced endothelial damage by increasing endothelial progenitor cells in DOCA-salt hypertensive rats (Athiroh et al., 2014). *Scurrula oortiana* showed significant cytotoxicity against WEHI-164 cells and increased tumour cell sensitivity to TNFalpha mediated lysis (Murwani, 2003). Quercetin from *Scurrula ferruginea* potentially inhibited U251 (human glioblastoma cells) (Lohézic-Le et al., 2002). Polysaccharides from *Scurrula parasitica* down-regulated the expression of Ki-67, CyclinD1 and Bcl2 proteins and up-regulated the expression of Bax protein which inhibited the cancer cell proliferation and enhanced apoptosis (Xiao et al., 2010).

2.10 *Symplocos lucida* (Thunberg) Siebold & Zuccarini

Symplocos lucida (Thunberg) (Plate 3) belongs to a unigeneric family Symplocaceae consisting of only one genus named *Symplocos* (Cronquist, 1981; Takhtajan, 1997). The genus *Symplocos* consists of about 300–500 species. These plants are distributed in tropical and subtropical regions of Asia, Australia and America (Cronquist, 1981; Hegnauer, 1973; Takhtajan, 1997). Traditionally, plants of the genus *Symplocos* have been widely used in the treatment of

various diseases. *S. racemosa* is used in Ayurvedic medicine for the treatment of diarrhea, dysentery, eye diseases, gum bleeding, menorrhagia and uterine disorders (Watt, 1972). *S. chinensis* is a potent remedy for malaria, tumefaction, enteritis, nephritis, and snake bite (Li et al., 2003). *S. cochinchnensis* is used in the treatment of leprosy, tumors, diarrhea, dysentery, menorrhagia, inflammation, and uterine disorders (CCTHD, 1992).

The phytochemical investigation of *Symplocos* species had resulted in isolation of confusoside, trilobatin, *β*-sitosterol, *β*-amyrin, symplocoside, salireposide, benzoylsalireposide, oleanolic acid and stigmasterol (Abbasi, 2004). Compounds such as (-)-pinoresinol-*β*-D-glucoside and (-)-pinoresinol monomethyl ether-*β*-D-glucoside were isolated from *Symplocos lucida* (Inouye et al., 1973). *Symplocos racemosa* afforded the isolation of flavans, symposide and its glycoside, (–)-epiafzelechin (Dhaon et al., 1989), *n*- hexacosanol, *n*-octacosanol, nonaeicosanol, *n*-hentriacontanol, methyl triacontanoate, tricontyl palmitate (Gupta & Sharma, 2015), 28-hydroxy-20*α*-urs-12,18(19)-dien-3*β*-yl acetate, 3-oxo-urs-20*α*-12,18(19)-dien-28-oic acid, 24-hydroxyolean-12-en-3-one, betulin and oleanolic acid (Ali et al., 1990). Symplocososides A-F were isolated from *Symplocos chinensis* (Tang et al., 2004). Octacos-1-ene, stigmasterol and lupeol were isolated from *Symplocos paniculata* (Kumar & Jangwan, 2012).

Recently much attention has been paid to the genus *Symplocos* due to their diverse biological activity in treating various disorders. *Symplocos racemosa* exhibited antioxidant, and antitumor properties (Vijayabaskaran et al., 2010), antifibrinolytic activity (Dhaon et al., 1989), anti-inflammatory, and analgesic effects (Mehjabeen et al., 2014), and antibacterial activities against *Propionibacterium acnes* and *Staphylococcus epidermidis* (Devmurari, 2010; Kumar et al., 2007). *S. racemosa* exerted anticancer effects on HL 60, and HeLa cancer cell lines (Bhuvan et al., 2009). Symconoside A and symconoside B from *Symplocos racemosa* inhibited phosphodiesterase-I (Ahmad et al., 2005). Locoracemosides A, B and C from *Symplocos racemosa* inhibited *α*-chymotrypsin (Rashid et al., 2008). *S. chinensis* root extract and an ursane triterpenoid, 2*β*,3*β*,19*α*,24-tetrahydroxy-23-norurs-12-en-28-oic acid, from it inhibited B16 and BGC-823 cells (Li et al., 2003). Triterpenoid saponins from *Symplocos chinensis* were found cytotoxic against different cells such as symplocososide A against KB, HCT-8, A549, and HELF cells, symplocososide C against HCT-8 and BGC-823 cells, and symplocososide F against HCT-8 cells (Tang et al., 2004). Matairesinol and harman from *Symplocos setchuensis* inhibited HIV replication in H9 lymphocyte cells (Ishida et al., 2001). *S. cochinchinensis* showed antidiabetic and antilipidemic effects in streptozotocin induced diabetic rats (Sunil et al., 2012).

2.11 *Terminalia bellirica* (Gaertner) Roxburgh

Terminalia bellirica Roxb (Combretaceae) (Plate 3) is a large deciduous tree distributed in Nepal, India, Sri Lanka, Southeast Asia (Manandhar, 2002), and equally in Africa and America (Sastri, 1952). It is widely used in Ayurveda, Siddha and Chinese systems of medicine(Saraswathi et al., 2012) for the treatment of anaemia, asthma, cancer, constipation, diarrhoea, dysuria, headache, hypertension, inflammation and rheumatism (Duke et al., 2002). *T. bellirica* is effective in diarrhoea, leucoderma, impotence, bronchitis, cold, cholera, respiratory tract infections, anaemia, haemorrhoids, eye infections, as a brain tonic and as a diuretic to remove kidney stones (Chopra et al., 1956; Sharma et al., 2001; Singh et al., 2002).

Terminalia bellirica contains alkaloids, flavonoids, tannins, saponin, glycosides, terpenoids and polyphenols (Arulmozhi & Surya, 2016). *Terminalia bellirica* afforded the isolation of termilignan, thannilignan, 7-hydroxy-3',4'-(methylenedioxy) flavone, anolignan B, gallic acid, ellagic acid, *β*-sitosterol, arjungenin, belleric acid, bellericoside and cannogenol 3-O-*β*-D-galactopyranosyl-(1→4)-O-*α*-L-rhamnopyranoside (Khan & Gilani, 2008; Nandy et al., 1989). It also contains numerous phytoconstituents such as gallo-tannic acid, bellericanin, ellagic acid, gallic acid , termilignan, flavone and anolignan B, tannins, ellargic acid, galloyl glucose and chebulaginic acid, phenyllemblin, mannitol, glucose, fructose and rhamnose, flavonoids (Khan & Gilani, 2008).

Terminalia bellirica showed antidiabetic and hypolipidemic activity (Arulmozhi & Surya, 2016). It was effective against obesity, insulin resistance, and hyperlipidemia (Makihara et al., 2012). *Terminalia bellirica* suppressed absorption of triacylglycerol and inhibited pancreatic lipase activity (Makihara et al., 2012). *Terminalia bellirica* stimulates the secretion of insulin and enhance its action and inhibits starch digestion (Kasabri et al., 2010). *Terminalia bellirica* showed better glucose uptake potential in *Vero*, L-6 and 3T3 cell lines (Das & Devi, 2015). Ethnolic extract of *Terminalia bellirica* triggers both non-specific and specific cellular immunity (Manjunatha et al., 2011). Ethyl acetate and methanolic extract of *Terminalia bellirica* fruits displayed antiviral activity at very low concentrations (Nagendraswamy et al., 2013). *Terminalia bellirica* exhibits anti-asthmatic, antitussive, anti-spasmodic, anti-mutagenic and anti-HIV properties (Wadekar & Patil, 2008). *T. bellirica* exhibited antioxidant, antimicrobial, and antiinflammatory activities (Manohar et al., 2012; Upadhyay et al., 2015). *Terminalia bellirica* Roxb. fruits extracts have shown nephroprotective effect in ethylene glycol induced urolithiatic model (Upadhyay et al., 2015). *Terminalia bellirica* are therapeutically effective for the treatment of CaOx stones (Upadhyay et al., 2015). Alcoholic extract of *Terminalia bellirica* showed anti-colitic activity in dextran sulfate sodium induced

colitis (Desai et al., 2014). *Terminalia bellirica* leaf showed a strong angiogenic activity (Prabhu & Gopal, 2011; Vinoth et al., 2012). Ethanolic and aqueous extracts of *Terminalia bellirica* possessed significant analgesic and antipyretic in mice and rats (Sharma et al., 2010).

2.12 *Tsuga dumosa* (D. Don) Eichler

Tsuga dumosa D. Don (Pinaceae) (Plate 2) is an economically as well as medicinally important conifer (Fang et al., 1985) found in Nepal, India and Myanmar (Manandhar, 2002). In Nepal, it is found in the northern belt at an altitude of 2000–3600 m in association with other conifers like *Taxus, Abies* and *Picea* (Bhattacharyya et al., 1992). It is commonly known as "Hemlock Spruce" and locally called as "Chune Salla", "Gobre Salla" or "Thingre Salla" (Manandhar, 2002). The plant has been extensively used for timbering and lumber products because of its resistance to decay. The bark of this plant is a rich source of tannin, hence can be used for dying (Zhao et al., 2005). A solid self bioadhesive herbal compound isolated from *T. dumosa* was used for the treatment of the oral mucosal disorder and for patients exhibiting herpetic stomatities lesions, aphthous ulcers, mucosal inflammation, toothache and lesions on the lips and gingival (Domb & Wolnerman, 2002).

Phytochemical screening of *T. dumosa* showed the presence of volatile oil, fatty acids, triterpenes, sterols, carotenoids, polyphenols, reducing compounds, anthracenocoids, coumarins, flavone aglycones, tannins and saponins (Dhakal et al., 2011). *Tsuga dumosa* afforded the isolation of nonacosan-10-ol, stigmasterol, α-sitosterol, α-sitosterol glucoside (Dhakal et al., 2011), dumosaol elucidated as 3-(4-hydroxy-3-methoxy-benzyl)-5-[2-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydrobenzofuran-5-yl]-4-hydroxymethyl-dihydro-furan-2-one (Zhao et al., 2005), 4,8-dihydroxyepipinoresinol (Zhao et al., 2004b), (+)-parryin-15,16-o-isopropylidene, (-)-isopimara-8 (14)-en-15,16-O-isopropylidene, isopimara-8-en-15,16-O-isopropylidene and isopimara-8-en-15,16-diol (Zhao et al., 2004a).

3 Materials and Methods

3.1 Collection of Plant Materials

Plants were selected based on their ethnomedicinal use in the study area. During sample collection, habitat features of plant species like GPS location, associated species, habitat type and forest type were recorded. Ethnobotanical information was documented on the basis of field based primary data collection through semi-structured and unstructured questionnaires and secondary information was collected from published and unpublished literatures. Information of plant specimens like local names, vernacular name, plant parts used, purpose of use, mode of preparation and administration for medicinal and other purposes were collected. The plant specimens were collected from the study area during the period of June – October 2014. The specimens were identified in the field with the help of the local people for the vernacular name. The voucher specimens were collected and dried which were then authenticated by Ethnobotanical society of Nepal (ESON) with the help of taxonomic literatures (Grierson & Long, 1984-1999; Polunin & Stainton, 1987; Press et al., 2000) along with consultation of taxonomists and thus prepared herbarium specimens were deposited at Tribhuvan University Central Herbarium (TUCH).

Table 3.1 Name, collection site, parts used and ethnobotanical information of 12 medicinal plants.

Scientific name	Family	Vernacular name	Collection site	GPS coordinates	Elevation (m)	Plant parts	Ethnobotanical information
Loxogramme involuta	Polypodiaceae		Phulkharka-3, Dhading	N 28° 04.510', E 84° 55.104'	1750	Whole plant	Cuts and wounds
Hydrocotyle javanica	Apiacea	Ghortapre	Baseri-5, Dhading	28° 06' 14.7'' 84 51 42.7	1378	Whole plant	Fever
Phyllanthus emblica	Euphorbiaceae	Amala	Budhathum-9, Dhading	N 28° 04' 39.9'', E 84° 50' 22.4''	850	Bark	Stomach uneasiness

Chrysopogon aciculatus	Poaceae	Kuro ghans	Baseri-5, Dhading	N 28° 06' 14.8'', E 84° 51' 42.9''	1380	Whole plant	Wound, cut delivery problems
Rhododendron lepidotum	Ericaceae	Sundhup	Phulkharka-2 Dhading	N 28° 06.286', E 84° 54.436'	2890	Leaf, Stem	Rheumatic pain and Syphilis
Geranium wallichianum	Geraniaceae	Urmen	Phulkharka-3, Dhading	N 28° 05.456', E 84° 55.379'	2350	Root	Gastritis and female problems
Tsuga dumosa	Pinaceae	Gobre salla	Phulkharka-3, Dhading	N 28° 06.290', E 84° 54.512'	2870	Bark	NA
Anemone rivularis	Ranunculaceae	Hatti paile	Phulkharka-3, Dhading	N 28° 05.459', E 84° 55.455'	2366	Whole plant	Cough and fever
Scurrula elata	Loranthaceae	Liso	Phulkharka-3 Dhading	N 28° 05.292', E 84° 55.285'	2275	Branches	NA
Terminalia bellerica	Combretaceae	Barro	Amalachour-8, Baglung	N 28° 12.475', E 83° 38.778'	952	Fruit	Fever, Anthihelmintic
Ranunculus scleratus	Ranunculaceae	Mudule jhar	Amalachour-8, Baglung	N 28° 12.475', E 83° 38.778'	952	Whole plant	Blisters, Gastric
Symplocos lucida	Symplocaceae	Tebba	Phulkharka-4 Dhading	N 28° 06.180', E 84° 54.916'	2805	Bark	NA

3.2 Methanol Extraction of Plant Samples

Air-dried plant samples were grinded to fine powder using an electric grinder. 100g of plant powder was weighed and soaked in 750 ml of HPLC grade methanol and then subjected to intermittent sonication at 40 kHz and 45°C for 75 minutes each time for three consecutive days to complete the extraction. The resulting solution was filtered through Whatmann No.1 filter paper. The filtrate was then subjected to rotoevaporation with water bath at 45°C, and round bottom flask at 60-80 rpm for 20-45 minutes. Extract was thus concentrated to 50-60ml and transferred to clean and weighed petriplates and kept in an incubator at 37°C for about 3 days to remove excess methanol. The dried extracts in petriplates were weighed to estimate the percentage yield using following formula,

$$Percentage\ Yield = \frac{Dry\ Weight\ of\ Extract}{Dry\ Weight\ of\ Plant\ Material} \times 100$$

The dried extracts were collected in cryovials and stored at -20°C until use.

3.3 Total Phenolic Content

Total phenolic content (TPC) of methanolic extracts were determined by Folin-Ciocalteu method (Singleton & Rossi, 1965). A hundred microliter of extract (2.5mg/ml) was separately mixed with 1 ml Folin Ciocalteu phenol reagent (Merck Ltd, India) (1:10 dilution with the distilled water) and 0.8ml of aqueous 1M Na_2CO_3 solution. The reaction mixture was allowed to stand for about 15 minutes at room temperature. After completion of incubation the absorbance of the reaction mixture (Blue/violet solution) was measured at 765nm using the UV-Visible Spectrophotometer (Thermo fisher scientific, Genesystem- 10-5). For blank, 0.1 ml of absolute methanol was added instead of extract solution in above reaction mixture. A callibration curve was obtained using gallic acid (Moly chem, Mumbai, india) as standard in methanol and water mixture (50:50 v/v) with the concentration ranging from 5-350µg/ml. Total phenolic content of plant extract were then quantified by using standard calibration curve generated. The result was expressed in terms of milligram of gallic acid equivalent per gm extract (mgGAE/g). For each extract, triplicates were performed to get more accurate result.

3.4 Total Flavonoid Content

Total flavonoid content (TFC) of methanol extracts were determined using spectrophotometric assay based on aluminium-flavonoid complex formation in presence of

potassium acetate as suggested by Pękal & Pyrzynska (2014) with slight modification. Two hundred and fifty microliters of each extract (10mg/ml) was separately mixed with the 0.75 ml of methanol, 0.05ml of 10% aluninium chloride, 0.05 ml of 1M potassium acetate (CH3COOK) and 1.4 ml of distilled water. The reaction mixtures were shaken and incubated for 30 minutes at room temperature. The absorbance of the mixture (yellow coloured complex) was measured at 415nm using the UV-Visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5). Blank was prepared adding all the reagents except the plant sample.

Callibration curve was obtained with the help of quercetin (Sigma) as standard solution in methanol with the concentration ranging from 10-100µg/ml. The total flavonoid content was then expressed in terms of milligram of quercetin equivalent per gram extract (mgQE/g). All the tests were carried in triplicates to get more accurate result.

3.5 Antioxidant Activity

The antioxidant activity of plant extracts and standard ascorbic acid in methanol were determined based on their ability to scavenge 1, 1-diphenyl-2-picrylhyrazyl (DPPH) free radical. The method was carried out as described by Xu & Chang (2007) and Chen & Ho (1995) with slight modification. Different concentration of plant extract (4-300µg/ml) and ascorbic acid (1-25 µg/ml) were prepared in methanol on clean test tubes to which equal volume (0.5 ml) of the 0.2mM DPPH solution was added. Tubes were shaken uniformly for proper mixing and incubated in dark for half an hour. The control was prepared as above but without the plant extract or ascorbic acid. Methanol was taken as blank. Absorbance was taken at 517nm using UV-Visible spectrophotometer (Thermo Fisher Scientific, genesystem- 10-5).

Free radical scavenging activity of the plant extracts and ascorbic acid were calculated by using the given formula:

$$\%\ Radical\ Scavenging\ Activity\ (RSA) = \frac{Abs.control - Abs.sample}{Abs.control} \times 100$$

IC_{50} value of plant extracts and ascorbic acid was calculated by using the given formula:

$$IC50 = EXP\left(LN(conc. > 50\%) - \left(\frac{pi{>}50\% - 50}{pi{>}50\% - pi{<}50\%} \times LN\left(\frac{conc.{>}50\%}{\text{conc.}{<}50\%}\right)\right)\right)$$

EXP: Exponential function; LN: Natural log function, both used in Microsoft Excel 2016; pi>50%: RSA value just above 50%; pi<50%: RSA value just below 50% conc.>50%: Concentration of Signal>50%; conc.<50%: Concentration of Signal <50%.

3.6 GC-MS Analysis

GC-MS analyses of methanol extracts of 12 medicinal plants were performed in Nepal Forensic Science Laboratory, Khumaltar, Lalitpur and Advanced Instrumentation Research Facility, JNU, New Delhi using GCMS-QP2010 Plus and GCMS-QP2010 Ultra Shimadzu systems each comprising an automatic operation controller (AOC-20i, 20s) and Gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) fused silica column (30 m × 0.25 mm ID × 0.25 µm df). For GCMS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1.35 ml/min, and an injection volume of 1 µl was employed (a split ratio of 2:1). The injection temperature was maintained at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 50 °C (isothermal for 0 min), with an increase of 10 °C/min to 250°C (isothermal for 2 min), and 15 °C/min to 280°C (isothermal for 16 min). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 60 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Interpretation on mass spectrum GCMS was conducted using the software database of NIST08, NIST08s, NIST11 and WILEY8 libraries. The spectrum of the unknown components was compared with the spectrum of known components stored in the library. The name, molecular weight, and structure of the components of the plant extracts were ascertained.

3.7 Antimicrobial activity

3.7.1 Nutrient agar (NA)

NA plates were prepared for bacterial culture in antibacterial tests. About 28 gram of NA powder (Hi Media Laboratories Pvt. Ltd, Mumbai, India) was carefully weighed and poured in distilled water. The contents were dissolved on the water completely and the final volume was maintained to 1000 ml followed by boiling for uniform mixing. This media was sterilized on an autoclave at 15lbs pressure at 121°C for 15 minutes. The autoclave tape was used as an indicator for the completeness of sterilization. After cooled to about 45-50°C, the media was poured on sterilized and properly labeled petridishes. About 25 ml of the media was poured on each petridish of 9 cm diameter in sterile conditions under a laminar flow hood. All of the plates were left for the solidification of media. For the preparation of the NB broth, screw tight bottles were filled with NB media and autoclaved. It was then cooled and used.

3.7.2 Mueller Hinton Agar (MHA)

The Mueller Hinton Agar (MHA) media was required for antimicrobial tests. About 38 gram of MHA powder (Hi Media Laboratories Pvt. Ltd, Mumbai, India) was weighed and transferred on a conical flask. The content was dissolved in distilled water and final volume was maintained to 1000 ml. The media was sterilized by autoclaving at 15 lbs pressure and 121°C for 15 minutes. Autoclave tape was used for the indication of the completeness of the sterilization. The media was cooled in laminar airflow and dispensed on sterile and dry petridishes.

3.7.3 Standard bacterial culture Inoculums

The individual pure culture of *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* (MDR), and *Salmonella* Typhi (MDR) were obtained from Tribhuvan University Teaching Hospital, Kathmandu and were streaked on NB broth with the help of the sterilized inoculating loop. The inoculated culture bottles were kept on the shaking incubator at 37°C and 120 rpm for overnight. The turbidity of the sub-cultured bacterial suspension was adjusted at the 0.5 McFarland standards freshly prepared on the other day for antibacterial tests. These bacterial inoculums were used for the swabbing on the MHA plates to test the antimicrobial effects of the plant extracts.

3.7.4 Antibacterial test

The antimicrobial activities of medicinal plant extracts were tested by using agar well diffusion method. The sterile and dry MHA plates were properly labeled with name of bacteria, name of the plant species and the concentration of the plant extract to be added. The plates were inoculated with the appropriate bacterial culture by a sterile cotton swab aseptically. These plates were then allowed to dry for about 15 to 20 minutes.

Five wells were prepared on the solid MHA media with the help of the sterile cork borer (4 mm diameter). Four different concentrations (100 mg/ml, 50 mg /ml, 25 mg /ml, and 12.5 mg /ml) of the plant sample were prepared on DMSO. With the help of the sterile pipette the 30 µl of each individual plant extract were poured in the above prepared wells. The DMSO was taken as negative control while the antibiotic disc – Piperacillin (PI100), Methicillin (MET5), or Piperacillin/Tazobactam (PIT100/10) – was taken as the positive control. The plates were incubated on the microbial incubator overnight at 37°C and the zone of inhibition was observed against each type of bacteria at different concentrations of each plant extract.

3.7.5 Potato Dextrose Agar (PDA) and Potato Dextrose Broth

About 25 gram of PDA powder (Hi Media Laboratories Pvt. Ltd, Mumbai, India) was carefully weighed, dissolved in distilled water and final volume was maintained to 1000 ml. This media was autoclaved at 15 lbs pressure and 121°C for 15 minutes, was cooled in laminar airflow and dispensed in sterile petriplates. Likewise, PD broth was prepared in culture tubes excluding agar.

3.7.6 Preparation of the standard fungal cultures

The individual pure and characterized cultured of *Saccharomyces cerevesiae*, and *Pichia sp.* was obtained from CDBT, TU and were sub-cultured in PD broth with the help of the sterilized inoculating loop and kept on the shaking incubator at 37°C and 120 rpm for overnight. The turbidity of the sub-cultured fungal suspension was adjusted to 0.5 McFarland standards. These bacterial inoculums were used for the swabbing on the MHA plates to test the antimicrobial effects of the plant extracts.

3.7.7 Antifungal test

The antifungal activities of medicinal plant extracts were tested by using agar well diffusion method. The sterile and dry PDA plates were properly labeled with name of fungi, name of the plant species and the concentration of the plant extract to be added. The plates were inoculated with the appropriate fungal culture by a sterile cotton swab aseptically. These plates were then allowed to dry for about 15 to 20 minutes.

Six wells were prepared in solid agar in PDA plates with the help of the sterile cork borer (4 mm diameter). Four different concentrations (100 mg/ml, 50 mg /ml, 25 mg /ml, and 12.5 mg/ml) of the plant extracts were prepared on DMSO. With the help of the sterile pipette, 30 µl of different concentrations of each plant extract were dispensed in their respective wells. The DMSO was taken as negative control. Fluconazole (2.5 mg/ml) was taken as the positive control. The plates were incubated on the microbial incubator overnight at 28°C and the zone of inhibition was observed against different type of fungi at different concentrations of each plant extract.

3.8 Brine Shrimp Cytotoxicity

Brine shrimp lethality bioassay was carried out as previously described by Solis et al. (1993) with some modification. Artificial sea water was prepared by dissolving 38 gm of pure NaCl in one litre of distilled water supplemented with 6 mg of dried yeast. *Artemia salina* (brine shrimp) eggs, obtained from Central Department of Chemistry, were left to hatch in artificial

sea water for 48 hours at room temperature (25-29°C). Brine shrimp larvae (nauplii) were attracted to one side of the vessel exposing them to a light source and separated from eggs by pipetting them 2-3 times in small beakers containing saline water. Plant extracts were dissolved in artificial sea water and made up to 2 mg/ml. Water insoluble compounds were dissolved in 50 µl DMSO prior to adding sea water. Plant extracts were pipetted into the wells of 96-well microplates (Nunc, Denmark) and serial dilutions were made so that the final concentration of each extract after adding 100 µl of suspension containing 10-12 nauplii to form the final volume of 200 µl would be 1000 µg/ml, 100 µg/ml, and 10 µg/ml in triplicate. Control wells with Potassium dichromate, DMSO and artificial sea water were included in each experiment. The plates were covered and incubated at room temperature (25-29°C) for 24 hours. Plates were then examined under the binocular steromicroscope and the numbers of dead (non-motile) nauplii in each well were counted. One hundred microliter of methanol were added to each well to immobilize the nauplii and after 15 minutes the total numbers of brine shrimp in each well were counted. Percentage mortality was calculated as given by the formula:

$$\%Mortality = \frac{Observed\ Propertion - Propertion\ at\ Zero\ Concentration}{1 - Propertion\ at\ Zero\ Concentration} * 100$$

Here the proportion is meant for the number of dead to the total number of brine shrimps.

Probit analysis was performed to determine the lethal concentration to half of the test organisms (LC_{50}) on Excel 2016 as described by Currell (2015). Plant extracts were classified for their toxicity based on the benchmark created by Clarkson according to which extracts with LC_{50} >1000 µg/ml are non-toxic, 500 - 1000 µg/ml are low toxic, 100 - 500 µg/ml are medium toxic, and <100 µg/ml are highly toxic (Clarkson et al., 2004).

3.9 MTT Cell Proliferation

Cytotoxicity of plant extracts on Hela (human cervical adenocarcinoma) and MDCK (normal canine kidney) cell lines obtained from NCCS, Pune was determined by a rapid colorimetric assay, using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), which is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable cells into an insoluble colored formazan product. Five hundred milliliter of Eagle's Minimum Essential Medium (EMEM) was supplemented with 10% of fetal calf serum, 5 ml of penicillin/streptomycin (50 IU/ml and 50 µg/ml respectively), 5 ml of sodium pyruvate (1 mM), $NaHCO_3$ (1 g) and 5 ml of L-glutamine (2 mM). The final medium was then sterilized using 0.22µ microfilters and stored at 4°C before use. Methanolic extracts of twelve medicinal

plants were dissolved in DMSO and further diluted with cell culture medium so that the final DMSO concentration was ≤ 0.3% of total volume of the medium in all treatments. The monolayer cell culture in T175 flask was trypsinized and the cell count was adjusted to 1×10^4 cells per ml using medium containing 10% FBS. 100 µl of cell suspension (1×10^3 cells per well) were seeded into 96-well microplate and incubated for 24 hours at 37°C in 5% CO_2 incubator. The medium was flicked off and 100µl of each plant extract was added to the respective well. Cells with no treatment and 5-fluroruracil treatment were examined as negative and positive controls, respectively. Microplates containing cells and extracts were incubated for another 24 hours. The cells were checked for granularity, shrinkage, swelling. 20 µL of MTT reagent (Merck, Germany) (5mg/ml in PBS) was added to each well and incubated for 3 hours. The medium was removed and 100 µl of DMSO (culture grade) was added to each well and gently shaken for 15 minutes to dissolve any formazan crystals formed. The formazan salts were then quantified by measuring the absorbance at 595nm and 655nm on a microplate reader. Cell viability was calculated as a percentage of untreated cells.

$$\%\ Cell\ Survival = \frac{At-Ab}{Ac-Ab} \times 100$$

At: Absorbance of tested concentration, Ab: Absorbance of blank, Ac: Absorbance of negative control

The cytotoxicity of each plant extract was presented as EC_{50} using GraphPad Prism 6 Software.

3.10 Statistical Analysis

The data were expressed as mean ± standard deviation of three independent experiments. The data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P<0.05$. Statistical analysis was performed using the Microsoft Excel 2016 and the GraphPad Prism 6.

4 Results

4.1 Methanol Extraction of Plant Materials

Among the twelve medicinal plants selected, the extract yield in methanol varied from 4.39% to 32.48%. The highest yield was obtained from *Terminalia bellerica* and lowest yield was found in *Chrysopogan aciculatus*. Extracts varied in color from yellowish green to reddish brown which were either greasy (sticky) or powdery in consistency. The percentage yield and characteristics of the extracts are shown in Table 4.1.

Table 4.1 Percentage yield and physical characteristics of the crude methanol extracts.

Plant Sample	Dry Weight (gm)	Weight of extract (gm)	Percentage Yield	Characteristics of Extract	
				Color	Consistency
Loxogramme involuta	100	10.3	10.3	Yellowish green	Sticky
Hydrocotyle javanica	100	9.85	9.85	Yellowish green	Sticky
Phyllanthus emblica	100	20.76	20.76	Greenish brown	Powdery
Chrysopogan aciculatus	100	4.39	4.39	Yellowish green	Sticky
Rhododendron lepidotum	100	7.12	7.12	Yellowish green	Sticky
Geranium wallichianum	100	19.34	19.34	Reddish brown	Powdery
Tsuga dumosa	100	9.18	9.18	Reddish brown	Powdery
Anemone rivularis	100	5.75	5.75	Greenish brown	Sticky
Scurrula elata	100	10.19	10.19	Greenish brown	Powdery
Terminalia bellerica	100	32.48	32.48	Greenish yellow	Powdery
Ranunculus scleratus	100	7.41	7.41	Brownish green	Sticky
Symplocos lucida	100	30.17	30.17	Yellowish red	Sticky

4.2 Determination of Total Phenolic Content

Standard curve of gallic acid offered an equation of y = 0.0047x + 0.1819, R^2 = 0.9884 (Figure 4.1). It helped determine the total phenolic content of methanolic extracts of each plant whose values were located at or somewhere within the range in between 6.74 mgGAE/g and 55.66 mgGAE/g (Figure 4.2). Highest amount of total phenolic content was found in *P. emblica* (55.66 ± 0.84 mgGAE/g) followed by *G. wallichianum* (54.99 ± 0.74 mgGAE/g), *S. elata* (54.71 ± 0.68 mgGAE/g), *T. bellerica* (54.69 ± 0.77 mgGAE/g), and *T. dumosa* (54.19 ± 0.93 mgGAE/g). The lowest amount of total phenolic content was found in *R. scleratus* (6.74 ± 0.62 mgGAE/g) (Figure 4.2).

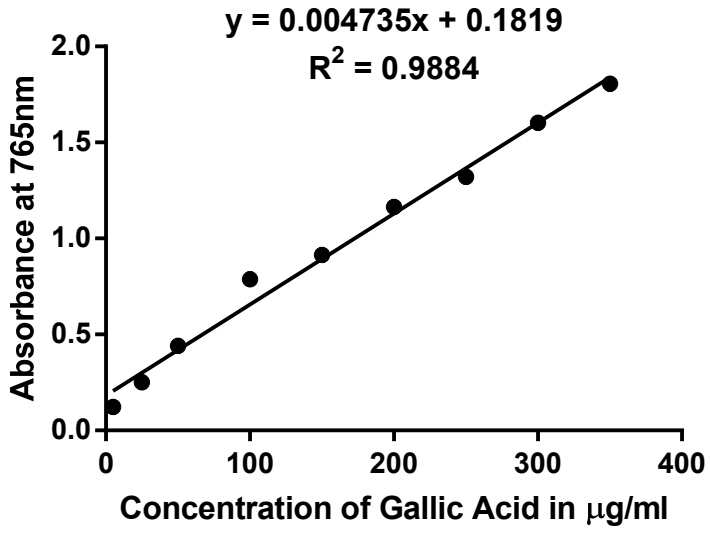

Figure 4.1 Standard graph of gallic acid.

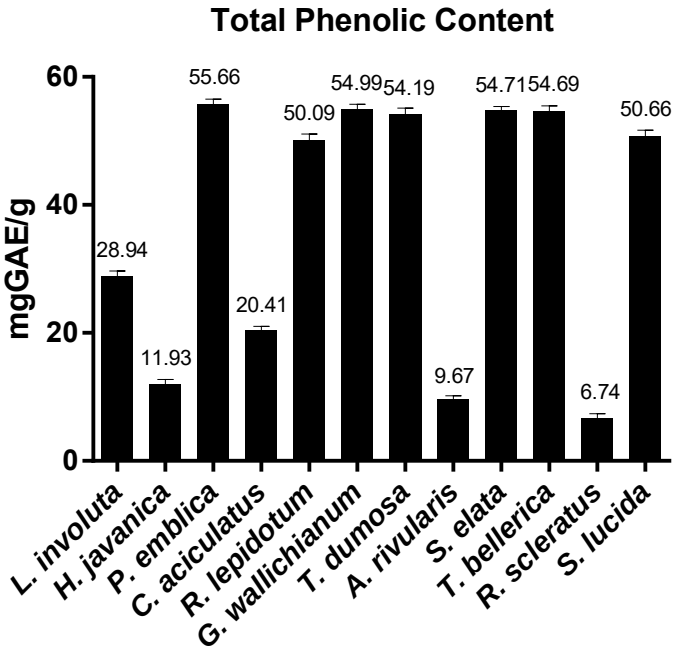

Figure 4.2 Total phenolic content in methanolic extracts of 12 medicinal plants.

4.3 Determination of Total Flavonoid Content

Standard curve of quercetin presented itself with an equation of y = 0.0198x - 0.1337, R^2 = 0.9740 (Figure 4.3). It helped determine the total flavonoid content of each extract whose value was positioned at or somewhere within the narrow range between 7.51 mgQE/g and 25.65 mgQE/g (Figure 4.4). The highest amount of total flavonoid content was found in *P. emblica* (25.65 ± 0.77 mgQE/g) followed by *S. elata* (22.26 ± 0.69 mgQE/g), *C. aciculatus* (19.83 ± 0.94 mgQE/g), *G. wallichianum* (18.53 ± 0.67 mgQE/g), *R. lepidotum* (18.35 ± 0.76 mgQE/g), and *H. javanica* (18.24 ± 0.83 mgQE/g). The lowest amount of total flavonoid content was found in *T. bellerica* (7.51 ± 0.54 mgQE/g) (Figure 4.4).

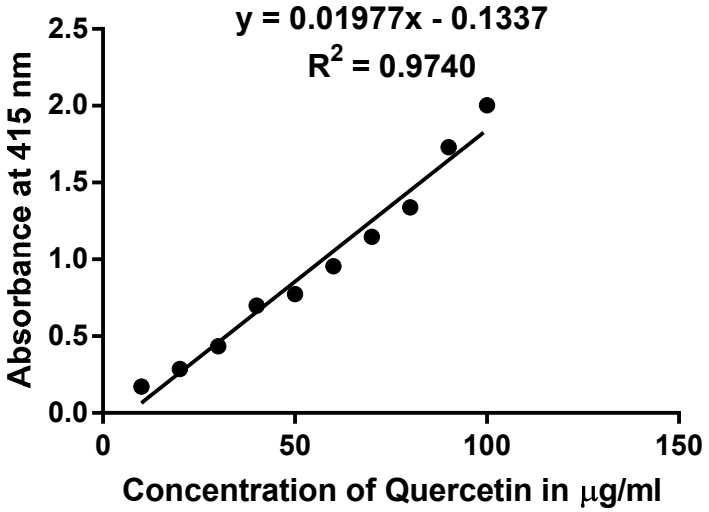

Figure 4.3 Standard graph of quercetin.

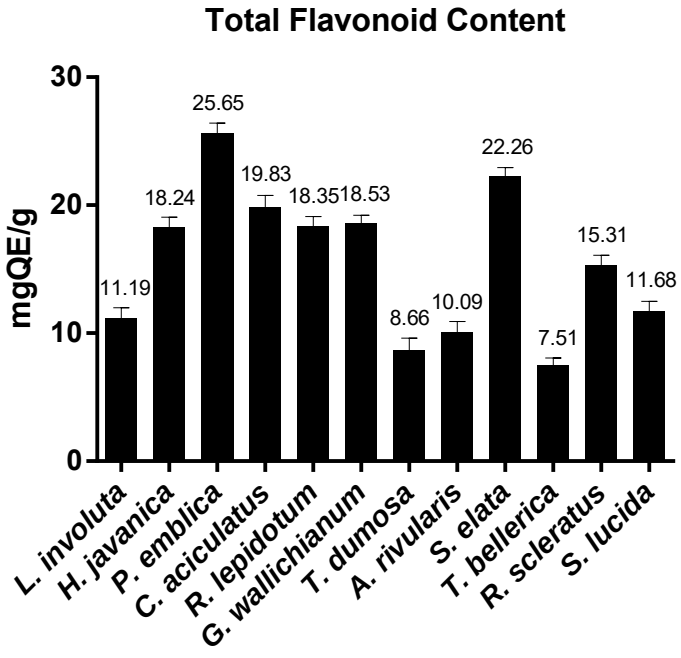

Figure 4.4 Total flavonoid content in methanolic extracts of 12 medicinal plants.

4.4 Estimation of Antioxidant Activity

Plant extracts exerted a dose dependent DPPH free radical scavenging activity with their IC_{50} values ranging from 4.54 µg/ml to 195.55 µg/ml (Figure 4.6 and Figure 4.7). *T. dumosa* (4.54 ±

0.18 µg/ml) showed the highest antioxidant activity with the lowest IC_{50} value – lower than that observed in ascorbic acid (4.57 ± 00 µg/ml) – followed by *P. emblica* (6.64 ± 0.71 µg/ml), *G. wallichianum* (7.24 ± 0.48 µg/ml), *T. bellerica* (12.08 ± 0.76 µg/ml), and *S. elata* (14.20 ± 0.94 µg/ml). *R. scleratus* (195.55 ± 2.45 µg/ml) showed the highest IC_{50} value with the lowest antioxidant activity (Figure 4.7).

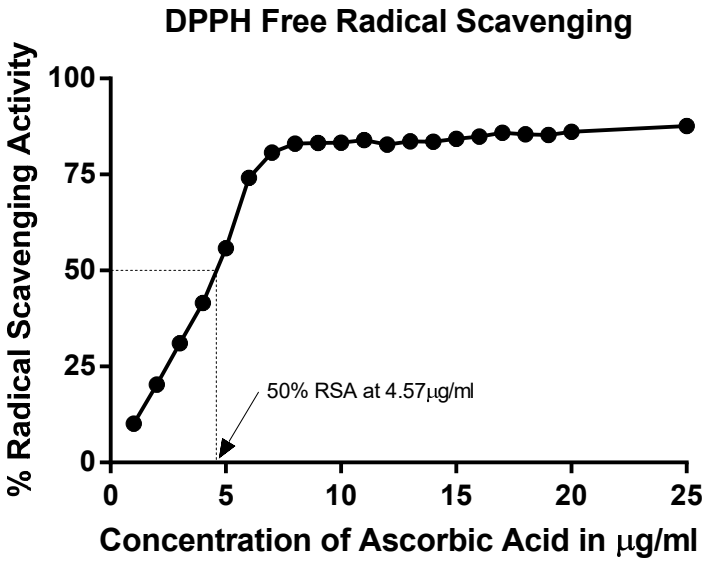

Figure 4.5 DPPH free radical scavenging activity of ascorbic acid.

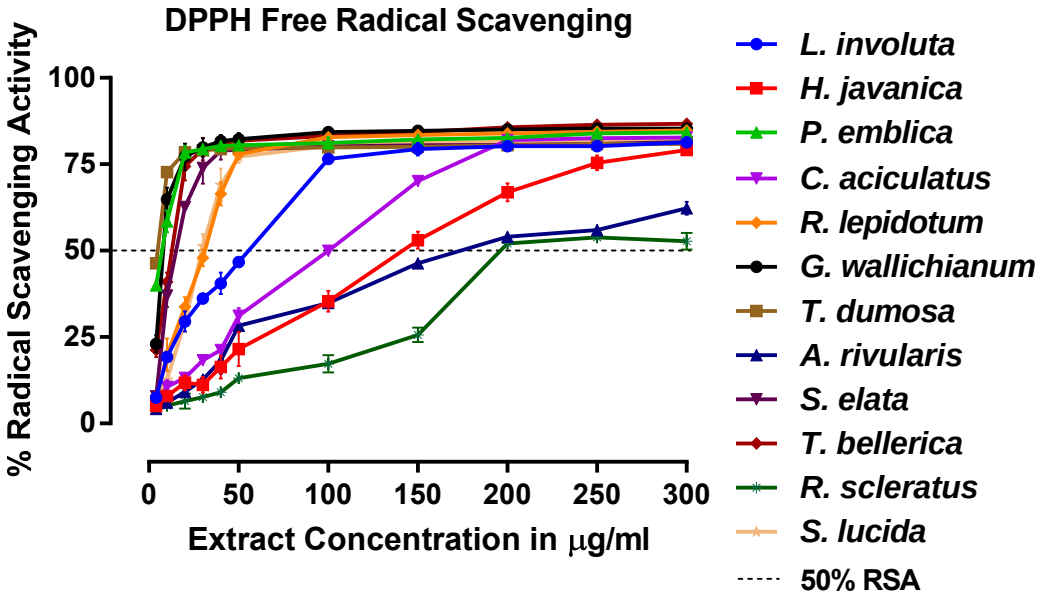

Figure 4.6 DPPH free radical scavenging activity of methanolic extracts of 12 medicinal plants.

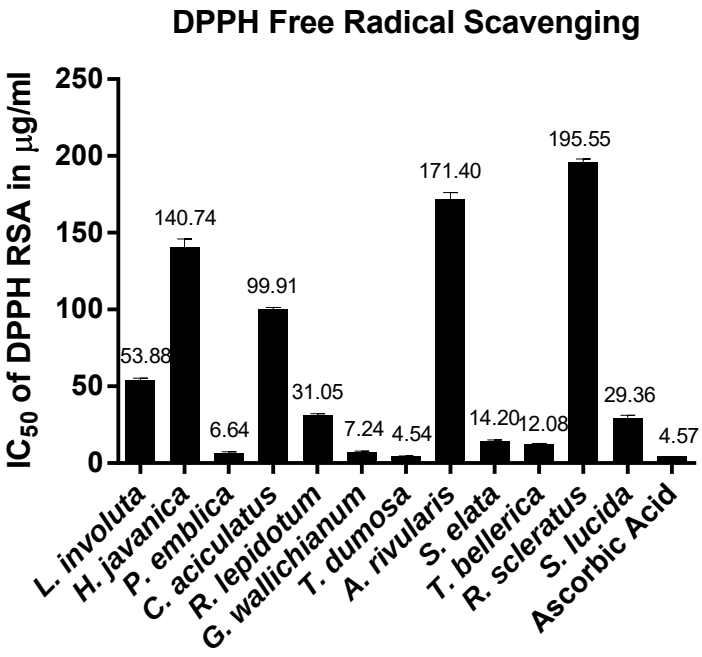

Figure 4.7 IC_{50} of DPPH free RSA of methanolic extracts of 12 medicinal plants.

4.5 Correlation between Total Phenolic Content and Antioxidant Activity

A significant correlation was observed between the antioxidant activity and the total phenolic content while comparing the TPC with the IC_{50} values of DPPH free radical scavenging activity of twelve medicinal plants ($r = -0.9684$, $p<0.0001$).

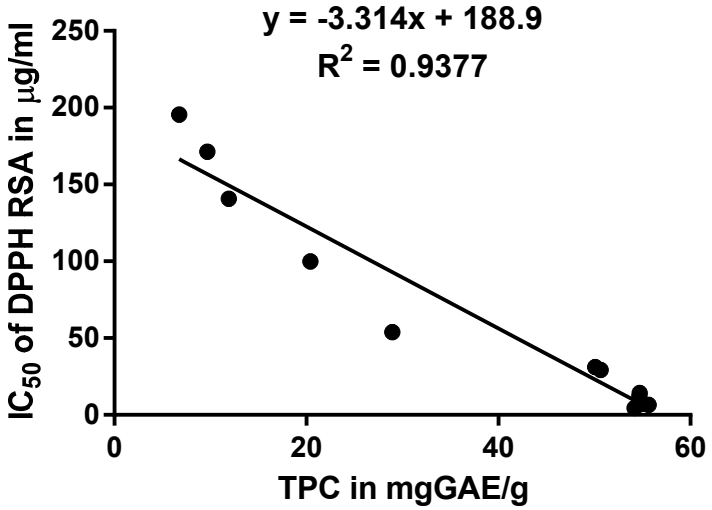

Figure 4.8 Correlation between total phenolic content and IC_{50} of DPPH free RSA of methanolic extracts of 12 medicinal plants.

4.6 Correlation between Total Flavonoid Content and Antioxidant activity

No significant correlation could be established between antioxidant activity and total flavonoid content while comparing the TFC with the IC_{50} values of DPPH free radical scavenging activity of twelve medicinal plants (r = -0.09653, p = 0.7654).

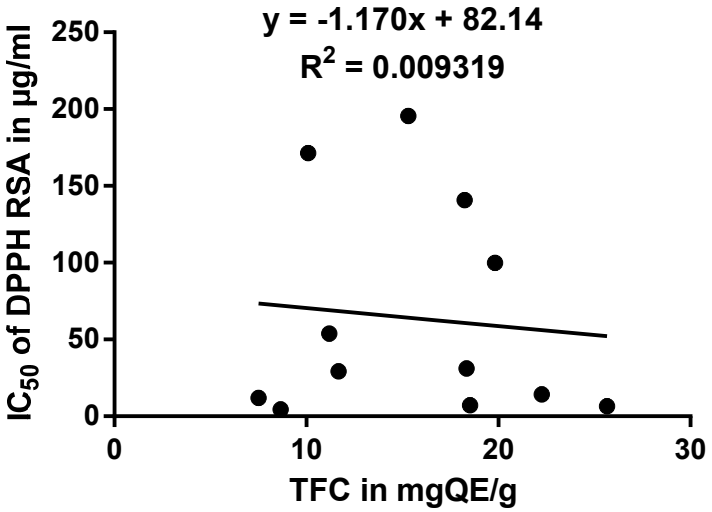

Figure 4.9 Correlation between total flavonoid content and IC_{50} of DPPH free RSA of methanolic extracts of 12 medicinal plants.

4.7 GC-MS Analysis

GC-MS analyses presented an excellent seperation of phyto-compounds as shown by the chromatograms in Figure 4.10 to Figure 4.21. It permitted the inspection of each peak for their identification by comparison of the observed spectra of each constituent with that from NIST and WILEY software database libraries. The active principals with their retention time, molecular formula, molecular weight, and concentration percentage in the methanol extract are presented in

Table 4.2 to Table 4.13.

4.7.1 GC-MS Analysis of *Loxogramme involuta*

Loxogramme involuta afforded the identification of compounds such as 2,4-Bis[4-chloro-*trans*-styryl]-6-[(3-pyrrolidinomethyl-4-hydroxyphenyl)amino]pyrimidine (20.19%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (9%), Cyclopentane, 1-acetyl-1,2-epoxy- (7.13%), Benzaldehyde, 2-hydroxy-6-methyl- (4%), 1,2-Benzenediol (3.87%), 9-Octadecynoic acid (3.81%), Octadecane, 2,2,4,15,17,17-hexamethyl-7,12-bis(3,5,5-trimethylhexyl)- (3.75%), Crypto dioxmine diacetate (3.66%), 2-Deoxy-D-galactose (3.41%), and 2-(Heptyl-methyl-carbamoyl)-hexanedioic acid, bis-(heptyl-methyl-amide) (2.96%).

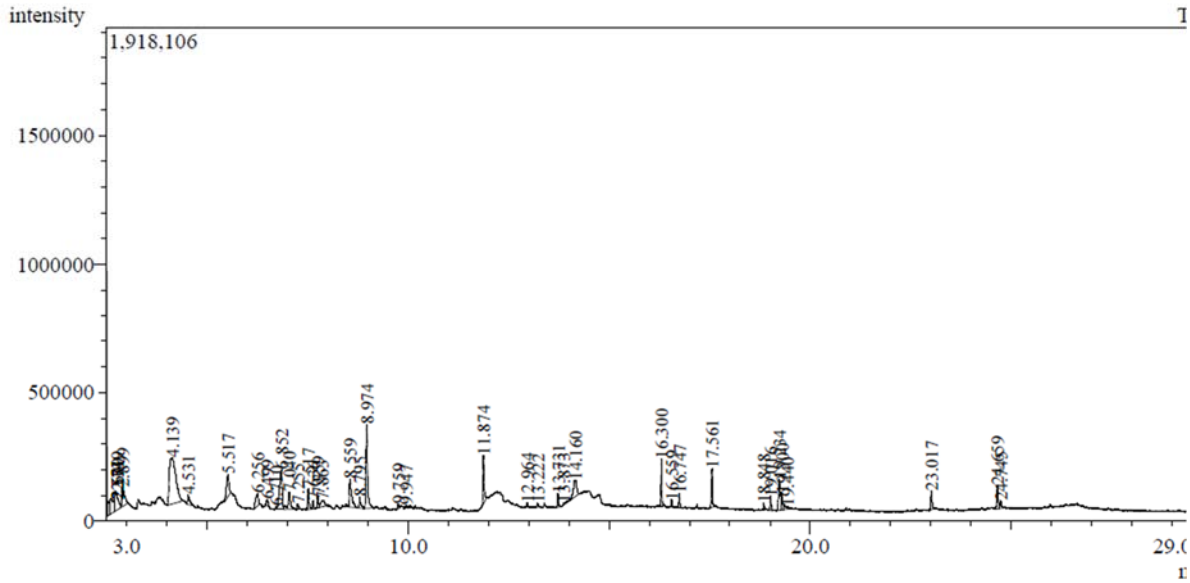

Figure 4.10 GC-MS Chromatogram of methanolic extract of *Loxogramme involuta* whole plant.

Table 4.2 Phytocomponents identified in the methanolic extract of *Loxogramme involuta* whole plant by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	2.533	2.13	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-		
2	2.6	2.96	2-(Heptyl-methyl-carbamoyl)-hexanedioic acid, bis-(heptyl-methyl-amide)	$C_{31}H_{61}N_3O_3$	523
3	2.899	3.66	Crypto dioxmine diacetate	$C_{31}H_{48}N_2O_6$	544
4	4.139	20.19	2,4-Bis[4-chloro-*trans*-styryl]-6-[(3-pyrrolidinomethyl-4-hydroxyphenyl)amino]pyrimidine	$C_{31}H_{28}Cl_2N_{4O}$	542
5	4.531	1.32	7-Chloro-1,3,4,10-tetrahydro-10-hydroxy-1-[[3-[1-piperidinyl]propyl]imino]-3-[3,4,5(trimethoxy)phenyl]-9(2H)-acridinone	$C_{30}H_{36}ClN_3O_5$	553
6	5.517	3.75	Octadecane, 2,2,4,15,17,17-hexamethyl-7,12-bis(3,5,5-trimethylhexyl)-	$C_{42}H_{86}$	590
7	6.256	2.27	Tetratriacontyl trifluoroacetate	$C_{36}H_{69}F_3O_2$	590
8	6.499	0.71	Decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononyl ester	$C_{19}H_{22}F_{16}O_2$	586
9	6.71	0.25	2,4,7-Trinitro-9-fluorenone 2,4-dinitrophenylhydrazone	$C_{19}H_9N_7O_{10}$	495
10	6.852	7.13	Cyclopentane, 1-acetyl-1,2-epoxy-	$C_7H_{10}O_2$	126
11	7.04	2.86	Maytansine	$C_{34}H_{46}ClN_3O_{10}$	691
12	7.255	0.37	N-(4,6-Dipiperidino-1,3,5-triazin-2-yl)-N-{4,6-bis[2,2,2-trifluoro-1,1-bis(trifluoromethyl)ethyl]-1,3,5-triazin-2-yl}amine	$C_{24}H_{21}F_{18}N_9$	777
13	7.517	2	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-		
14	7.636	0.63	2-[4-Chloro-*trans*-styryl]-6-chloro-5-[4-chlorophenyl]-4-[3,5-bis[pyrrolidinomethyl]-4-hydroxyanilino]pyrimidine	$C_{34}H_{34}Cl_3N_5O$	633
15	7.759	1	*α*-d-Glucofuranose, 3-O-(2,3,4-tri-O-acetyl-*α*-d-ribopyranosyl)-1,2-5,6-di-O-isopropylidene-	$C_{23}H_{34}O_{13}$	518

16	7.863	1.59	Acetamide, N-phenyl-		
17	8.559	3.87	1,2-Benzenediol	$C_6H_6O_2$	110
18	8.795	1.07	Rhodium, bis[5,6-bis(.eta.2-ethenyl)cyclooctene]di-.mu.-chlorodi-, stereoisomer	$C_{24}H_{36}Cl_2Rh_2$	600
19	8.974	9	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126
20	9.947	0.35	Card-20(22)-enolide, 1,5,11,14,19-pentahydroxy-3-[(3,4,5-tryhydroxy-6-methyltetrahydropyran-2-yl)oxy]-	$C_{29}H_{44}O_{12}$	584
21	11.874	4	Benzaldehyde, 2-hydroxy-6-methyl-	$C_8H_8O_2$	136
22	12.964	0.33	Pseduotomatidin-5,20-dien diacetate	$C_{31}H_{47}NO_4$	497
23	13.222	0.35	Pseudosolasodine diacetate	$C_{31}H_{49}NO_4$	499
24	13.731	1.11	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
25	14.16	3.41	2-Deoxy-D-galactose	$C_6H_{12}O_5$	164
26	16.3	2.68	Phytol	$C_{20}H_{40}O$	296
27	16.559	0.49	Triacontane, 1,30-dibromo-	$C_{30}H_{60}Br_2$	578
28	16.747	0.93	6-Octadecenoic acid, methyl ester, (*Z*)-		
29	17.561	2.78	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
30	18.848	0.34	9,12-Octadecadienoic acid, methyl ester, (*E,E*)-	$C_{19}H_{34}O_2$	294
31	19.016	0.92	1-Hexadecene		
32	19.234	3.81	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280
33	19.3	1.22	Acetamide, N-(2-methoxyphenyl)-		
34	19.44	0.82	Beryllium, hexakis[.mu.-(2,2-dimethylpropanoato-O:O')]-.mu.4-oxotetra-	$C_{30}H_{54}Be_4O_{13}$	658
35	23.017	1.66	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330
36	24.659	2.17	9,12-Octadecadienoic acid (*Z,Z*)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354
37	24.745	0.71	4-Hexadecen-6-yne, (*E*)-	$C_{16}H_{28}$	220

4.7.2 GC-MS Analysis of *Hydrocotyle javanica*

Hydrocotyle javanica afforded the identification of compounds such as 9,12-Octadecadienoic acid (*Z,Z*)- (29.21%), *n*-Hexadecanoic acid (15.53%), Acetic acid, fluoro-, ethyl ester (7.65%), Ethane, 1,1,1-trimethoxy- (5.03%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (3.96%), Phytol (2.75%), 2-[[4-Chlorophenyl]thio]-4-[[7-chloro-4-quinolinyl]amino]-6-[[diethylamino]methyl]phenol (2.31%), 9,12-Octadecadienoic acid (*Z,Z*)-, 2-hydroxy-1-

(hydroxymethyl)ethyl ester (2.26%), Diacetamate (2.04%), and 2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-, (3R-*trans*)- (2%).

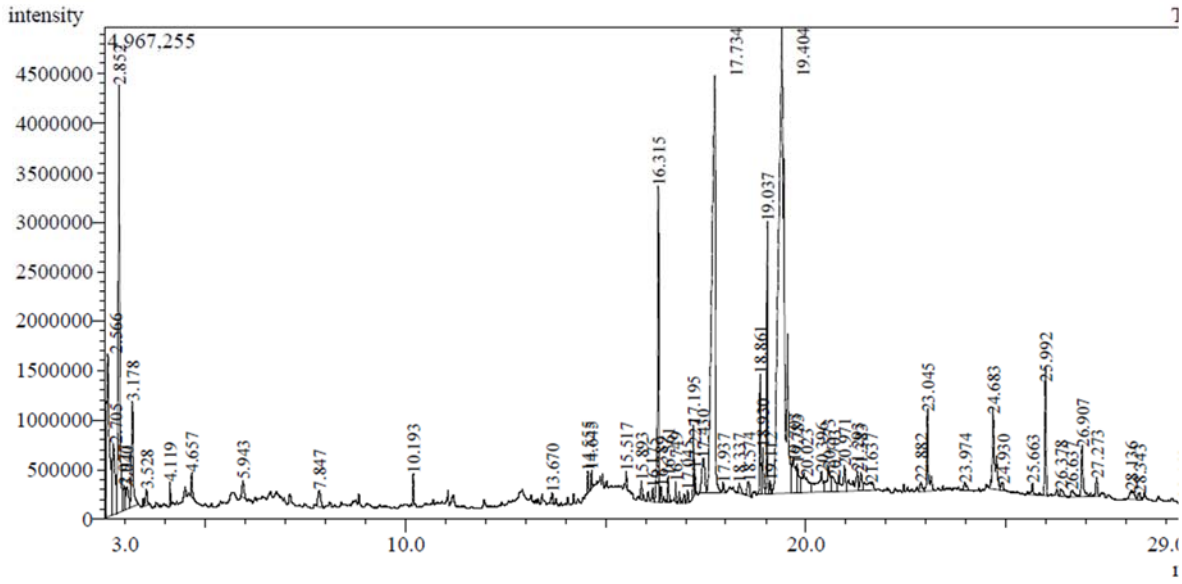

Figure 4.11 GC-MS Chromatogram of methanolic extract of *Hydrocotyle javanica* whole plant.

Table 4.3 Phytocomponents identified in the methanolic extract of *Hydrocotyle javanica* whole plant by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	2.566	5.03	Ethane, 1,1,1-trimethoxy-	$C_5H_{12}O_3$	120
2	2.705	2.31	2-[[4-Chlorophenyl]thio]-4-[[7-chloro-4-quinolinyl]amino]-6-[[diethylamino]methyl]phenol	$C_{26}H_{25}Cl_2N_3OS$	497
3	2.852	7.65	Acetic acid, fluoro-, ethyl ester	$C_4H_7FO_2$	106
4	2.977	0.53	Tetratriacontyl trifluoroacetate	$C_{36}H_{69}F_3O_2$	590
5	3.04	0.64	Glycerin	$C_3H_8O_3$	92
6	3.178	2.04	Diacetamate		0
7	4.119	0.22	2-Cyclopentene-1,4-dione	$C_5H_4O_2$	96
8	4.657	0.33	Cyclohexanone	$C_6H_{10}O$	98
9	5.943	0.35	4-Heptanol, 4-methyl-	$C_8H_{18}O$	130
10	7.847	0.49	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
11	10.193	0.33	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150

12	13.67	0.22	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a*α*,4a*α*,7*β*,7a*β*,7b*α*)]-	$C_{15}H_{24}O$	220
13	14.555	0.24	Bicyclo[4.3.0]nonan-1-ol, 7,9-bis(methylene)-2,2,6-trimethyl-	$C_{14}H_{22}O$	206
14	15.517	0.16	Octadecanoic acid	$C_{18}H_{36}O_2$	284
15	15.893	0.28	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	$C_{10}H_{16}O_2$	168
16	16.175	0.2	8-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294
17	16.315	3.96	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
18	16.389	0.27	2-Undecanone, 6,10-dimethyl-	$C_{13}H_{26}O$	198
19	16.561	0.3	Oleic Acid	$C_{18}H_{34}O_2$	282
20	17.045	0.18	*Z,Z,Z*-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	260
21	17.195	0.72	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
22	17.43	1.13	9,12,15-Octadecatrienoic acid, methyl ester, (*Z,Z,Z*)-	$C_{19}H_{32}O_2$	292
23	17.734	15.53	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
24	17.937	0.18	Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene)methyl]-	$C_{15}H_{20}O_2$	232
25	18.337	0.24	Falcarinol	$C_{17}H_{24}O$	244
26	18.574	0.36	2-Aziridinone, 1-(1-adamantyl)-3-(1-methylcyclopentyl)-	$C_{18}H_{27}NO$	273
27	18.861	1.35	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
28	19.037	2.75	Phytol	$C_{20}H_{40}O$	296
29	19.112	0.16	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
30	19.404	29.21	9,12-Octadecadienoic acid (*Z,Z*)-	$C_{18}H_{32}O_2$	280
31	19.703	1.4	Cyclopropane, 1-methoxy-2,2-dimethyl-3-(3,3-dimethyl-1-propynyl)-	$C_{12}H_{20}O$	180
32	19.783	0.77	3,9-Dodecadiyne	$C_{12}H_{18}$	162
33	20.023	1.32	Methyl *trans*-2-octadecenoate	$C_{19}H_{36}O_2$	296
34	20.396	1.23	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296
35	20.573	0.93	Cyclopentolate	$C_{17}H_{25}NO_3$	291
36	20.703	0.72	Acetamide, N-(2-methoxyphenyl)-		0
37	20.971	0.98	*cis*-9-Hexadecenal	$C_{16}H_{30}O$	238
38	21.293	1.06	Benzene, [(butylsulfonyl)ethynyl]-	$C_{12}H_{14}O_2S$	222
39	21.383	0.43	Eicosanoic acid	$C_{20}H_{40}O_2$	312
40	21.637	0.72	6-Phenylhexanoic acid	$C_{12}H_{16}O_2$	192
41	22.882	0.27	*E,E*-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266

42	23.045	1.49	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
43	23.974	0.24	6-Monoacetylmorphine	$C_{19}H_{21}NO_4$	327
44	24.683	2.26	9,12-Octadecadienoic acid (*Z*,*Z*)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354
45	24.93	0.22	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382
46	25.663	0.24	4-Pentenoic acid, 2,2-diethyl-3-oxo-5-phenyl-, ethyl ester	$C_{17}H_{22}O_3$	274
47	25.992	1.77	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-*E*)-	$C_{30}H_{50}$	410
48	26.378	0.25	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy)carbonyl]-*α*-nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxyphenyl ester, [S-(R*,R*)]-	$C_{28}H_{44}N_2O_7$	520
49	26.637	0.26	1-Triacontanol	$C_{30}H_{62}O$	438
50	26.907	1	1,3-Octanediol	$C_8H_{18}O_2$	146
51	27.273	0.41	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-*E*)-	$C_{30}H_{50}O$	426
52	28.136	0.42	*β*-Eudesmol, trimethylsilyl ether	$C_{18}H_{34}OSi$	294
53	28.343	0.19	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-, (*E,E,E*)-	$C_{20}H_{34}O$	290
54	29.463	0.29	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666
55	29.784	2	2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-, (3R-*trans*)-	$C_{20}H_{18}O_6$	354

4.7.3 GC-MS Analysis of *Phyllanthus emblica*

Phyllanthus emblica afforded the identification of compounds such as 1,2,3-Benzenetriol (57.39%), Phosphoric acid, bis(trimethylsilyl)monomethyl ester (6.79%), *β*-D-Glucopyranose, 1,6-anhydro- (5.49%), 2-Butenal, 2-methyl-, (*E*)- (5.47%), 4-Dimethylsilyloxytridecane (2.59%), 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl- (2.47%), 2H-Pyran-2,6(3H)-dione (2.21%), Levoglucosenone (1.95%), Benzene-1,3-diamine, 5-[3,4,4,4-tetrafluoro-1,3-di(trifluoromethyl)-2-heptafluoroisopropyl-1-butenyloxy- (1.82%), and *β*-Allyloxypropionic acid (0.97%).

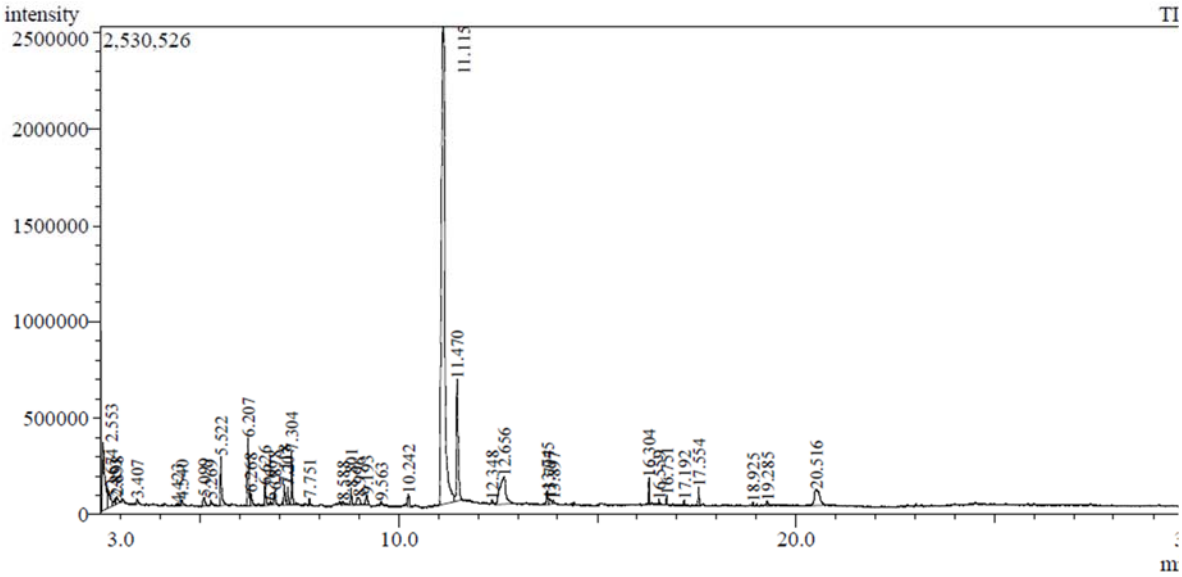

Figure 4.12 GC-MS Chromatogram of methanolic extract of *Phyllanthus emblica* bark.

Table 4.4 Phytocomponents identified in the methanolic extract of *Phyllanthus emblica* bark by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	2.553	5.47	2-Butenal, 2-methyl-, (*E*)-	C_5H_8O	84
2	2.674	1.82	Benzene-1,3-diamine, 5-[3,4,4,4-tetrafluoro-1,3-di(trifluoromethyl)-2-heptafluoroisopropyl-1-butenyloxy-	$C_{15}H_7F_{17}N_2O$	554
3	2.898	0.5	N,N-Dicyclohexyl-2-[2-(2-{2-[(dicyclohexylcarbamoyl)-methoxy]-ethoxy}-ethoxy)-ethoxy]-acetamide	$C_{34}H_{60}N_2O_6$	592
4	3.407	0.22	L-Phenylalanine, N-[N-[N2,N5-bis(trifluoroacetyl)-L-ornithyl]-O-(trifluoroacetyl)-D-allothreonyl]-, methylester	$C_{25}H_{27}F_9N_4O_8$	682
5	4.54	0.3	9-Amidinohydrazono-3,6-dichloro-2,7-bis-[2-piperidinoethoxy]fluorine	$C_{28}H_{36}Cl_2N_6O_2$	558
6	5.099	0.53	Diacetamate		0
7	5.269	0.16	Hexadecanoic acid, methyl ester		0
8	5.522	2.21	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	112
9	6.207	2.47	5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-	$C_7H_{12}O_2$	128
10	6.626	0.97	*β*-Allyloxypropionic acid	$C_6H_{10}O_3$	130

11	6.766	0.17	2,4-Bis[4-chloro-*trans*-styryl]-6-[(3-pyrrolidinomethyl-4-hydroxyphenyl)amino]pyrimidine	$C_{31}H_{28}Cl_2N_4O$	542
12	6.878	0.58	3-Carbethoxy-6-*n*-butyl-7-octadecylmercapto-4-quinolone	$C_{34}H_{55}NO_3S$	557
13	7.118	0.93	1-Hexadecene		0
14	7.304	1.95	Levoglucosenone	$C_6H_6O_3$	126
15	7.751	0.29	Cholestano[3,2-c]isoquinolin-1'(2'H)-one, 3',4'-dihydro-6',7'-dimethoxy-	$C_{36}H_{55}NO_3$	549
16	8.588	0.16	Cyclohexane, (1-hexadecylheptadecyl)-	$C_{39}H_{78}$	546
17	8.801	0.67	Benzofuran, 2,3-dihydro-	C_8H_8O	120
18	8.996	0.83	Triacontyl pentafluoropropionate	$C_{33}H_{61}F_5O_2$	584
19	9.193	0.75	Hexatriacontyl trifluoroacetate	$C_{38}H_{73}F_3O_2$	618
20	10.242	0.71	iso-Valeraldehyde propyleneglycol acetal 2	$C_8H_{16}O_2$	144
21	11.115	57.39	1,2,3-Benzenetriol	$C_6H_6O_3$	126
22	11.47	6.79	Phosphoric acid, bis(trimethylsilyl)monomethyl ester	$C_7H_{21}O_4PSi_2$	256
23	12.348	0.21	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518
24	12.656	5.49	*β*-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162
25	13.745	0.75	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
26	13.897	0.15	Triacontyl trifluoroacetate	$C_{32}H_{61}F_3O_2$	534
27	16.304	0.75	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
28	16.751	0.27	6-Octadecenoic acid, methyl ester, (*Z*)-		0
29	17.192	0.21	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270
30	17.554	0.65	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
31	18.925	0.17	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320
32	19.285	0.26	9,12,15-Octadecatrien-1-ol, (*Z*,*Z*,*Z*)-	$C_{18}H_{32}O$	264
33	20.516	2.59	4-Dimethylsilyloxytridecane	$C_{15}H_{34}OSi$	258

4.7.4 GC-MS Analysis of *Chrysopogan aciculatus*

Chrysopogan aciculatus afforded the identification of compounds such as 2-Propanone, 1,3-dihydroxy- (29.07%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (8.65%), *β*-D-Glucopyranose, 1,6-anhydro- (4.53%), L-Proline, N-(3-fluoro-5-trifluoromethylbenzoyl)-, octadecyl ester (4.29%), Cyclopentane, 1-acetyl-1,2-epoxy- (4.01%), Sucrose (3.97%), 2-Methoxy-4-vinylphenol (3.74%), *n*-Hexadecanoic acid (2.97%), 2,2'-Bis[N-[5-

diethylaminopent-2-yl]carbamyl]diphenyl disulfide (2.65%), and Palladium(II), [(S)-O,N-bis(dicyclohexylphosphino)-2-pyrrolidinemethanol](dichloro)- (2.59%).

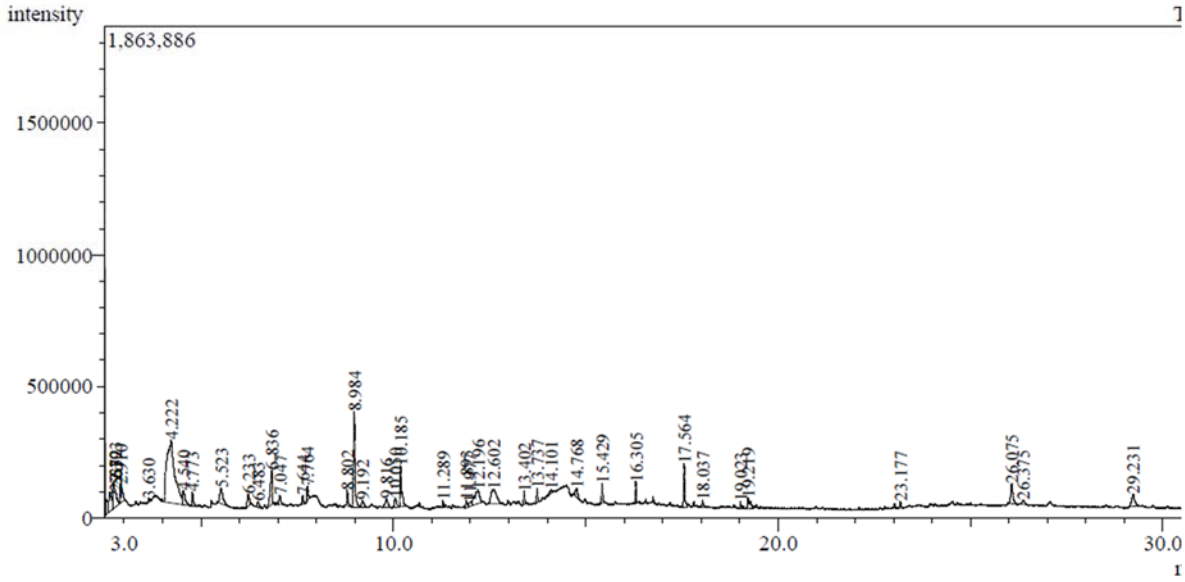

Figure 4.13 GC-MS Chromatogram of methanolic extract of *Chrysopogan aciculatus* whole plant.

Table 4.5 Phytocomponents identified in the methanolic extract of *Chrysopogan aciculatus* whole plant by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	2.533	2.07	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-		0
2	2.619	2.59	Palladium(II), [(S)-O,N-bis(dicyclohexylphosphino)-2-pyrrolidinemethanol](dichloro)-	$C_{29}H_{53}Cl_2NOP_2Pd$	669
3	2.723	4.29	L-Proline, N-(3-fluoro-5-trifluoromethylbenzoyl)-, octadecyl ester	$C_{31}H_{47}F_4NO_3$	557
4	2.91	2.42	Acetamide, N-(2-methoxyphenyl)-		0
5	3.63	0.43	2-(Allylamino)-4,6-bis[2,2,2-trifluoro-1,1-bis(trifluoromethyl)ethyl]-1,3,5-triazine	$C_{14}H_6F_{18}N_4$	572
6	4.222	29.07	2-Propanone, 1,3-dihydroxy-	$C_3H_6O_3$	90
7	4.54	2.1	4,7-Bis(4-morpholino-1,2,5-thiadiazol-3-yloxymethyl)-1,3-dioxepane-5,6-diol	$C_{19}H_{28}N_6O_8S_2$	532
8	4.773	1.25	6-Octadecenoic acid, methyl ester, (*Z*)-		0
9	5.523	2.65	2,2'-Bis[N-[5-diethylaminopent-2-yl]carbamyl]diphenyl disulfide	$C_{32}H_{50}N_4O_2S_2$	586

10	6.233	1.34	Maytansine	$C_{34}H_{46}ClN_3O_{10}$	691
11	6.483	0.62	Phenanthrene-10-ethanamine, 3-bromo-*β*-hydroxy-N,N-diheptyl-, hydrosulfate	$C_{30}H_{42}BrNO_4S$	591
12	6.836	4.01	Cyclopentane, 1-acetyl-1,2-epoxy-	$C_7H_{10}O_2$	126
13	7.047	0.97	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	604
14	7.764	1.08	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
15	8.802	1.16	Benzene, 1-isocyanato-2-methoxy-		0
16	8.984	8.65	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126
17	9.192	0.93	14-Oxa-1,11-diazatetracyclo[7.4.1.0(2,7).0(10,12)]tetradeca-2,4,6-triene, 11-acetyl-6,9-bis(acetyloxy)-4-formyl-8-[(aminocarbonyloxy)methyl]-	$C_{20}H_{21}N_3O_9$	447
18	9.816	1.55	Rhodium, chloro[ethenylidene(2,2-dimethylpropylidyne)phosphinidyne]bis[tris(1-methylethyl)phosphine]-,(tb-5-22)-	$C_{25}H_{53}ClP_3Rh$	584
19	10.05	1.28	3-Heptanol	$C_7H_{16}O$	116
20	10.185	3.74	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
21	11.289	0.53	Isoquinoline, 3-methyl-, 2-oxide	$C_{10}H_9NO$	159
22	11.893	0.7	3,5-Dimethylanisole	$C_9H_{12}O$	136
23	11.977	0.68	1,2-Benzenedicarboxylic acid, diisooctyl ester		0
24	12.196	3.97	Sucrose	$C_{12}H_{22}O_{11}$	342
25	12.602	4.53	*β*-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162
26	13.402	0.78	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180
27	13.737	0.87	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
28	14.101	0.43	1-Hexadecene		0
29	14.768	1.14	9*α*-Hydroxysolasodine, o,N-diacetate	$C_{31}H_{47}NO_5$	513
30	15.429	1.28	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
31	16.305	0.98	Octadecanal	$C_{18}H_{36}O$	268
32	17.564	2.97	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
33	18.037	0.44	Phenol, 2,6-dimethyl-4-nitro-	$C_8H_9NO_3$	167
34	19.023	0.44	Phytol	$C_{20}H_{40}O$	296
35	19.219	1.62	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280
36	23.177	0.42	13-Methyltritriacontane	$C_{34}H_{70}$	478
37	26.075	2.58	*β*-Sitosterol	$C_{29}H_{50}O$	414
38	26.375	0.63	Androst-4,6-dien-3,11,17-trione, 9-mercapto-	$C_{19}H_{22}O_3S$	330

39	29.231	2.06	4H-1-Benzopyran-4-one, 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-	$C_{16}H_{12}O_5$	284

4.7.5 GC-MS Analysis of *Rhododendron lepidotum*

Rhododendron lepidotum afforded the identification of compounds such as Benzoyl chloride, 4-pentyl- (13.12%), Verrucarol (8.4%), 2H-1-Benzopyran, 7-methoxy-2,2-dimethyl- (7.42%), alpha.-D-Glucopyranoside, O-*α*-D-glucopyranosyl-(1.fwdarw.3)-*β*-D-fructofuranosyl (5.27%), 1-[3-(1-Adamantyl)-1-methylpropylidene]thiosemicarbazide (5.04%), Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]- (3.64%), Acetate, (2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl) ester (3.25%), 1-Buten-1-ol, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, formate, (*E*)- (2.91%), *n*-Hexadecanoic acid (2.64%), and Octadecanal (2.49%).

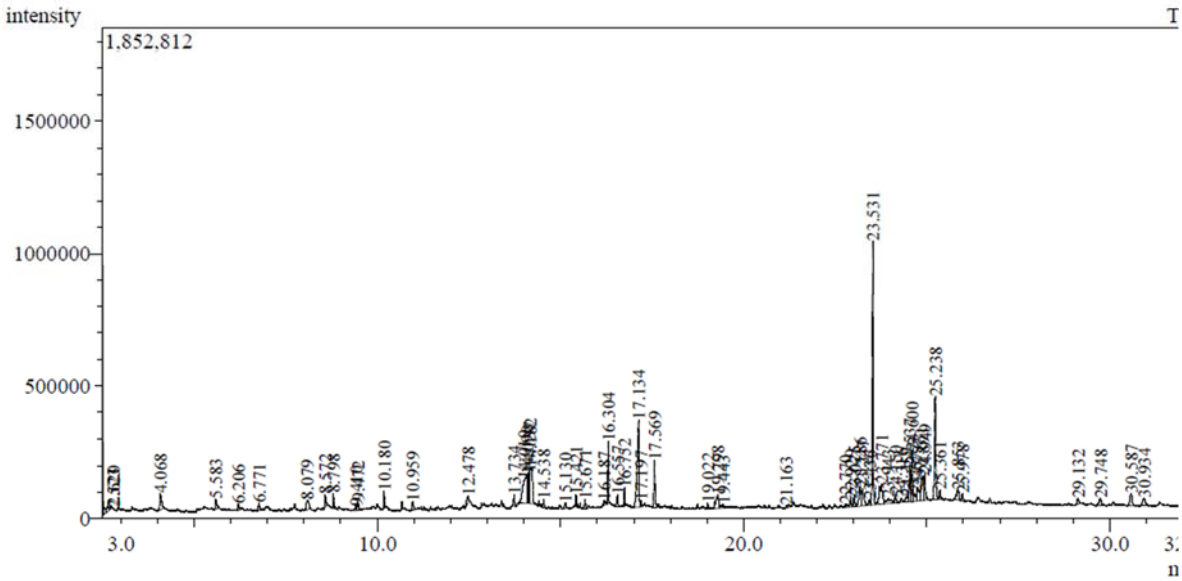

Figure 4.14 GC-MS Chromatogram of methanolic extract of *Rhododendron lepidotum* leaf and stem.

Table 4.6 Phytocomponents identified in the methanolic extract of *Rhododendron lepidotum* leaf and stem by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	2.523	0.73	3-Carbethoxy-6-*n*-butyl-7-octadecylmercapto-4-quinolone	$C_{34}H_{55}NO_3S$	557
2	2.62	0.52	2-(N-Methyl-N-nitroamino)-4,6-bis[2,2,2-trifluoro-1,1-bis(trifluoromethyl)ethyl]-1,3,5-triazine	$C_{12}H_3F_{18}N_5O_2$	591

3	4.068	1.57	Methylperfluoro[3-(4-ethylpiperazinyl)butyrate]	$C_{11}H_3F_{19}N_2O_2$	556
4	5.583	0.89	2-Methyliminoperhydro-1,3-oxazine	$C_5H_{10}N_2O$	114
5	6.206	0.43	Benzene-1,3-diamine, N,N-diacetyl-5-[3,4,4,4-tetrafluoro-2-(1,2,2,2-tetrafluoro-1-trifluoromethylethyl)-1,3-di(trifluoromethyl)-1-butenyloxy]-	$C_{19}H_{11}F_{17}N_2O_3$	638
6	6.771	0.49	Pseudosolasodine diacetate	$C_{31}H_{49}NO_4$	499
7	8.079	1.37	Octadecanoic acid, octadecyl ester	$C_{36}H_{72}O_2$	536
8	8.572	0.96	1,2-Benzenediol	$C_6H_6O_2$	110
9	8.798	0.76	Benzofuran, 2,3-dihydro-	C_8H_8O	120
10	9.41	0.39	Hexatriacontane	$C_{36}H_{74}$	506
11	9.472	0.55	1,2-Benzenediol, 3-methoxy-	$C_7H_8O_3$	140
12	10.18	0.91	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
13	10.959	0.76	1,4-Benzenediol, 2-methyl-	$C_7H_8O_2$	124
14	12.478	0.81	Hexatriacontyl trifluoroacetate	$C_{38}H_{73}F_3O_2$	618
15	13.734	0.59	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
16	14.01	5.27	alpha.-D-Glucopyranoside, O-*α*-D-glucopyranosyl-(1.fwdarw.3)-*β*-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504
17	14.09	1.45	1-Hexadecene		0
18	14.137	2.48	*n*-Decanoic acid	$C_{10}H_{20}O_2$	172
19	14.182	5.04	1-[3-(1-Adamantyl)-1-methylpropylidene]thiosemicarbazide	$C_{15}H_{25}N_3S$	279
20	14.538	0.46	.tau.-Muurolol	$C_{15}H_{26}O$	222
21	15.13	0.42	Serverogenin acetate	$C_{29}H_{36}O_{10}$	544
22	15.421	0.54	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
23	15.671	0.42	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	220
24	16.187	0.52	2-Hydroxy-5-methylisophthalaldehyde	$C_9H_8O_3$	164
25	16.304	2.49	Octadecanal	$C_{18}H_{36}O$	268
26	16.752	0.79	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
27	17.134	8.4	Verrucarol	$C_{15}H_{22}O_4$	266
28	17.197	0.41	Hexadecanoic acid, methyl ester		0
29	17.569	2.64	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
30	19.022	0.41	Phytol	$C_{20}H_{40}O$	296
31	19.298	2.07	3-Tetradecyn-1-ol	$C_{14}H_{26}O$	210
32	22.77	0.51	1-Heptacosanol	$C_{27}H_{56}O$	396

33	23.025	0.84	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330
34	23.186	2.2	Methylphosphonic acid, anhydride, bis(2,2-dimethylcyclohexyl) ester	$C_{18}H_{36}O_5P_2$	394
35	23.247	2.05	6-Octadecenoic acid, methyl ester, (*Z*)-		0
36	23.436	0.42	1,1-Propanedicarbonitrile, 1,2-dicyclohexyl-	$C_{17}H_{26}N_2$	258
37	23.531	13.12	Benzoyl chloride, 4-pentyl-	$C_{12}H_{15}ClO$	210
38	23.771	3.64	Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-	$C_{13}H_{18}O$	190
39	23.943	1.36	Cyclononasiloxane, octadecamethyl-		0
40	24.15	0.57	Succinimide, N-phenyl-3-(2-vinylcyclohexyl)-	$C_{18}H_{21}NO_2$	283
41	24.537	2.18	2-Pentenamide, N-phenyl-	$C_{11}H_{13}NO$	175
42	24.6	2.91	1-Buten-1-ol, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, formate, (*E*)-	$C_{15}H_{24}O_2$	236
43	24.757	1.48	Neoisolongifolene, 8-oxo-	$C_{15}H_{22}O$	218
44	24.891	3.25	Acetate, (2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl) ester	$C_{16}H_{26}O_2$	250
45	25.238	7.42	2H-1-Benzopyran, 7-methoxy-2,2-dimethyl-	$C_{12}H_{14}O_2$	190
46	25.361	0.55	Di-*n*-octyl phenyl phosphate	$C_{22}H_{39}O_4P$	398
47	25.978	0.5	Squalene	$C_{30}H_{50}$	410
48	29.132	0.58	*γ*-Tocopherol	$C_{28}H_{48}O_2$	416
49	29.748	0.9	Stigmasteryl tosylate	$C_{36}H_{54}O_3S$	566
50	30.587	1.4	Vitamin E	$C_{29}H_{50}O_2$	430
51	30.934	1.22	4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl-, (*E*)-	$C_{16}H_{28}O$	236

4.7.6 GC-MS Analysis of *Geranium wallichianum*

Geranium wallichianum afforded the identification of compounds such as 1,2,3-Benzenetriol (57.73%), 1,2-Benzenediol, 3-methoxy- (3.81%), 1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid (3.5%), Pentadecanoic acid (3.48%), D-Allose (3.15%), Stigmast-5-en-3-ol, (3*β*)- (2.94%), 9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester (2.72%), Hexadecanoic acid, methyl ester (2.33%), *cis*-Vaccenic acid (2.25%), and 9-Octadecenoic acid (*Z*)-, methyl ester (2.19%).

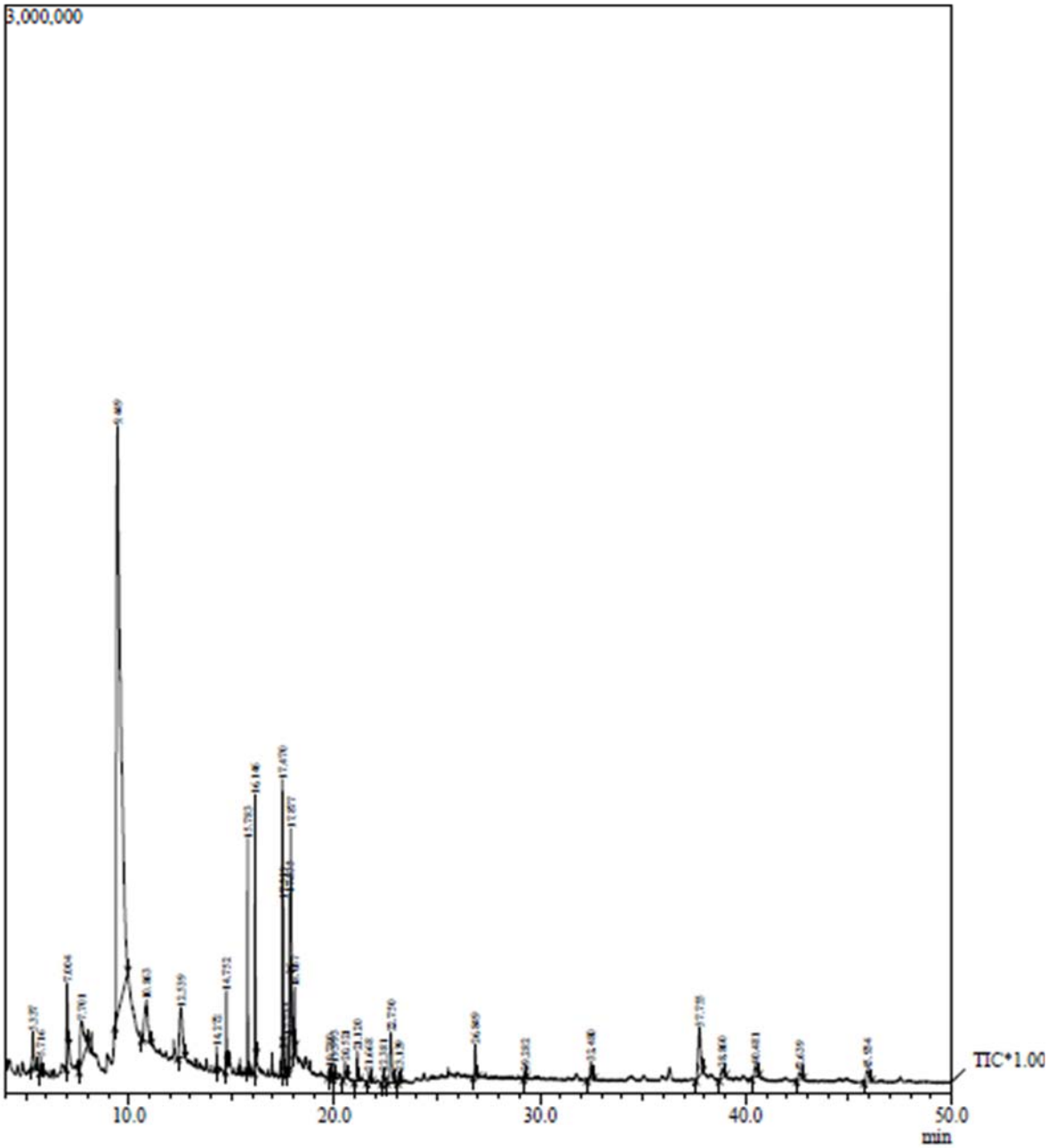

Figure 4.15 GC-MS Chromatogram of methanolic extract of *Geranium wallichianum* root.

Table 4.7 Phytocomponents identified in the methanolic extract of *Geranium wallichianum* root by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	5.337	1.11	Dimethyl dl-malate	$C_6H_{10}O_5$	162
2	5.716	0.37	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144

3	7.004	2	5-Hydroxymethylfurfural	$C_6H_6O_3$	126
4	7.701	3.81	1,2-Benzenediol, 3-methoxy-	$C_7H_8O_3$	140
5	9.449	57.73	1,2,3-Benzenetriol	$C_6H_6O_3$	126
6	10.863	3.15	D-Allose	$C_6H_{12}O_6$	180
7	12.539	3.5	1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192
8	14.272	0.24	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	$C_9H_{12}N_2O_4$	212
9	14.752	1.35	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_5$	198
10	15.783	2.33	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
11	16.146	3.48	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
12	17.47	2.72	9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester	$C_{19}H_{34}O_2$	294
13	17.519	2.19	9-Octadecenoic acid (*Z*)-, methyl ester	$C_{19}H_{36}O_2$	296
14	17.735	0.36	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
15	17.835	1.19	2-Chloroethyl linoleate	$C_{20}H_{35}ClO_2$	342
16	17.877	2.25	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282
17	18.067	0.79	Octadecanoic acid	$C_{18}H_{36}O_2$	284
18	19.78	0.3	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466
19	19.993	0.24	Cyclohexanone, 3-methyl-2-(1-methylethyl)-, *cis-trans*	$C_{10}H_{18}O$	154
20	20.521	0.49	1,3-Benzenediol, 5-pentadecyl-	$C_{21}H_{36}O_2$	320
21	21.12	0.72	Succinic acid, dec-4-enyl dodecyl ester	$C_{26}H_{48}O_4$	424
22	21.668	0.3	Abieta-8,11,13-trien-18-oic acid	$C_{20}H_{28}O_2$	300
23	22.381	0.25	Abietic acid	$C_{20}H_{30}O_2$	302
24	22.75	1.87	9-Octadecenal, (*Z*)-	$C_{18}H_{34}O$	266
25	23.139	0.23	Cyclopentane, 1-hexyl-3-methyl-	$C_{12}H_{24}$	168
26	26.849	0.7	Squalene	$C_{30}H_{50}$	410
27	29.282	0.12	9-Oxabicyclo[3.3.1]nonane, 2,6-diiodo-	$C_8H_{12}I_2O$	378
28	32.48	0.61	Vitamin E	$C_{29}H_{50}O_2$	430
29	37.755	2.94	Stigmast-5-en-3-ol, (3*β*)-	$C_{29}H_{50}O$	414
30	38.86	0.6	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-octadecahydro-2H-picen-3-one $$ Olean-12-en-3-one	$C_{30}H_{48}O$	424
31	40.481	0.77	Urs-12-ene	$C_{30}H_{50}$	410
32	42.639	0.47	5*β*-Androstan-3*α*,11*α*,17*β*-triol	$C_{19}H_{32}O_3$	308
33	45.934	0.84	Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-methyl-1,3-butadienyl)-	$C_{14}H_{24}O$	208

4.7.7 GC-MS Analysis of *Tsuga dumosa*

Tsuga dumosa afforded the identification of compounds such as 4-O-Methylmannose (10.18%), Mome inositol (10%), 1-Naphthalenepropanol, *α*-ethenyldecahydro-*α*,5,5,8a-tetramethyl-2-methylene-, [1S-[1*α*(R*),4a*β*,8a*α*]]- (9.55%), Phenol, 2-methoxy-4-(2-propenyl)-, acetate (8.89%), Benzoic acid (8.58%), 1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid (7.96%), *cis*-Vaccenic acid (6.97%), D-Allose (4.56%), Pentadecanoic acid (3.78%), and Stigmast-5-en-3-ol, (3*β*)- (3.66%).

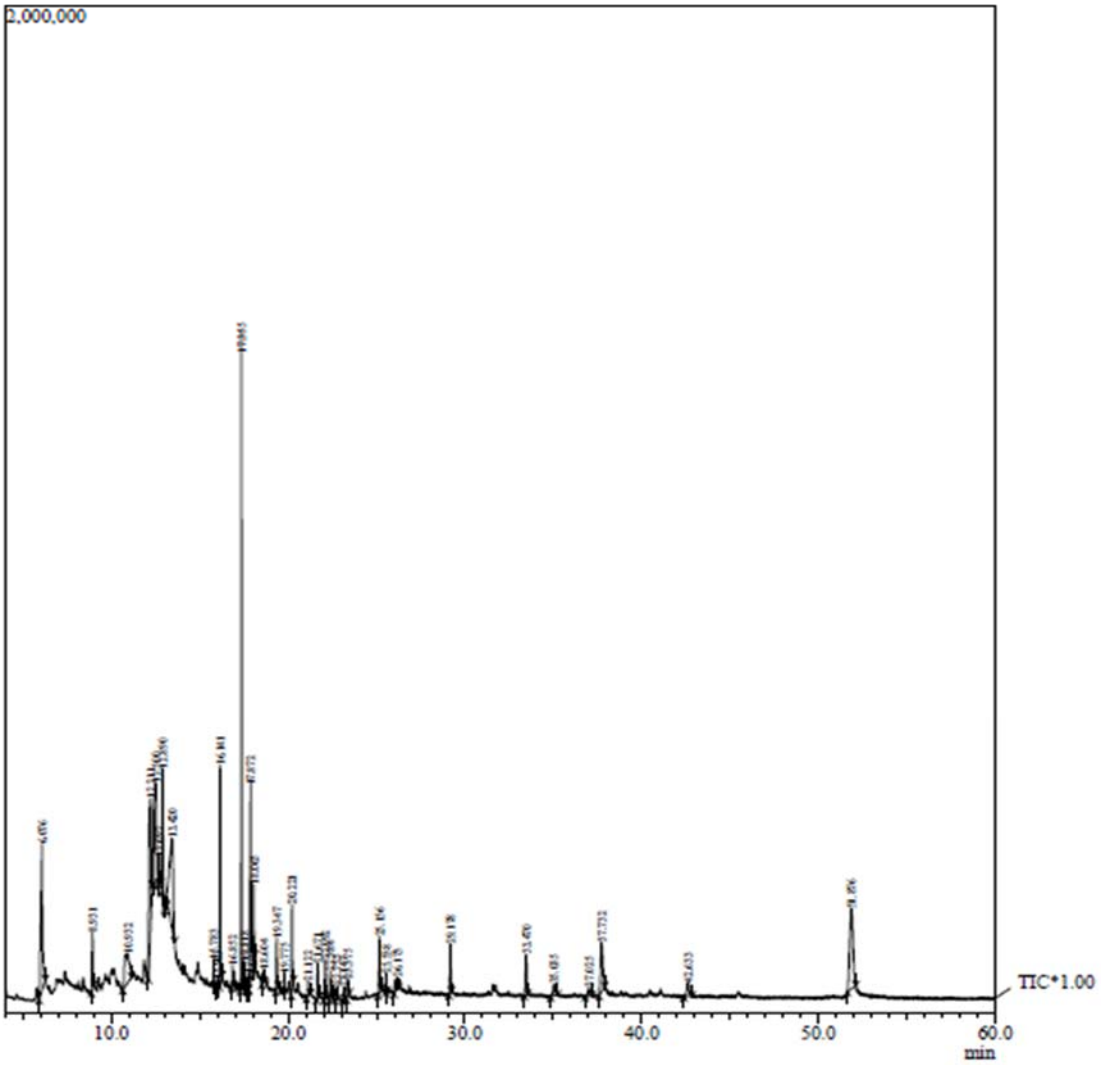

Figure 4.16 GC-MS Chromatogram of methanolic extract of *Tsuga dumosa* bark.

Table 4.8 Phytocomponents identified in the methanolic extract of *Tsuga dumosa* bark by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	6.076	8.58	Benzoic acid	$C_7H_6O_2$	122
2	8.931	1.57	6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-carboxylic acid	$C_{10}H_{14}O_2$	166
3	10.932	4.56	D-Allose	$C_6H_{12}O_6$	180
4	12.211	10.18	4-O-Methylmannose	$C_7H_{14}O_6$	194
5	12.5	7.96	1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192
6	12.692	0.76	Phloroglucinol, trimethylsilyl ether	$C_9H_{14}O_3Si$	198
7	12.89	3.11	Methyl-(2-hydoxy-3-ethoxy-benzyl)ether	$C_{10}H_{14}O_3$	182
8	13.42	10	Mome inositol	$C_7H_{14}O_6$	194
9	15.783	0.36	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326
10	15.963	0.24	Cyclohexane, (2-methylpropyl)-	$C_{10}H_{20}$	140
11	16.141	3.78	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
12	17.355	9.55	1-Naphthalenepropanol, α-ethenyldecahydro-α,5,5,8a-tetramethyl-2-methylene-, [1S-[1α(R*),4aβ,8aα]]-	$C_{20}H_{34}O$	290
13	17.518	0.32	9-Octadecenoic acid (*Z*)-, methyl ester	$C_{19}H_{36}O_2$	296
14	17.872	6.97	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282
15	18.065	1.17	Octadecanoic acid	$C_{18}H_{36}O_2$	284
16	18.604	0.35	Undecanoic acid, 11-fluoro-, trimethylsilyl ester	$C_{14}H_{29}FO_2Si$	276
17	19.347	1.31	*n*-Nonadecanol-1	$C_{19}H_{40}O$	284
18	19.775	0.31	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
19	20.221	1.82	Eicosanoic acid	$C_{20}H_{40}O_2$	312
20	21.122	0.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
21	21.671	1.02	Abieta-8,11,13-trien-18-oic acid	$C_{20}H_{28}O_2$	300
22	22.384	0.87	Abietic acid	$C_{20}H_{30}O_2$	302
23	22.755	0.69	16-Heptadecenal	$C_{17}H_{32}O$	252
24	23.147	0.34	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390
25	25.156	1.74	1-Eicosanol	$C_{20}H_{42}O$	298
26	26.173	0.74	Hexanal, 3,3-dimethyl-	$C_8H_{16}O$	128
27	29.178	1.35	Docosanoic acid	$C_{22}H_{44}O_2$	340
28	33.47	1.72	Tetrahydro geranyl acetate	$C_{12}H_{24}O_2$	200

29	35.035	0.83	(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,12-trimethyl-9-methylene-, [1R-(1R*,4R*,6R*,10S*)]-	$C_{15}H_{24}O$	220
30	37.025	0.51	Dodecanoic acid, 2-phenylethyl ester	$C_{20}H_{32}O_2$	304
31	37.732	3.66	Stigmast-5-en-3-ol, (3*β*)-	$C_{29}H_{50}O$	414
32	42.633	1.07	Androst-4-en-3-one, 17-hydroxy-, (17*β*)-	$C_{19}H_{28}O_2$	288
33	51.876	8.89	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206

4.7.8 GC-MS Analysis of *Anemone rivularis*

Anemone rivularis afforded the identification of compounds such as Diethyl Phthalate (7.35%), Cyclohexanecarboxylic acid, 2-hydroxy-1,6-dimethyl-, [1S-(1*α*,2*β*,6*α*)]- (6.01%), Decanoic acid, octyl ester (5.93%), 2(3H)-Furanone, dihydro-4-hydroxy- (4.7%), 3-Phenylpropanoic acid, 1-adamantylmethyl ester (4.69%), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- (4.21%), Pentadecanoic acid (3.65%), *β*-D-Glucopyranose, 1,6-anhydro- (3.46%), Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (2.81%), 1,4-Benzenedicarboxylic acid, diethyl ester (2.69%).

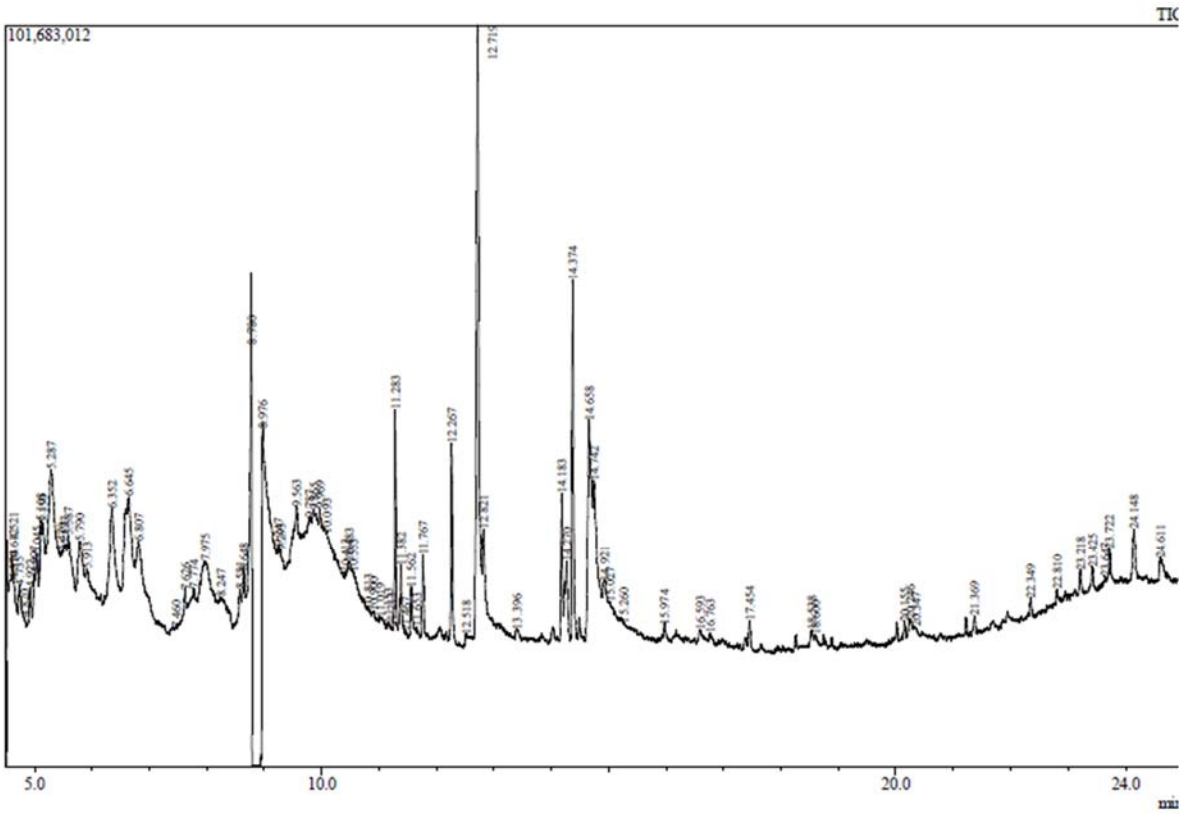

Figure 4.17 GC-MS Chromatogram of methanolic extract of *Anemone rivularis* whole plant.

Table 4.9 Phytocomponents identified in the methanolic extract of *Anemone rivularis* whole plant by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	4.521	0.85	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236
2	4.57	0.68	Acetic acid, 2-(N-methyl-N-phosphonatomethyl)amino-	$C_4H_{10}NO_5P$	183
3	4.612	2.4	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128
4	4.735	1.63	Pentanal	$C_5H_{10}O$	86
5	4.82	0.51	Butanamine, 2,2-dinitro-N-methyl-	$C_5H_{11}N_3O_4$	177
6	4.922	1.43	3-Ethyl-1,3-dimethyldiaziridine (*trans*)	$C_5H_{12}N_2$	100
7	5.045	1.03	*n*-Pentanol, 5-[2-methyl-1-cycloazapropyl]-	$C_8H_{17}NO$	143
8	5.108	1.01	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
9	5.15	1.05	3-Heptanone, 5-ethyl-4-methyl-	$C_{10}H_{20}O$	156
10	5.287	4.7	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	102
11	5.47	0.68	Ethanedial, bis(dimethylhydrazone)	$C_6H_{14}N_4$	142
12	5.527	0.77	2-Butanone, 4-hydroxy-3-methyl-	$C_5H_{10}O_2$	102
13	5.587	1.59	1-Oxaspiro[3.5]nona-5,8-dien-7-one, 3-methylene-	$C_9H_8O_2$	148
14	5.79	1.25	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126
15	5.913	0.41	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134
16	6.352	1.37	2-Methyl-9-*β*-d-ribofuranosylhypoxanthine	$C_{11}H_{14}N_4O_5$	282
17	6.645	2.15	Propanoic acid, 2-[(tetrahydro-2H-pyran-2-yl)oxy]-	$C_8H_{14}O_4$	174
18	6.807	1.42	1,2:5,6-Dianhydrogalactitol	$C_6H_{10}O_4$	146
19	7.46	0.1	1-(4-Hydroxy-6-methyl-2-pyrimidinyl)-2-aziridinone	$C_7H_7N_3O_2$	165
20	7.626	0.88	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518
21	7.774	1.07	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	$C_{13}H_{18}O$	190
22	7.975	4.21	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151
23	8.247	3.46	*β*-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162
24	8.581	2.24	2,5-Dimethoxy-4-ethylamphetamine	$C_{13}H_{21}NO_2$	223
25	8.78	2.69	1,4-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
26	8.976	7.35	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
27	9.247	1.71	Cyclohexanone, 2,3-dimethyl-2-(3-oxobutyl)	$C_{12}H_{20}O_2$	196

28	9.293	2.16	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592
29	9.563	5.93	Decanoic acid, octyl ester	$C_{18}H_{36}O_2$	284
30	9.787	4.69	3-Phenylpropanoic acid, 1-adamantylmethyl ester	$C_{20}H_{26}O_2$	298
31	9.865	2.58	4,6-Dimethyl-2(1H)pyridone, 3-(4,6-dibromo-2-hydroxyphenylmethylenamino)	$C_{14}H_{12}Br_2N_2O_2$	398
32	9.969	2.43	Hexadecanal	$C_{16}H_{32}O$	240
33	10.093	6.01	Cyclohexanecarboxylic acid, 2-hydroxy-1,6-dimethyl-, [1S-(1α,2β,6α)]-	$C_9H_{16}O_3$	172
34	10.413	0.68	2-Amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one	$C_{10}H_{13}N_5O_5$	283
35	10.483	1.7	Butanoic acid, 4-(2-oxocyclopentyl)- $$ 4-(2-Oxocyclopentyl)butanoic acid	$C_9H_{14}O_3$	170
36	10.553	2.81	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	$C_{10}H_{12}O_3$	180
37	10.813	0.64	Carbamodithioic acid, diethyl-, ethyl ester	$C_7H_{15}NS_2$	177
38	10.9	0.66	3-Buten-2-one, 4-(6,6-dimethyl-1-cyclohexen-1-yl)-	$C_{12}H_{18}O$	178
39	11.019	0.89	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666
40	11.153	0.37	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242
41	11.213	0.16	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-acridinedione	$C_{19}H_{13}BrClNO_3$	417
42	11.283	1.01	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
43	11.382	0.62	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268
44	11.467	0.12	2-Butanone, 4-(2,2-dimethyl-6-methylenecyclohexyl)-	$C_{13}H_{22}O$	194
45	11.653	0.13	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370
46	12.267	0.72	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
47	12.719	3.65	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
48	12.821	0.79	Dibutyl phthalate	$C_{16}H_{22}O_4$	278
49	13.396	0.08	Methyl 10-methyl-hexadecanoate	$C_{18}H_{36}O_2$	284
50	14.183	0.55	10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
51	14.27	0.54	9,12,15-Octadecatrienoic acid, methyl ester, (*Z*,*Z*,*Z*)-	$C_{19}H_{32}O_2$	292
52	14.374	1.39	Phytol	$C_{20}H_{40}O$	296
53	14.658	1.89	9,12-Octadecadienoic acid (*Z*,*Z*)-	$C_{18}H_{32}O_2$	280
54	14.742	1.64	9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)-	$C_{18}H_{30}O_2$	278

55	14.921	0.61	Octadecanoic acid	$C_{18}H_{36}O_2$	284
56	15.027	0.38	2-Isopropenyl-4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-benzofuran-6-ol	$C_{14}H_{22}O_2$	222
57	15.26	0.13	Tricyclo[4.3.1.1(3,8)]undecan-3-amine	$C_{11}H_{19}N$	165
58	15.974	0.12	1-Bromo-4-bromomethyldecane	$C_{11}H_{22}Br_2$	312
59	16.593	0.12	1-Tetradecanol, 14-chloro-	$C_{14}H_{29}ClO$	248
60	16.763	0.11	Retinoic acid	$C_{20}H_{28}O_2$	300
61	17.454	0.14	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	220
62	18.538	0.11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
63	18.6	0.09	Octadecanoic acid, 5,9,13,17-tetramethyl-, methyl ester, [5R-(5R*,9R*,13R*)]-	$C_{23}H_{46}O_2$	354
64	20.155	0.11	1-Nonadecene	$C_{19}H_{38}$	266
65	20.246	0.17	*E,Z*-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262
66	20.347	0.09	7-Octynoic acid, methyl ester	$C_9H_{14}O_2$	154
67	21.369	0.09	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-*E*)-	$C_{30}H_{50}$	410
68	22.81	0.1	Anthraergostatetraenol hexahydrobenzoate	$C_{35}H_{52}O_2$	504
69	23.218	0.12	Stigmasta-4,7,22-trien-3*β*-ol	$C_{29}H_{46}O$	410
70	23.425	0.15	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	888
71	23.647	0.1	Isophthalic acid, allyl pentadecyl ester	$C_{26}H_{40}O_4$	416
72	23.722	0.26	7-Dehydrodiosgenin 3-acetate	$C_{29}H_{42}O_4$	454
73	24.148	0.36	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	$C_{27}H_{46}O$	386

4.7.9 GC-MS Analysis of *Scurrula elata*

Scurrula elata afforded the identification of compounds such as 1,2,3-Benzenetriol (25.12%), Diethyl Phthalate (18.14%), Benzoic acid, 2-amino-4-hydroxymethyl-, methyl ester (8.56%), Phthalic acid, di-(1-hexen-5-yl) ester (3.76%), *γ*-Sitosterol (3.15%), D-Allose (2.98%), 2-Cyclopenten-1-one, 2-butyl-3-methoxy- (2.34%), *trans*-3-Cyclopentene-1,2-dicarboxylic acid, 3-acetyl- 4-methyl-, diethyl ester (2.19%), 1-(3,3,3-Trifluoro-2-oxopropyl)-2(1H)-pyridinone (1.86%), and 3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one (1.65%).

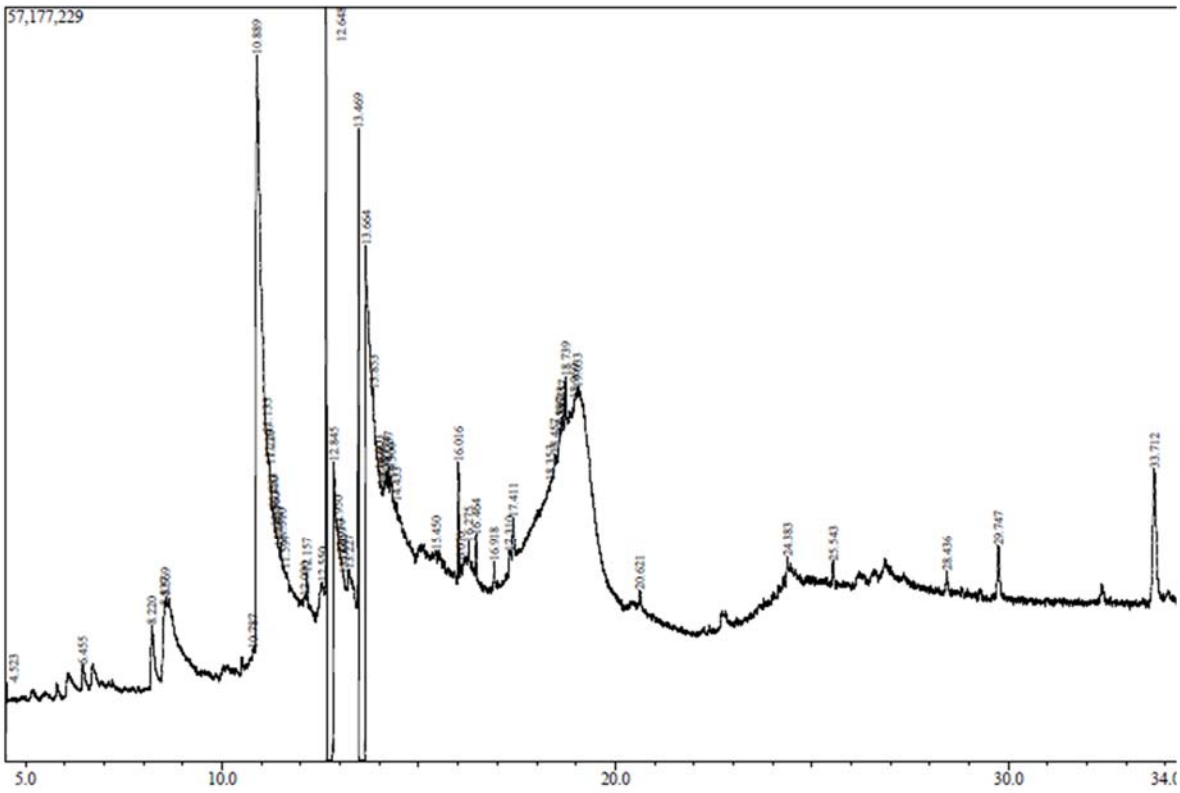

Figure 4.18 GC-MS Chromatogram of methanolic extract of *Scurrula elata* branches.

Table 4.10 Phytocomponents identified in the methanolic extract of *Scurrula elata* branches by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	4.523	0.28	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153
2	6.455	0.22	5-O-Methyl-d-gluconic acid dimethylamide	$C_9H_{19}NO_6$	237
3	8.22	1.2	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151
4	8.537	0.47	3-Chloropropionic acid, 2-isopropoxyphenyl ester	$C_{12}H_{15}ClO_3$	242
5	8.569	0.27	2-Amino-2-[3-methyl-2-furyl]acetic acid	$C_7H_9NO_3$	155
6	10.787	0.11	Phthalic acid, 2-ethoxyethyl pentadecyl ester	$C_{27}H_{44}O_5$	448
7	10.889	25.12	1,2,3-Benzenetriol	$C_6H_6O_3$	126
8	11.133	2.34	2-Cyclopenten-1-one, 2-butyl-3-methoxy-	$C_{10}H_{16}O_2$	168
9	11.197	1.32	Imidazole, 5-methoxymethyl-4-nitro-	$C_5H_7N_3O_3$	157
10	11.22	1.86	1-(3,3,3-Trifluoro-2-oxopropyl)-2(1H)-pyridinone	$C_8H_6F_3NO_2$	205
11	11.283	0.67	1-(2-Pyrazinyl)-3-methyl-1-butanone	$C_9H_{12}N_2O$	164
12	11.36	0.87	Pyrazole-5-carboxylic acid, 3-methyl-	$C_5H_6N_2O_2$	126

13	11.427	0.62	3-[(Cyclohexyl-methyl-amino)-methyl]-3H-benzooxazol-2-one	$C_{15}H_{20}N_2O_2$	260
14	11.457	0.44	5-Furfurylidene hydantoin	$C_8H_6N_2O_3$	178
15	11.51	0.53	2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester	$C_{14}H_{22}O_2$	222
16	11.597	0.17	Nicotinyl Alcohol	C_6H_7NO	109
17	12.09	0.08	Furazanamine, 4-azido-	$C_2H_2N_6O$	126
18	12.157	0.32	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	$C_{13}H_{18}O$	190
19	12.55	2.98	D-Allose	$C_6H_{12}O_6$	180
20	12.648	3.76	Phthalic acid, di-(1-hexen-5-yl) ester	$C_{20}H_{26}O_4$	330
21	12.95	1.65	3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one	$C_9H_{10}N_2O_2$	178
22	13.057	0.87	1-Propanone, 1-(1,3-benzodioxol-5-yl)-3-(dimethylamino)-	$C_{12}H_{15}NO_3$	221
23	13.097	0.2	Tricyclo[4.3.1.1(3,8)]undecane-1-carboxylic acid	$C_{12}H_{18}O_2$	194
24	13.227	0.22	Phthalic acid, monoamide, N-ethyl-N-phenyl-, ethyl ester	$C_{18}H_{19}NO_3$	297
25	13.664	18.14	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
26	13.853	8.56	Benzoic acid, 2-amino-4-hydroxymethyl-, methyl ester	$C_9H_{11}NO_3$	181
27	14.003	1.08	Benzonitrile, 2-amino-4,5-diethoxy-	$C_{11}H_{14}N_2O_2$	206
28	14.117	2.19	*trans*-3-Cyclopentene-1,2-dicarboxylic acid, 3-acetyl- 4-methyl-, diethyl ester	$C_{14}H_{20}O_5$	268
29	14.174	0.89	Tricyclo[4.3.1.1(3,8)]undecane-1-carboxylic acid, methyl ester	$C_{13}H_{20}O_2$	208
30	14.237	0.97	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol	$C_{20}H_{34}O$	290
31	14.3	0.59	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	$C_{13}H_{18}O_2$	206
32	14.433	0.1	Acetic acid, 2,6,6-trimethyl-3-methylene-7-(3-oxobutylidene)oxepan-2-yl ester	$C_{16}H_{24}O_4$	280
33	15.45	0.12	Furan-2-carboxylic acid, 5-(1-hexynyl)-	$C_{11}H_{12}O_3$	192
34	16.016	0.79	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
35	16.07	0.1	2-(1-Cyclohexenyl)cyclohexanone	$C_{12}H_{18}O$	178
36	16.464	0.33	1,4-Eicosadiene	$C_{20}H_{38}$	278
37	16.918	0.21	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270

38	17.31	0.36	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334
39	17.411	0.47	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278
40	18.353	0.1	Carbonic acid, 2-ethoxyethyl neopentyl ester	$C_{10}H_{20}O_4$	204
41	18.457	0.23	Pentadecyl trifluoroacetate	$C_{17}H_{31}F_3O_2$	324
42	18.587	0.24	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	$C_{22}H_{38}O_2$	334
43	18.623	0.19	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652
44	18.657	0.25	9,12,15-Octadecatrienoic acid, methyl ester, (*Z*,*Z*,*Z*)-	$C_{19}H_{32}O_2$	292
45	18.739	0.52	Phytol	$C_{20}H_{40}O$	296
46	18.96	0.13	2R,3S-9-[[1,3-Dihydroxy-4-fluoro-3-butoxy]methyl]guanine	$C_{10}H_{14}FN_5O_4$	287
47	19.033	0.03	L-Lyxose	$C_5H_{10}O_5$	150
48	20.621	0.21	*trans*-2-Dodecenoic acid	$C_{12}H_{22}O_2$	198
49	24.383	0.16	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl)bis-, (R*,R*)-(.+/-.)-	$C_{16}H_{30}$	222
50	25.543	0.26	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-*E*)-	$C_{30}H_{50}$	410
51	28.436	0.22	*β*-Tocopherol	$C_{28}H_{48}O_2$	416
52	29.747	0.9	dl-*α*-Tocopherol succinate	$C_{33}H_{54}O_5$	530
53	33.712	3.15	*γ*-Sitosterol	$C_{29}H_{50}O$	414
54	34.652	0.62	*β*-Amyrin	$C_{30}H_{50}O$	426

4.7.10 GC-MS Analysis of *Terminalia bellerica*

Terminalia bellerica afforded the identification of compounds such as 2-Methyl-9-*β*-d-ribofuranosylhypoxanthine (18.73%), Diethyl Phthalate (16.72%), 1,2,3-Benzenetriol (14.43%), Phenol, 4,4'-methylenebis[2,6-dimethyl- (10.94%), 1,4-Benzenedicarboxylic acid, diethyl ester (10.63%), Spiro[cyclopenta[d]-1,3,2-dioxaborin-4(5H),1'-cyclopentane], 2-ethyl-6,7-dihydro- (5.12%), Phthalic acid, ethyl 2-pentyl ester (2.73%), Phenol, 2-[(1-methylpropyl)thio]- (2.07%), Phthalic acid, ethyl non-5-yn-3-yl ester (1.87%), and 1-(6-Hydroxyimidazo[2,1-b]thiazol-5-yl)ethanone (1.06%).

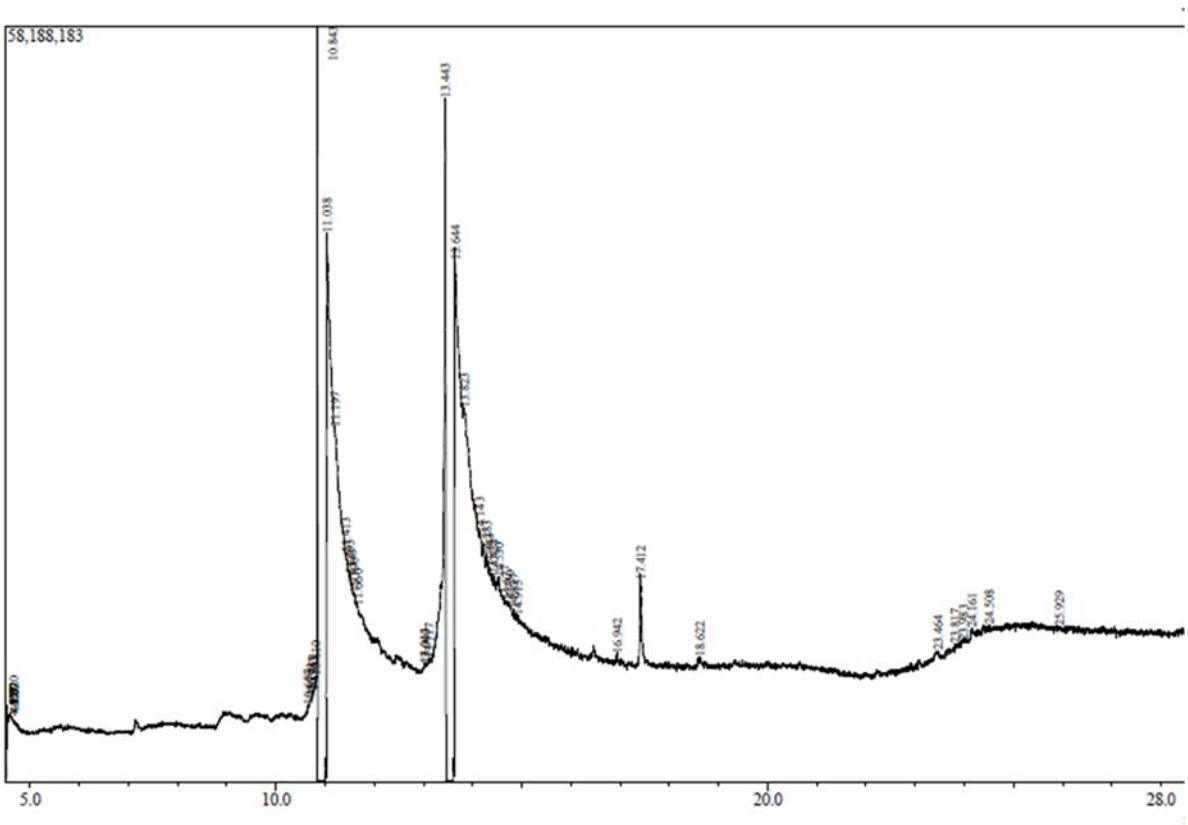

Figure 4.19 GC-MS Chromatogram of methanolic extract of *Terminalia bellerica* fruit.

Table 4.11 Phytocomponents identified in the methanolic extract of *Terminalia bellerica* fruit by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	4.52	0.32	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153
2	4.557	0.28	N-[2,2,2-Trifluoro-1-(isopropylamino)-1-(trifluoromethyl)ethyl]isovaleramide	$C_{11}H_{18}F_6N_2O$	308
3	4.589	0.66	Tricyclo[4.3.1.1(3,8)]undecane-1-carboxylic acid	$C_{12}H_{18}O_2$	194
4	10.657	0.22	Menthone (2,4-dinitrophenyl)hydrazine	$C_{16}H_{22}N_4O_4$	334
5	10.743	0.36	4-Hexenoic acid, 6-hydroxy-4-methyl-, methyl ester, (*E*)-	$C_8H_{14}O_3$	158
6	10.757	0.33	Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-	$C_{14}H_{22}O_2$	222
7	10.81	1.06	1-(6-Hydroxyimidazo[2,1-b]thiazol-5-yl)ethanone	$C_7H_6N_2O_2S$	182
8	11.038	14.43	1,2,3-Benzenetriol	$C_6H_6O_3$	126
9	11.197	10.94	Phenol, 4,4'-methylenebis[2,6-dimethyl-	$C_{17}H_{20}O_2$	256
10	11.413	2.07	Phenol, 2-[(1-methylpropyl)thio]-	$C_{10}H_{14}OS$	182

11	11.493	0.61	2,2-Diethoxy-2-pyrazinyl-1-ethanol	$C_{10}H_{16}N_2O_3$	212
12	11.543	0.81	2,5-Cyclohexadiene-1,4-dione, 2-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-	$C_9H_{10}O_4$	182
13	11.59	0.34	1-(4-Ethoxy-2-nitroanilino)-1-deoxy-*β*-d-mannopyranose	$C_{14}H_{20}N_2O_8$	344
14	11.66	0.27	Pyrazole-5-carboxylic acid, 3-methyl-	$C_5H_6N_2O_2$	126
15	13.013	0.13	5,6,7,8-Tetrahydro-5-oxo-2-hydroxyquinolin-4-yl acetic acid	$C_{11}H_{11}NO_4$	221
16	13.04	0.28	Phenethylamine, 2,4,5-trimethoxy-*α*-methyl-	$C_{12}H_{19}NO_3$	225
17	13.117	0.87	1-Carbazol-9-yl-3-(2-phenoxy-ethylamino)-propan-2-ol	$C_{23}H_{24}N_2O_2$	360
18	13.443	10.63	1,4-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
19	13.644	16.72	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
20	13.823	18.73	2-Methyl-9-*β*-d-ribofuranosylhypoxanthine	$C_{11}H_{14}N_4O_5$	282
21	14.143	5.12	Spiro[cyclopenta[d]-1,3,2-dioxaborin-4(5H),1'-cyclopentane], 2-ethyl-6,7-dihydro-	$C_{12}H_{19}BO_2$	206
22	14.343	1.87	Phthalic acid, ethyl non-5-yn-3-yl ester	$C_{19}H_{24}O_4$	316
23	14.53	2.73	Phthalic acid, ethyl 2-pentyl ester	$C_{15}H_{20}O_4$	264
24	14.677	1.03	Perfluorooctanoic acid, 3,4-dimethylphenyl ester	$C_{16}H_9F_{15}O_2$	518
25	14.75	0.77	Benzonitrile, 2-amino-4,5-diethoxy-	$C_{11}H_{14}N_2O_2$	206
26	14.837	0.33	Thioxane-3-carbonitrile, 3-piperidyl-	$C_{11}H_{18}N_2S$	210
27	14.915	0.13	1,2-Benzenedicarboxylic acid, dihexyl ester	$C_{20}H_{30}O_4$	334
28	16.942	0.13	Dodecanoic acid, 10-methyl-, methyl ester	$C_{14}H_{28}O_2$	228
29	17.412	0.86	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278
30	18.622	0.19	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
31	23.464	0.15	2-Pentene, 3-diethylboryl-2-(methyl-1-propynylsilyl)-	$C_{13}H_{25}BSi$	220
32	23.817	0.15	[1,3,5]Triazine-2-carboxylic acid, 4-ethylamino-6-morpholin-4-yl-, amide	$C_{10}H_{16}N_6O_2$	252
33	23.983	0.17	Silane, dimethyl(dimethyl(but-3-enyloxy)silyloxy)isobutoxy-	$C_{12}H_{28}O_3Si_2$	276
34	24.161	0.32	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666
35	24.508	0.21	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280
36	25.929	0.13	Tricyclo[3.3.1.1(3,7)]decane-2-ol-1-carboxylic acid, methyl ester	$C_{12}H_{18}O_3$	210

4.7.11 GC-MS Analysis of *Ranunculus scleratus*

Ranunculus scleratus afforded the identification of compounds such as Diethyl Phthalate (19.41%), 2-Thiazolamine, 5-nitro- (18.4%), N-Aminopyrrolidine (8.07%), 1-Methylamino-1-deoxy-d-glucitol-N-thiocarboxylic acid 2-[1-[2-pyridyl]ethylidene]hydrazide (3.26%), 9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)- (3.08%), 1,2,3,4,5,6,7,8-Octahydro-2-naphthol, 4-methylene-2,5,5-trimethyl- (3.06%), 9,12-Octadecadienoic acid (*Z*,*Z*)-, 2,3-dihydroxypropyl ester (3.04%), 1,4-Benzenedicarboxylic acid, diethyl ester (3%), Pentadecanoic acid (2.99%), and Phthalic acid, 3-methylphenyl tridecyl ester (2.92%).

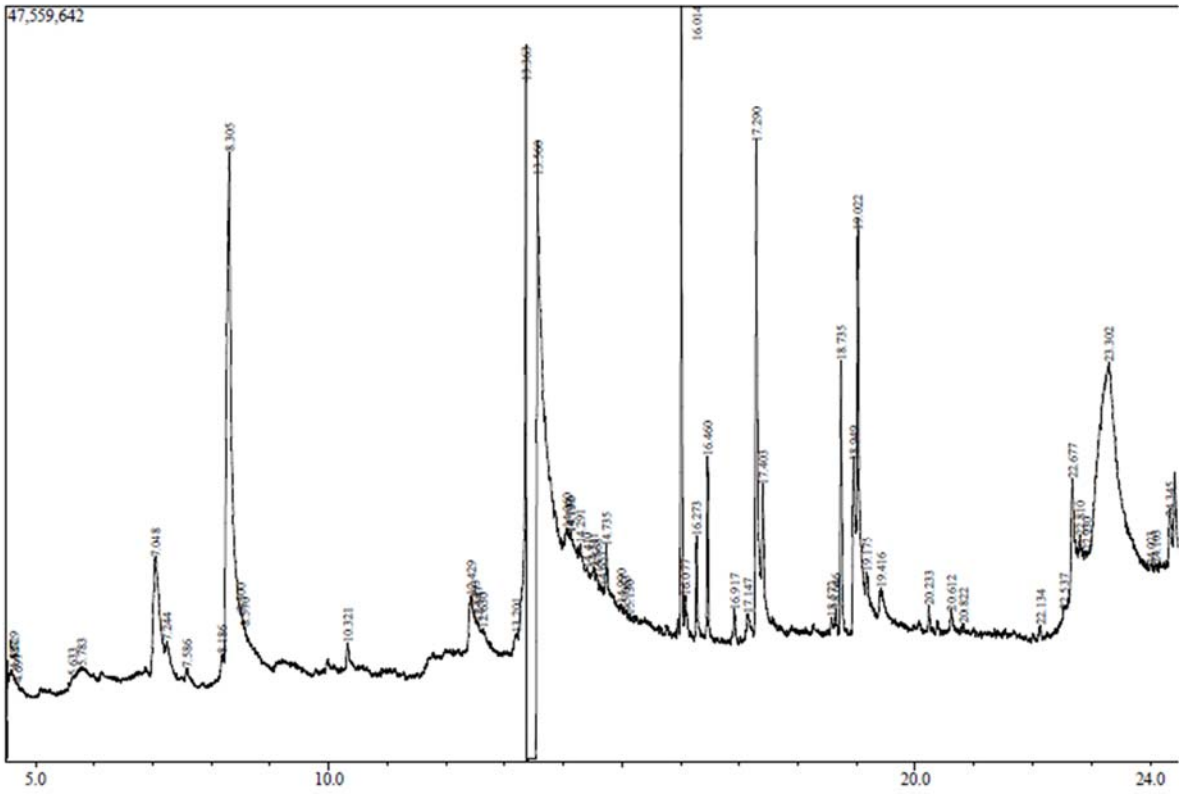

Figure 4.20 GC-MS Chromatogram of methanolic extract of *Ranunculus scleratus* whole plant.

Table 4.12 Phytocomponents identified in the methanolic extract of *Ranunculus scleratus* whole plant by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	4.529	0.36	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153
2	4.587	1.01	Diethylene glycol monododecyl ether	$C_{16}H_{34}O_3$	274
3	4.697	0.2	Isoxazolidine, 4-ethyl-2,5-dimethyl-, *trans*-	$C_7H_{15}NO$	129
4	5.633	0.12	1,2-Dideoxy-l-erythro-pentitol	$C_5H_{12}O_3$	120

5	5.783	0.12	2H-Azepin-2-one, hexahydro-1-methyl-	$C_7H_{13}NO$	127
6	7.048	2.25	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130
7	7.244	0.56	1,4-Dioxaspiro[2.4]heptan-5-one	$C_5H_6O_3$	114
8	7.586	0.11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144
9	8.186	0.15	7,8,9,10-Tetrahydro-4-hydroxy-3-piperidinomethyl-11H,13H-benzo[4',5']thieno[3',2':4,5]pyrimidino[1,2-a]azepin-13-one	$C_{21}H_{25}N_3O_2S$	383
10	8.305	8.07	N-Aminopyrrolidine	$C_4H_{10}N_2$	86
11	8.5	0.33	3(2H)-Furanone, dihydro-5-isopropyl-	$C_7H_{12}O_2$	128
12	8.57	0.13	Acetamide, N-(syn-7-chloro-exo-bicyclo[2.2.1]hept-2-yl)-	$C_9H_{14}ClNO$	187
13	10.321	0.14	Tripropylpropoxysilane	$C_{12}H_{28}OSi$	216
14	12.429	0.77	D-Allose	$C_6H_{12}O_6$	180
15	12.493	0.13	Adenine-9-propanoic acid, α-t-butoxycarbonylamino-	$C_{13}H_{18}N_6O_4$	322
16	12.547	0.21	3-(Dimethylhydrazono)-2,4-dimethylhexane-2,5-diol	$C_{10}H_{22}N_2O_2$	202
17	12.63	0.21	1-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3-(4-fluorocinnamoyl)-2-thiourea	$C_{19}H_{16}FN_3OS_2$	385
18	13.201	0.47	Benzo[b]dioxan, 4,4-dimethyl-5-nitro-3-methoxy-	$C_{11}H_{13}NO_5$	239
19	13.363	3	1,4-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
20	13.56	19.41	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
21	14.06	3.26	1-Methylamino-1-deoxy-d-glucitol-N-thiocarboxylic acid 2-[1-[2-pyridyl]ethylidene]hydrazide	$C_{15}H_{24}N_4O_5S$	372
22	14.103	0.53	Phthalic acid, 2-ethoxyethyl isobutyl ester	$C_{16}H_{22}O_5$	294
23	14.13	2.92	Phthalic acid, 3-methylphenyl tridecyl ester	$C_{28}H_{38}O_4$	438
24	14.291	3.06	1,2,3,4,5,6,7,8-Octahydro-2-naphthol, 4-methylene-2,5,5-trimethyl-	$C_{14}H_{22}O$	206
25	14.41	1.38	Pyridine-2,6-dicarboxylic acid, diethylboryl ester	$C_{11}H_{14}BNO_4$	235
26	14.511	1.2	Phthalic acid, 2-cyclohexylethyl ethyl ester	$C_{18}H_{24}O_4$	304
27	14.568	1.15	2-Decen-1-ol, (*Z*)-	$C_{10}H_{20}O$	156
28	14.657	0.9	5-Amino-1,4-dihydro-quinoxaline-2,3-dione	$C_8H_7N_3O_2$	177

29	14.735	2.42	Octadecanal	$C_{18}H_{36}O$	268
30	14.99	0.48	Tricyclo[4.3.1.1(3,8)]undecane-1-carboxylic acid	$C_{12}H_{18}O_2$	194
31	15.063	0.42	4-Pyridinecarboxamide, 2-ethyl-	$C_8H_{10}N_2O$	150
32	15.13	0.22	2(3H)-Benzofuranone, 3,3-dimethyl-5-[(methylsulfonyl)oxy]-	$C_{11}H_{12}O_5S$	256
33	16.014	2.43	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
34	16.077	0.25	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(*E*)]]-	$C_{20}H_{40}$	280
35	16.917	0.24	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
36	17.147	0.25	Gamolenic Acid	$C_{18}H_{30}O_2$	278
37	17.29	2.99	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
38	17.403	1.12	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278
39	18.572	0.11	10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
40	18.646	0.12	9,12,15-Octadecatrienoic acid, methyl ester,	$C_{19}H_{32}O_2$	292
41	18.735	1.27	Phytol	$C_{20}H_{40}O$	296
42	18.949	1.34	9,12-Octadecadienoic acid (*Z*,*Z*)-	$C_{18}H_{32}O_2$	280
43	19.022	3.08	9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)-	$C_{18}H_{30}O_2$	278
44	19.175	0.85	Octadecanoic acid	$C_{18}H_{36}O_2$	284
45	19.416	0.73	Hexanoic acid, 2-tetradecyl ester	$C_{20}H_{40}O_2$	312
46	20.233	0.1	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213
47	20.612	0.19	*E*-11-Hexadecenal	$C_{16}H_{30}O$	238
48	20.822	0.09	N,N'-Dicycloheptyl-1,2,4,5-tetrazine-3,6-diamine	$C_{16}H_{28}N_6$	304
49	22.134	0.1	Hexanoic acid, 2-dimethylaminoethyl ester	$C_{10}H_{21}NO_2$	187
50	22.537	0.34	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trimethyl-6-methylene-	$C_{15}H_{24}O$	220
51	22.677	1.97	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
52	22.93	0.98	Phosphorochloridic acid, diethyl ester	$C_4H_{10}ClO_3P$	172
53	23.302	18.4	2-Thiazolamine, 5-nitro-	$C_3H_3N_3O_2S$	145
54	24.023	0.27	Quinoline-1-carboxaldehyde, 1,2,3,4-tetrahydro-4,8-dimethyl-2-spirocyclohexane-	$C_{17}H_{23}NO$	257
55	24.103	0.98	Bicyclo[6.4.0]dodeca-1(8),4-diene-10,11-dicarboxylic acid	$C_{14}H_{18}O_4$	250

56	24.345	3.04	9,12-Octadecadienoic acid (*Z*,*Z*)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354
57	24.897	0.15	1H-1,2,3-Triazole-4-carboxylic acid, 1-(4-amino-1,2,5-oxadiazol-3-yl)-5-ethyl-, ethyl ester	$C_9H_{12}N_6O_3$	252

4.7.12 GC-MS Analysis of *Symplocos lucida*

Symplocos lucida afforded the identification of compounds such as 4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol (9.35%), Benzenemethanol, 2,5-dimethoxy-, acetate (6.88%), 4-Hydroxy-3,5-dimethylbenzoic acid (4.96%), *γ*-Sitosterol (4.8%), 3-(Prop-2-en-1-onyl)-2,4,4-trimethylcyclohex-2-en-1-one (4.26%), Benzoic acid, 3-formyl-4,6-dihydroxy-2,5-dimethyl-, methyl ester (3.71%), Cholest-5-en-3-ol, 24-propylidene-, (3*β*) (3.06%), Benzenemethanol, *α*-(1-phenylaminoethyl)- (2.96%), 1,4-Benzenedicarboxylic acid, diethyl ester (2.9%), and Stigmasta-4,22-dien-3*β*-ol (2.83%).

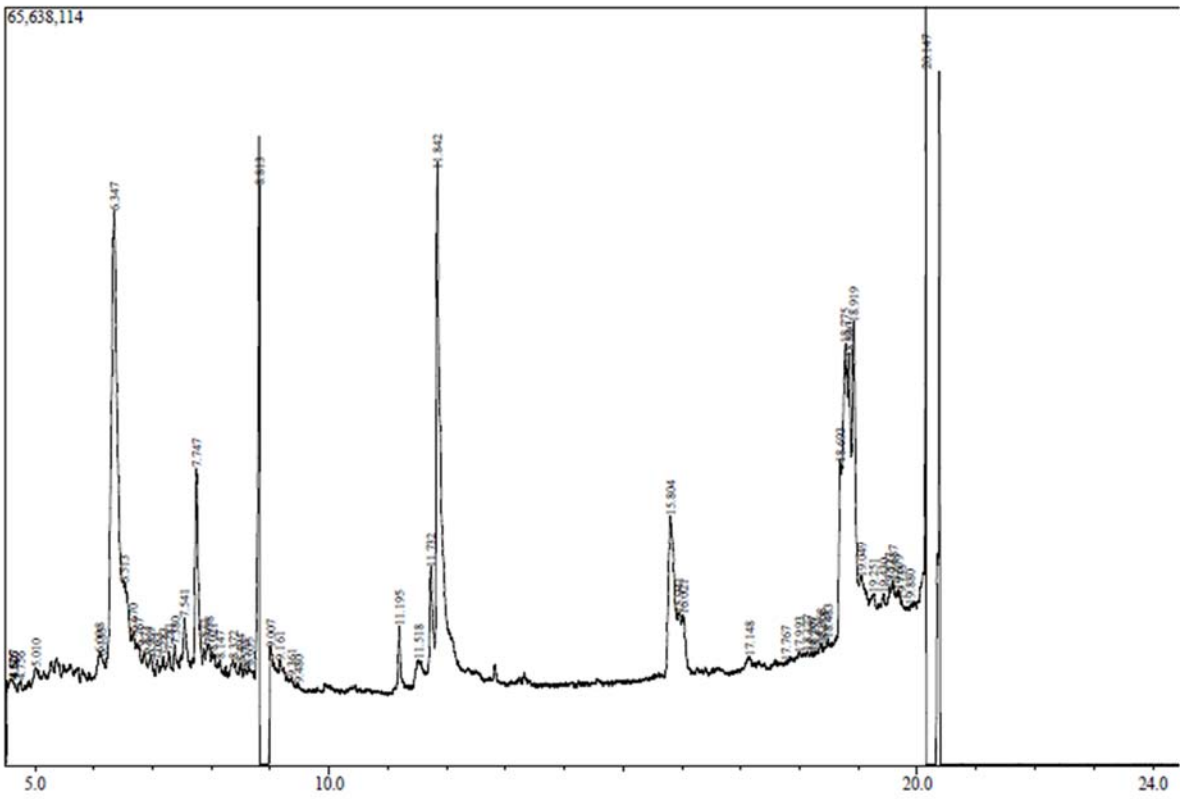

Figure 4.21 GC-MS Chromatogram of methanolic extract of *Symplocos lucida* bark.

Table 4.13 Phytocomponents identified in the methanolic extract of *Symplocos lucida* bark by GC-MS.

SN	R.Time	Area%	Name	Formula	MolWeight
1	4.526	0.3	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207
2	4.607	1.17	Phthalic acid, ethyl tridec-2-yn-1-yl ester	$C_{23}H_{32}O_4$	372
3	5.01	0.23	3-Phenpropanol, 2'-hydroxy-3',4',6'-trimethyl	$C_{12}H_{18}O_2$	194
4	6.098	0.5	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_{10}H_{12}O_4$	196
5	6.133	0.39	N-(4,6-Dimethyl-pyrimidin-2-yl)-N'-(3-methyl-butyl)-guanidine	$C_{12}H_{21}N_5$	235
6	6.347	9.35	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
7	6.767	0.93	Phthalic acid, ethyl 4-isopropylphenyl ester	$C_{19}H_{20}O_4$	312
8	7.541	2.07	2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-3-one	$C_{13}H_{20}O_2$	208
9	7.747	3.71	Benzoic acid, 3-formyl-4,6-dihydroxy-2,5-dimethyl-, methyl ester	$C_{11}H_{12}O_5$	224
10	7.958	1.2	Methyl tetra-p-nitrobenzate-*β*-O-galactopyranoside	$C_{35}H_{26}N_4O_{18}$	790
11	8.372	2.44	1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl	$C_{13}H_{20}O$	192
12	8.572	0.79	Benzonitrile, 2-amino-4,5-diethoxy-	$C_{11}H_{14}N_2O_2$	206
13	8.635	1.71	Phthalic acid, 2-ethoxyethyl dodecyl ester	$C_{24}H_{38}O_5$	406
14	8.813	2.9	1,4-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
15	9.007	1.9	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
16	9.161	1.36	2,5-Dimethoxy-4-propoxybenzaldehyde	$C_{12}H_{16}O_4$	224
17	9.361	0.85	Phthalic acid, decyl isobutyl ester	$C_{22}H_{34}O_4$	362
18	9.48	0.24	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370
19	11.195	0.48	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278
20	11.518	0.45	Methyl (3,4-dimethoxyphenyl)(hydroxy)acetate	$C_{11}H_{14}O_5$	226
21	11.732	1.1	3,5-Dimethoxy-4-hydroxycinnamaldehyde	$C_{11}H_{12}O_4$	208
22	11.842	6.88	Benzenemethanol, 2,5-dimethoxy-, acetate	$C_{11}H_{14}O_4$	210
23	15.804	2.83	Stigmasta-4,22-dien-3*β*-ol	$C_{29}H_{48}O$	412
24	15.941	0.53	Stigmasta-7,16-dien-3-ol, (3*β*,5*α*)-	$C_{29}H_{48}O$	412
25	16.021	0.8	Stigmasterol	$C_{29}H_{48}O$	412

26	17.148	0.16	Cedran-diol, 8S,14-	$C_{15}H_{26}O_2$	238
27	17.767	0.19	2-(1,3-Dihydro-3,3-dimethyl-1-phenyl-3,4-benzofuran-1-yl)acetic acid	$C_{18}H_{18}O_3$	282
28	18.127	0.48	Silanol, trimethyl-, phosphite (3:1)	$C_9H_{27}O_3PSi_3$	298
29	18.207	0.22	1-[3,4-Dichlorophenyl]-3-[4,6-dimethyl-2-pyrimidinyl]guanidine	$C_{13}H_{13}Cl_2N_5$	309
30	18.253	0.41	2-Isopropenyl-4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-benzofuran-6-ol	$C_{14}H_{22}O_2$	222
31	18.368	0.52	1-Bromo-3,7-dimethyloctane	$C_{10}H_{21}Br$	220
32	18.413	0.3	1-[N,N'-Bis(2-methylphenyl)amidino]aziridine	$C_{17}H_{19}N_3$	265
33	18.483	0.44	16-Heptadecenal	$C_{17}H_{32}O$	252
34	18.693	2.96	Benzenemethanol, *α*-(1-phenylaminoethyl)-	$C_{15}H_{17}NO$	227
35	18.775	4.8	*γ*-Sitosterol	$C_{29}H_{50}O$	414
36	19.251	2.39	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666
37	19.43	3.06	Cholest-5-en-3-ol, 24-propylidene-, (3*β*)	$C_{30}H_{50}O$	426
38	19.527	1.48	26,26-Dimethyl-5,24(28)-ergostadien-3*β*-ol	$C_{30}H_{50}O$	426
39	19.587	1.86	Tyramine, N-formyl-	$C_9H_{11}NO_2$	165
40	19.733	1.34	3*β*,5*α*,6*β*-Trihydroxyandrostan-17-one	$C_{19}H_{30}O_4$	322
41	19.88	4.26	3-(Prop-2-en-1-onyl)-2,4,4-trimethylcyclohex-2-en-1-one	$C_{12}H_{16}O_2$	192
42	20.147	4.96	4-Hydroxy-3,5-dimethylbenzoic acid	$C_9H_{10}O_3$	166

4.8 Antimicrobial Effects

The methanolic extracts of twelve medicinal plants displayed a dose dependent antimicrobial activity against one or more of the microorganisms tested (Table 4.14 to

Table 4.19). Maximum zone of inhibition against *S. aureus* and MRSA both were shown by *T. bellerica* while *K. pneumoniae* and *S.* Typhi were inhibited maximally by *L. involuta* and *T. dumosa* respectively. *L. involuta* did not show zone of inhibition against any of the gram positive bacteria taken. *S. elata* and *T. bellerica* were active against all the tested microorganisms while *S. lucida* only against MRSA. Ten of the twelve extracts inhibited at least three of the six organisms tested while eight extracts inhibited at least four organisms. *G. wallichianum* demonstrated effectiveness against five of the organisms tested except *K. pneumoniae*. *R. scleratus* did not inhibit any of the bacteria taken. Maximum zone of inhibition against *S. cerevisiae* and *Pichia sp*. were shown by *H. javanica* and *A. rivularis* respectively while *S. lucida* did not show the zone of inhibition against any of the fungi taken.

Most of the plant extracts were found active against gram positive bacteria and fungi while only some were found active to gram negative bacteria.

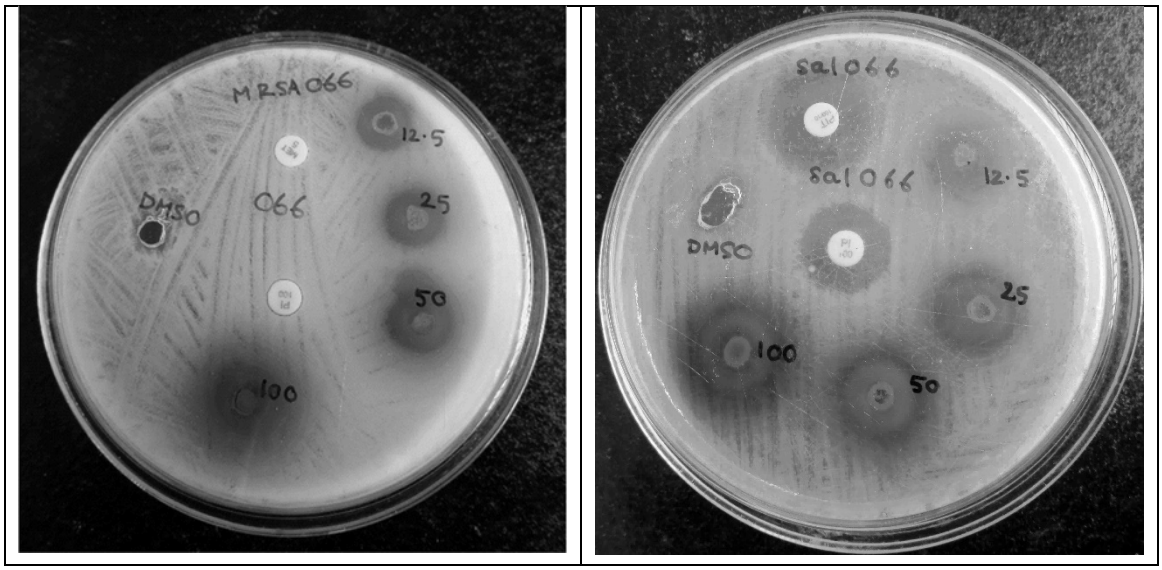

Figure 4.22 Antimicrobial activity of *T. dumosa* by agar well diffusion method as shown by the zone of inhibition against methicillin-resistant *Staphylococcus aureus* and *Salmonella* Typhi, respectively.

Table 4.14 Antibacterial activity of methanolic extracts of 12 medicinal plants as shown by zone of inhibition (mm) against *Staphyloccous aureus* using agar well diffusion method.

Plant extracts in mg/ml	100	50	25	12.5	DMSO	PI100
L. involuta	-	-	-	-	-	24
H. javanica	8	7	6	6	-	24
P. emblica	13	9	8	7	-	23
C. aciculatus	-	-	-	-	-	23
R. lepidotum	11	10	9	8	-	23
G. wallichianum	10	8	7	6	-	22
T. dumosa	11	10	9	8	-	22
A. rivularis	7	6	-	-	-	23
S. elata	10	9	8	7	-	23
T. bellerica	16	15	14	13	-	20
R. scleratus	-	-	-	-	-	21
S. lucida	-	-	-	-	-	22

Table 4.15 Antibacterial activity of methanolic extracts of 12 medicinal plants as shown by zone of inhibition (mm) against methicillin-resistant *Staphylococcus aureus* using agar well diffusion method.

Plant extracts in mg/ml	100	50	25	12.5	DMSO	MET5
L. involuta	-	-	-	-	-	-
H. javanica	7	6	-	-	-	-
P. emblica	20	18	17	15	-	-
C. aciculatus	8	7	6	-	-	-
R. lepidotum	11	10	9	7	-	-
G. wallichianum	13	12	11	9	-	-
T. dumosa	13	12	11	10	-	-
A. rivularis	-	-	-	-	-	-
S. elata	12	11	11	11	-	-
T. bellerica	21	20	19	17	-	-
R. scleratus	-	-	-	-	-	-
S. lucida	8	7	-	-	-	-

Table 4.16 Antibacterial activity of methanolic extracts of 12 medicinal plants as shown by zone of inhibition (mm) against *Klebsiella pneumoniae* using agar well diffusion method.

Plant extracts in mg/ml	100	50	25	12.5	DMSO	PIT100/10
L. involuta	10	8	7	7	-	
H. javanica	-	-	-	-	-	
P. emblica	-	-	-	-	-	
C. aciculatus	9	8	7	6	-	
R. lepidotum	-	-	-	-	-	
G. wallichianum	-	-	-	-	-	
T. dumosa	-	-	-	-	-	9
A. rivularis	-	-	-	-	-	
S. elata	8	7	6	-	-	
T. bellerica	8	7	6	-	-	
R. scleratus	-	-	-	-	-	
S. lucida	-	-	-	-	-	8

Table 4.17 Antibacterial activity of methanolic extracts of 12 medicinal plants as shown by zone of inhibition (mm) against *Salmonella* Typhi using agar well diffusion method.

Plant extracts in mg/ml	100	50	25	12.5	DMSO	PI100
L. involuta	-	-	-	-	-	15
H. javanica	-	-	-	-	-	15
P. emblica	-	-	-	-	-	15
C. aciculatus	11	10	9	8	-	15
R. lepidotum	-	-	-	-	-	13
G. wallichianum	8	7	-	-	-	15
T. dumosa	19	16	14	13	-	16
A. rivularis	8	7	-	-	-	12
S. elata	13	12	11	10	-	15
T. bellerica	11	9	7	6	-	16
R. scleratus	-	-	-	-	-	15
S. lucida	-	-	-	-	-	16

Table 4.18 Antifungal activity of methanolic extracts of 12 medicinal plants as shown by zone of inhibition (mm) against *Saccharomyces cerevisiae* using agar well diffusion method.

Plant extracts in mg/ml	100	50	25	12.5	DMSO	Fluconazole (2.5mg/ml)
L. involuta	7	6	-	-	-	
H. javanica	11	11	9	6	-	
P. emblica	9	8	7	6	-	
C. aciculatus	6	-	-	-	-	24
R. lepidotum	-	-	-	-	-	25
G. wallichianum	9	7	7	-	-	25
T. dumosa	-	-	-	-	-	25
A. rivularis	10	8	8	7	-	26
S. elata	7	6	-	-	-	27
T. bellerica	9	9	8	7	-	25
R. scleratus	8	7	-	-	-	24
S. lucida	-	-	-	-	-	25

Table 4.19 Antifungal activity of methanolic extracts of 12 medicinal plants as shown by zone of inhibition (mm) against *Pichia sp.* using agar well diffusion method.

Plant extracts in mg/ml	100	50	25	12.5	DMSO	Fluconazole (2.5 mg/ml)
L. involuta	12	11	10	9	-	16
H. javanica	16	11	9	8	-	12
P. emblica	9	9	8	7	-	11
C. aciculatus	-	-	-	-	-	11
R. lepidotum	8	7	6	6	-	13
G. wallichianum	9	8	7	6	-	10
T. dumosa	8	7	-	-	-	15
A. rivularis	22	16	13	9	-	10
S. elata	8	7	-	-	-	12
T. bellerica	8	7	6	6	-	12
R. scleratus	9	9	8	7	-	16
S. lucida	-	-	-	-	-	12

4.9 Brine Shrimp Cytotoxicity

The degree of lethality was found to be directly proportional to the concentration of the extract (Figure 4.23). Of the twelve medicinal plant extracts tested nine extracts exhibited significant toxicity towards *Artemia salina* (brine shrimp) in which *A. rivularis* (58.11 µg/ml), *L. involuta* (91.72 µg/ml) and *T. bellerica* (94.65 µg/ml) with LC_{50} value less than 100 µg/ml were highly toxic while *G. wallichianum* (135.90 µg/ml), *H. javanica* (195.68 µg/ml), *C. aciculatus* (252.82 µg/ml), *S. lucida* (292.19 µg/ml), *P. emblica* (306.27 µg/ml) and *S. elata* (400.35 µg/ml) with LC_{50} value less than 500 µg/ml were moderately toxic. *T. dumosa* (9523.10 µg/ml exhibited highest LC_{50} value followed by *R. scleratus* (2069.84 µg/ml) and *R. lepidotum* (1607.17 µg/ml) which were found to be practically non-toxic (Figure 4.24).

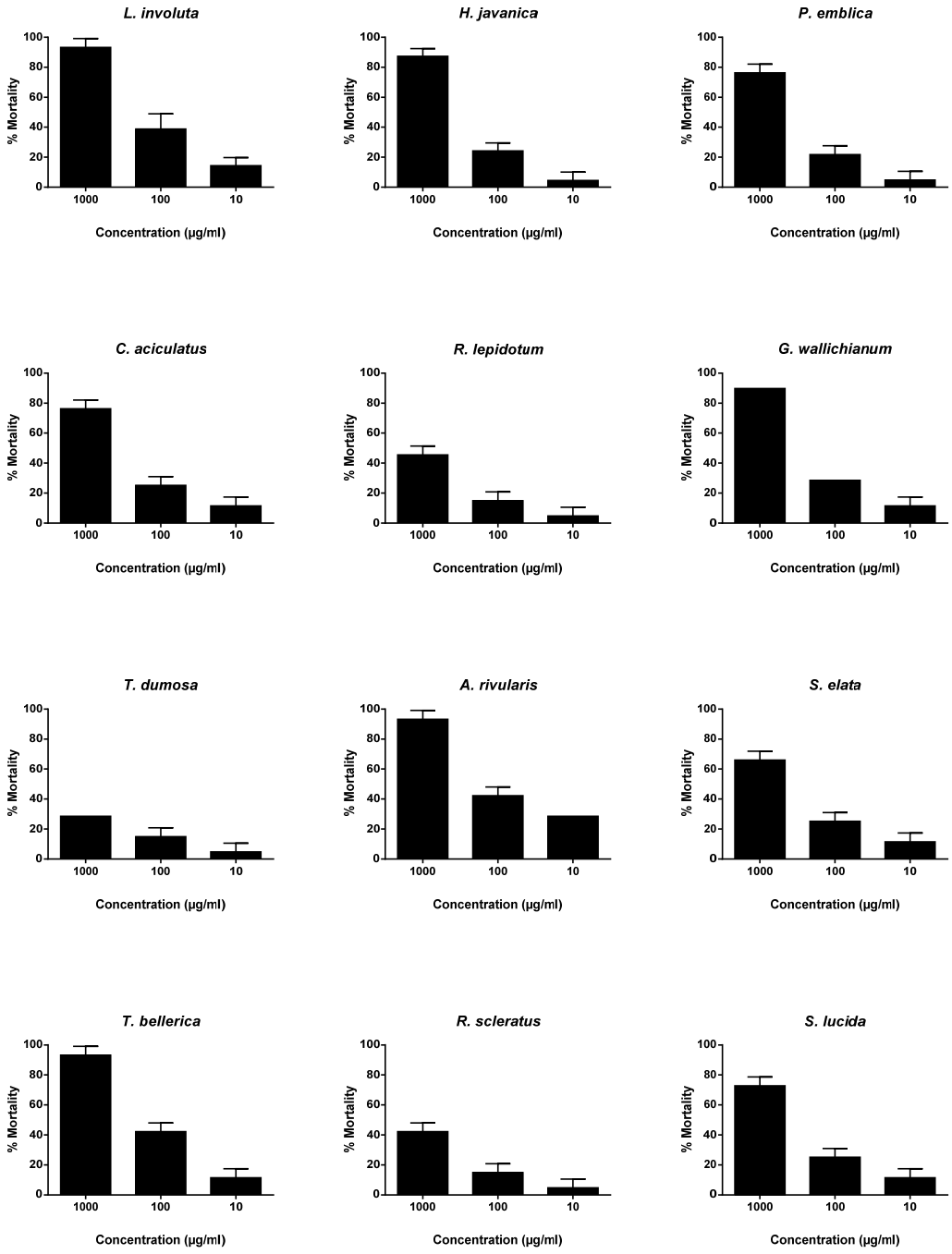

Figure 4.23 Percentage mortality of *Artemia salina* (brine shrimp) larvae on treatment of methanolic extracts of 12 medicinal plants.

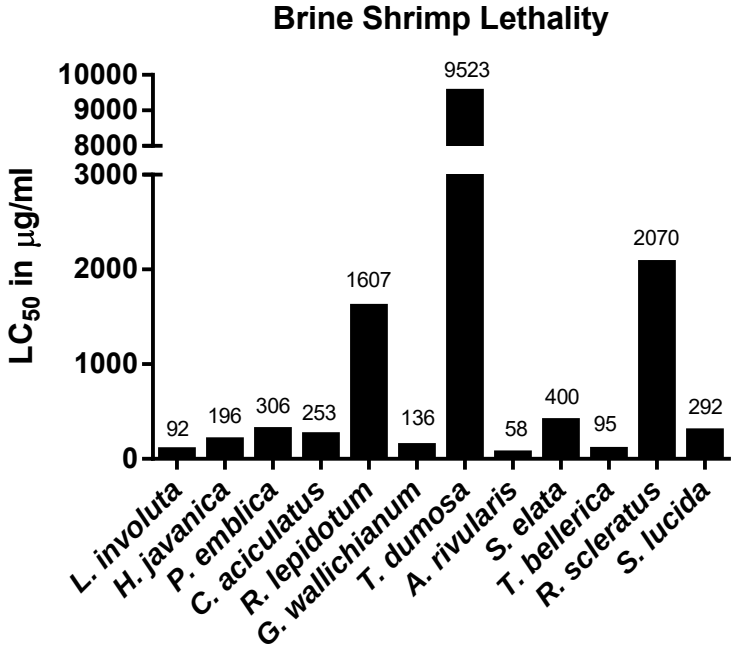

Figure 4.24 LC_{50} of methanolic extracts of 12 medicinal plants on *Artemia salina* (brine shrimp) larvae.

4.10 MTT Cell Proliferation Assay

The methanol extracts of twelve medicinal plants exerted a dose dependant cytotoxicity against HeLa and MDCK cell lines. Among the plant extracts tested for growth inhibition (EC_{50}) of HeLa cells, *S. lucida* (52.22 µg/ml) was found to be the most effective followed by *T. dumosa* (122.3 µg/ml), *C. aciculatus* (177.6 µg/ml), *R. scleratus* (249.4 µg/ml), *S. elata* (304 µg/ml), *A. rivularis* (345.6 µg/ml), and *R. lepidotum* (389.7 µg/ml) while *T. bellerica* (1048 µg/ml) was found to be the least effective. In case of MDCK cells, *L. involuta* (240 µg/ml) was found to be most effective followed by *T. dumosa* (305 µg/ml), *A. rivularis* (407.7 µg/ml), *S. elata* (751.1 µg/ml), and *P. emblica* (925.3 µg/ml) while others with EC_{50} value more than 1000 µg/ml were found to be less effective. Ten of the twelve medicinal plants including *S. lucida, C. aciculatus, R. scleratus, S. elata, R. lepidotum, G. wallichianum,* and *H. javanica* were found to be more effective against HeLa cells, however *L. involuta* and *P. emblica* inhibited MDCK cells more effectively.

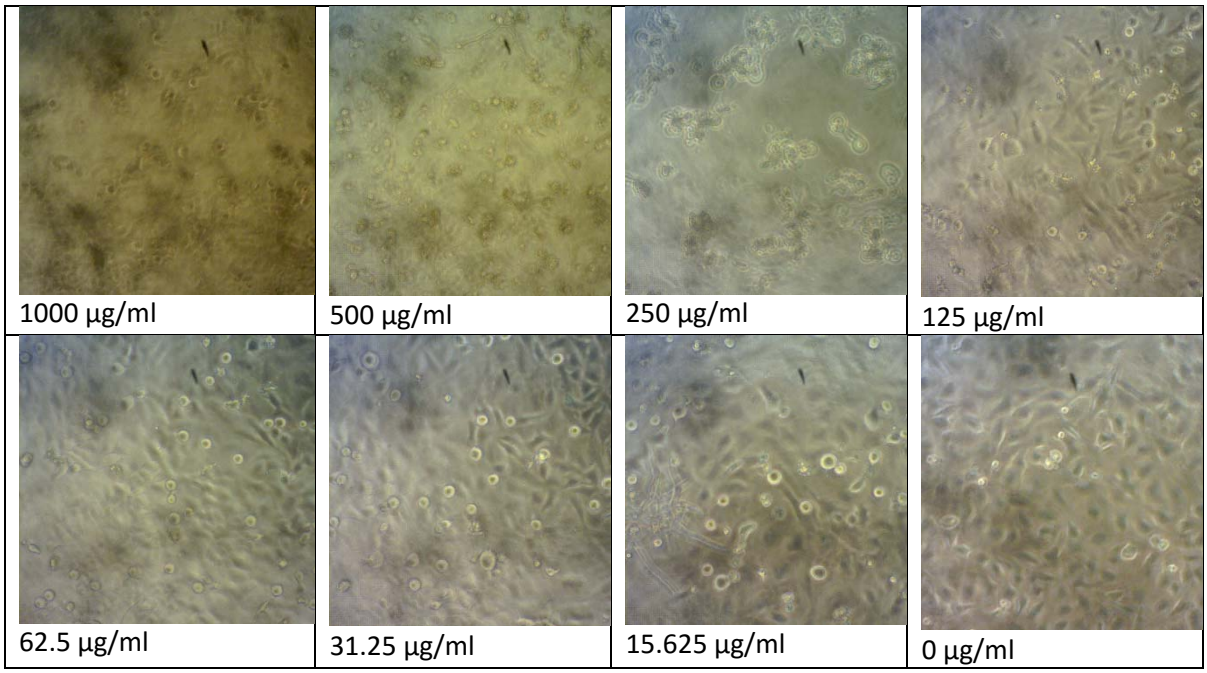

Figure 4.25 Effects of *H. javanica* methanol extract on HeLa cell line with its decreasing concentration.

4.10.1 HeLa Cell Survival

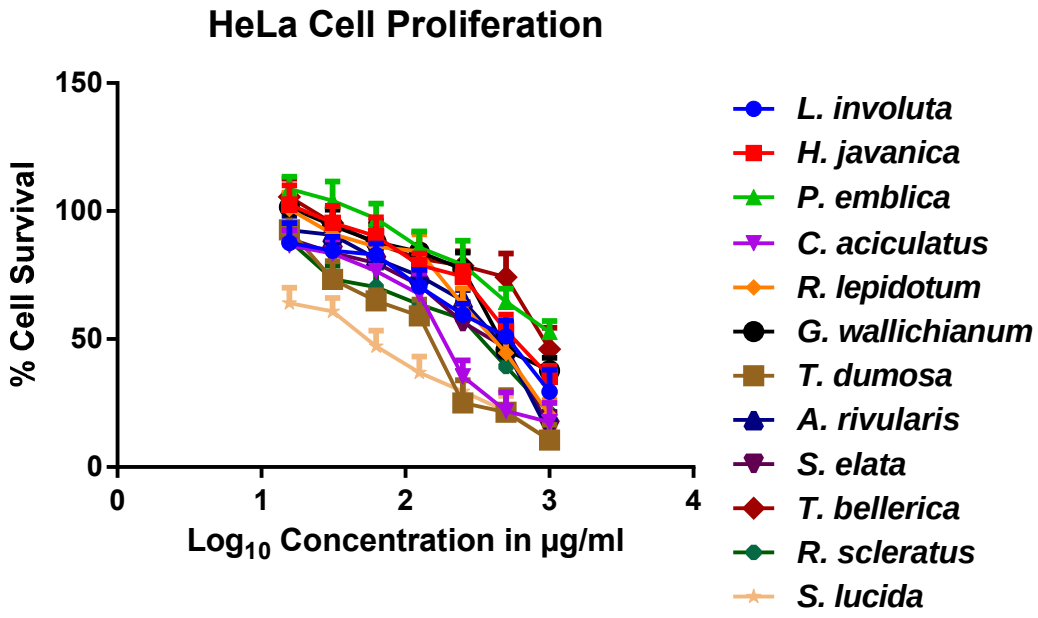

Figure 4.26 Effects of methanolic extracts of 12 medicinal plants on HeLa cell survival.

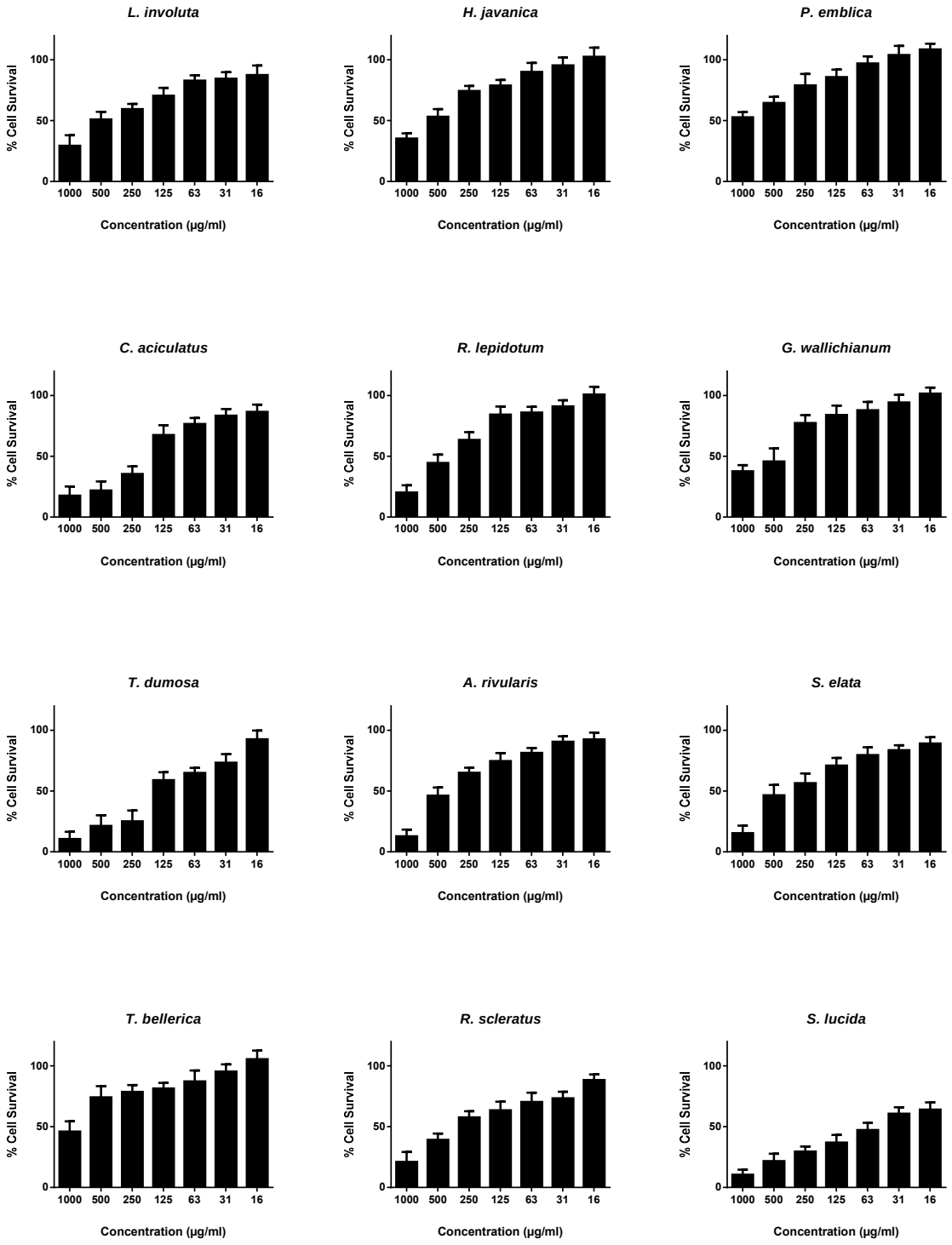

Figure 4.27 Effects of methanolic extracts of 12 medicinal plants on HeLa cell survival.

Table 4.20 EC_{50} of methanolic extracts of 12 medicinal plants on HeLa cell line.

Plant extracts	HeLa EC_{50} in µg/ml
L. involuta	412.9
H. javanica	574.9
P. emblica	991.6
C. aciculatus	177.6
R. lepidotum	389.7
G. wallichianum	560.1
T. dumosa	122.3
A. rivularis	345.6
S. elata	304
T. bellerica	1048
R. scleratus	249.4
S. lucida	52.22

4.10.2 MDCK Cell Survival

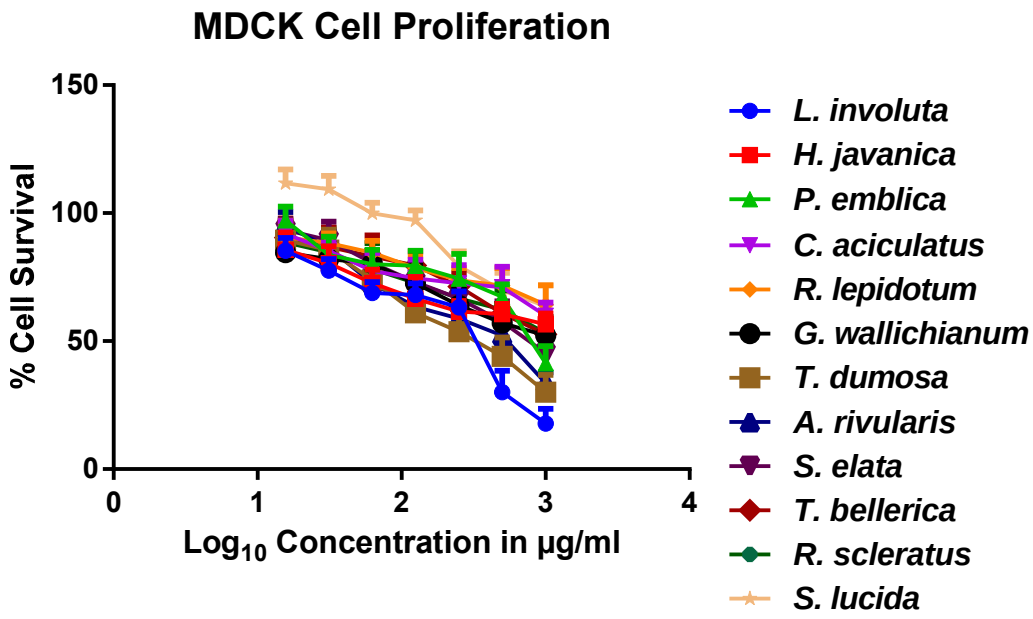

Figure 4.28 Effects of methanolic extracts of 12 medicinal plants on MDCK cell survival.

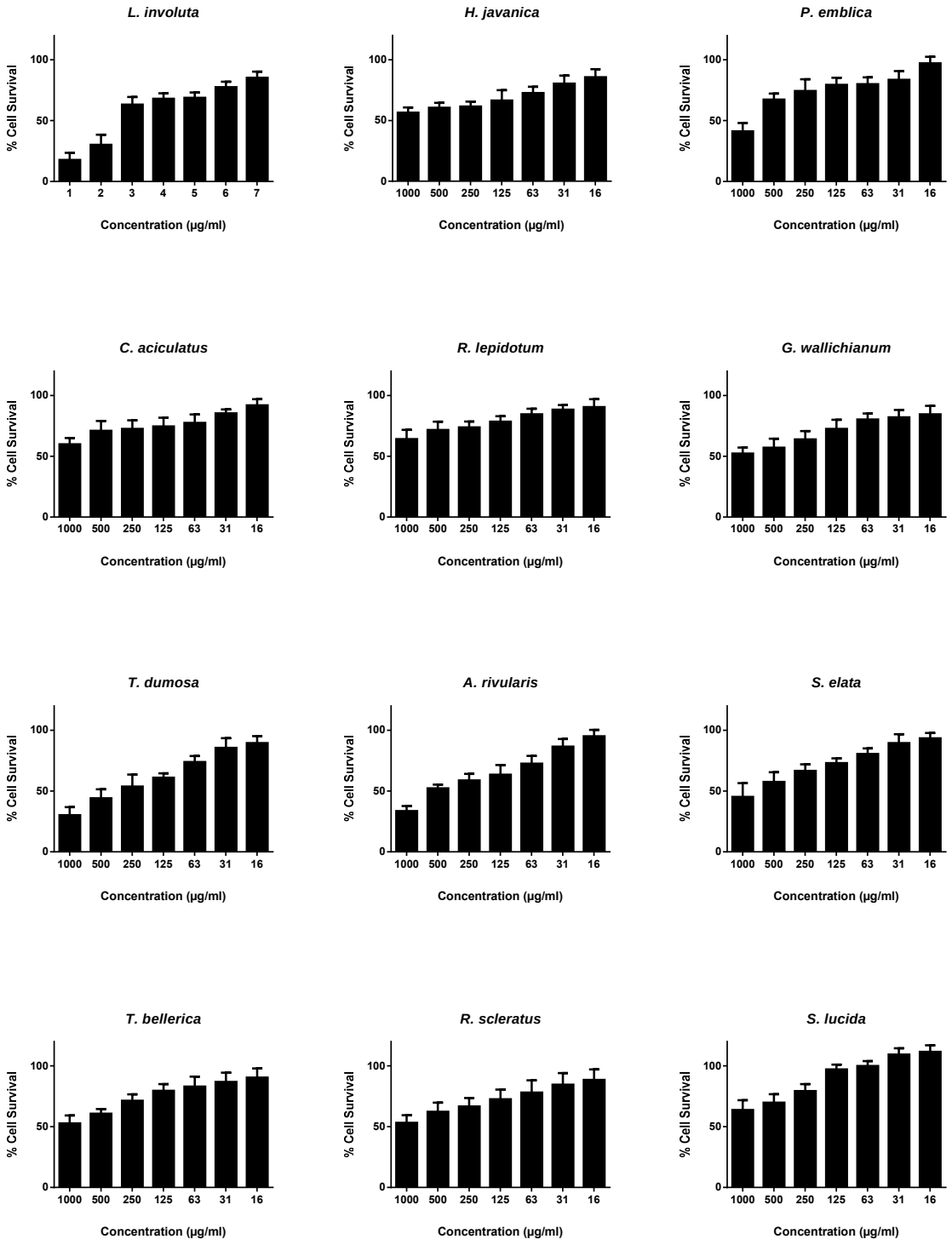

Figure 4.29 Effects of methanolic extracts of 12 medicinal plants on MDCK cell survival.

Table 4.21 EC_{50} of methanolic extracts of 12 medicinal plants on MDCK cell line.

Plant extracts	MDCK EC_{50} in µg/ml
L. involuta	240
H. javanica	>1000
P. emblica	925.3
C. aciculatus	3521
R. lepidotum	4448
G. wallichianum	1105
T. dumosa	305
A. rivularis	407.7
S. elata	751.1
T. bellerica	1239
R. scleratus	1360
S. lucida	1389

5 Discussion

Nepal enjoys the richness of highly valued medicinal plants with varieties of local uses in different parts of the country. Other parts of the world also report their use in one or the other ways. Some researches have been made globally to test for the presence of novel bioactive compounds and their effects *in vitro* and *in vivo*. Our part of the world, however, has remained far behind in the race. This excludes us from the the benefits mother nature has provided.

Nature has preserved the plants with the myriads of biochemicals evolved during their struggle for survival – either against the adverse environmental conditions or against the rival organisms. The plants are thus the self made biochemical factories. Each chemical can find their use somewhere for human wellbeing. In medicine, the phytochemicals have an immense value. They find wider applications in modern day health care system.

Phytomedicine is in transistion from fringe to the mainstream medicine due to the presence of active principles in plants capable of promoting human health. WHO has also recognized its importance in the safe treatment of infectious as well as non-infectious diseases. The global acceptance of phytopharmaceuticals can also be advocated by their higher rate of FDA approval.

Traditional use of medicinal plants need some scientific validation for their continued and diversified use. Present study is the preliminary one to identify the bioactive compounds and biological activities of some of the underutilized medicinal plants of Nepal. The presence of a diverse range of phytocompounds, as indicated by GC-MS analysis and other phytochemical tests, and the presentation of important biological activities – such as antioxidant and anticancer – by the crude extracts in different concentrations suggest the great prospects of our medicinal plants for their wider applications in phytotherapy and phytopharmaceuticals.

5.1 Methanol Extraction

Medicinal plants possess a vast number of compounds which differ in their polarities. Different solvents with different polarities could have been used for their extraction such as water, ethanol, or acetone. We used methanol as an extraction solvent. Methanol is polar than water and ethanol. It is known to extract most of the active constituents from the plant material. It has been reported as an effective solvent to extract bioactive compounds such as phenolics (Siddhuraju & Becker, 2003). Another advantage is its easy evaporation resulting in quick concentration of the extracts.

The extraction yield of twelve medicinal plants in methanol came in the range from 4.39% to 32.48%, the highest of which was obtained from *T. bellerica* and the lowest from *C. aciculatus*.

H. javanica whole plant in methanol (9.85 %) in our study presented an extract yield somewhat similar to that previously reported in other solvents such as water (11.55%) and ethanol (12.95%) (Huang et al., 2008). Percentage yield of methanol extract of *P. emblica* bark (20.76 %) in our study has been found to be similar to the minimum of that reported from methanol extract of *P. emblica* fruits from six regions in China (21.0% to 39.4%) (Liu et al., 2008). However, it was found to be higher than that reported from *P. emblica* fruit in water (8.76%) (Ngamkitidechakul et al., 2010), and 70% methanol (4.2%) (Hazra et al., 2010). Percentage yield of methanol extract of *R. lepidotum* leaf and stem (7.12 %) in our study has been found to be somewhat similar to that reported from methanol extract of *R. lepidotum* leaves and twigs (6.07%) (Bhattarai et al., 2010). However, it was lower than that reported from methanol extract of *R. lepidotum* aerial parts (18.9%) (Rajbhandari et al., 2009), and higher than that reported from chloroform:methanol extract of *R. lepidotum* leaves and twigs (4.397%) (Bhattarai et al., 2010). Extract yield of *G. wallichianum* root (19.34 %) in methanol in our study has been found to be higher than that reported from *G. wallichianum* in 70% ethanol (2%) (Ahmad et al., 2003). Extract yield of *T. bellerica* fruit in methanol (32.48 %) in our study has been found to be somewhat similar to that reported from *T. belerica* fruit in 70% ethanol (33% w/w) (Choudhary, 2008), and *T. bellirica* fresh leaves in 70% ethanol (30%) (Prabhu & Gopal, 2011; Vinoth et al., 2012). However it was found to be higher than that reported from *T. belerica* fruit in 70% methanol (3.7%) (Hazra et al., 2010), ethanol (15%) (Manjunatha et al., 2011).

The choice of the solvent influences the extraction yield of medicinal plants. Compound to be contained in the extract is determined by the solvent used which in turn affects the biological activities of plant extracts (Gong et al., 2012; Jang et al., 2007). Other factors that may influence the biological activities include plant parts used, geography, harvest season, drying process, storage conditions, and post-harvest processing (Wendakoon et al., 2012). These factors also affect the extraction yield.

5.2 Total Phenolic Content

Phenolics are the most abundant structures in plants. Antioxidant compounds in plants are usually in the phenolic form. These important plant components can give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals, hence play an important role in antioxidant activity. Phenolics from intake of fruit and vegetable has been

found to reduce the risk of oxidative stress associated diseases such as cardiovascular diseases, cancer or osteoporosis (Hollman & Katan, 1999; Scalbert et al., 2005). Phenolic compounds can exhibit many biological activities such as an antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial. They can exert chemopreventive effects due to their free radical scavenging and metal chelating properties (Kampa et al., 2007).

We determined the total phenolic content of plant extracts by Folin-Ciocalteau method. It is a preferred method for the determination of total phenolic content (Singleton et al., 1999). This method allows the estimation of all the phenolic compounds including flavonoids, anthocyanins and nonflavonoids (Benvenuti et al., 2004). This assay is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form the other type of blue complexes (possibly $(PMoW_{11}O_{40})^{4-}$) that are determined spectroscopically at approximately 765 nm (Singleton et al., 1999; Singleton & Rossi, 1965).

Other oxidation substrates present in the extract can interfere the total phenolics measurement in an inhibitory, additive or enhancing manner (Singleton et al., 1999; Singleton & Rossi, 1965). The inhibitory effects could be due to the oxidants competing with F-C reagent and/or air oxidation after the sample is made alkaline. For this reason, the F-C reagent is added ahead of alkali (Singleton et al., 1999). Additive effects occur from unanticipated phenols, aromatic amines, high sugar levels or ascorbic acid in the samples. However, despite these disadvantages, the F-C assay is simple and reproducible and has been widely used for quantification of phenolic compounds in plant extracts.

Total phenolic content shown by the plant extracts ranged from 6.74 mgGAE/g to 55.66 mgGAE/g. The highest amount of phenolic content was found in *P. emblica* (55.66 ± 0.84 mgGAE/g) followed by *G. wallichianum* (54.99 ± 0.74 mgGAE/g), *S. elata* (54.71 ± 0.68 mgGAE/g), *T. bellerica* (54.69 ± 0.77 mgGAE/g), and *T. dumosa* (54.19 ± 0.93 mgGAE/g) while the lowest was observed in *R. scleratus* (6.74 ± 0.62 mgGAE/g).

Total phenolic content in methanolic extract of *P. emblica* bark (55.66 ± 0.84 mgGAE/g) in our study has been found to be lower than that reported in methanolic extracts of *P. emblica* fruit from six regions of China (81.5 to 120.9 mgGAE/g) (Liu et al., 2008) in addition to that in water and 70% methanol extract of *P. emblica* fruits (34.22 g gallic acid/100g and 215.60 mg/ml GAE/100 mg respectively) (Charoenteeraboon et al., 2010; Hazra et al., 2010). Similarly, TPC observed in methanolic extract *T. bellerica* fruit (54.69 ± 0.77 mgGAE/g) in our study has been lower than that reported in 70% methanol extract of *T. belerica* fruit (133.00 mg/ml GAE/100

mg) (Hazra et al., 2010). Similarly, TPC in methanolic extract of *R. scleratus* whole plant (6.74 ± 0.62 mgGAE/g) in our study has been lower than that previously reported from whole plant of *R. sceleratus* ethyl acetate fraction (97.1 GAE /mg/g), *n*-butanol fraction (79.6 GAE/mg/g), chloroform fraction (73.3 GAE mg/g), aqueous fraction (64.6 GAE/mg/g), and *n*-hexane fraction (23.5 GAE/mg/g) (Shahid et al., 2015). Similarly, water and ethanol extract of *H. nepalensis* whole plant (115.77 and 74.53µg CE/mg respectively) (Huang et al., 2008) have been reported to contain higher amount of total phenolics.

We already know that phenolic compounds are responsible for antioxidant activities. Phenolic compounds present in our plant extracts may help prevent cancer, diabetes, heart disease, neurodegeneration, and other age related degenerative diseases. Further *in vitro* and *in vivo* tests after isolation and elucidation of specific phenolic compounds may open new doors for other infinite possibilities.

5.3 Total Flavonoid Content

Flavonoids are the most abundant polyphenolic compounds ubiquitously found in plants. Plants rich in flavonoids are good source of antioxidants which effectively scavenge the reactive oxygen species due to the structure and the substitution pattern of phenolic hydroxyl groups (Cao et al., 1997; Miller & Ruiz-Larrea, 2002; Sharififar et al., 2008; Subhasree et al., 2009). Flavonoids have been reported to exhibit a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-alergic (Anyasor et al., 2010; Ayoola et al., 2008; Barile et al., 2007; Chao et al., 2002; Hossain et al., 2011; Igbinosa et al., 2009; Kokate, 2008).

We determined the total flavonoid content in plant extracts by spectrophotometric method using aluminium chloride in the presence of potassium acetate. This is one of the most commonly used approaches for the determination of total flavonoid content in food or medicinal plant samples. It involves the formation of aluminium-flavonoid complexes, with flavonols (quercetin, morin, kaempferol and rutin) and flavones luteolin exhibiting the maximum absorbance at 415–425 nm (Pękal & Pyrzynska, 2014). The variation of this method in the presence of $NaNO_2$ shows specificity for rutin, luteolin and catechins (Pękal & Pyrzynska, 2014).

Total flavonoid content for each plant extract was observed at a point within the narrow range from 7.51 mgQE/g to 25.65 mgQE/g. The highest amount of flavonoid content was found in *P. emblica* (25.65 ± 0.77 mgQE/g) followed by *S. elata* (22.26 ± 0.69 mgQE/g), *C. aciculatus* (19.83 ± 0.94 mgQE/g), *G. wallichianum* (18.53 ± 0.67 mgQE/g), *R. lepidotum* (18.35

± 0.76 mgQE/g), and *H. javanica* (18.24 ± 0.83 mgQE/g) while the lowest was observed in *T. bellerica* (7.51 ± 0.54 mgQE/g).

Similar study on flavonoid content in methanol extracts of *P. emblica* fruit from six regions in China has reported it to vary from 20.3 to 38.7mgQE/g (Liu et al., 2008) which is similar to our result. However, TFC previously reported in 70% methanol extract of *P. emblica* fruit (176.00 mg/ml QE/100 mg) (Hazra et al., 2010), 70% methanol extract of *T. belerica* fruit (138.30 mg/ml QE/100 mg) (Hazra et al., 2010) have been found to be higher than that in our study. Similarly, water and ethanol extracts of *H. nepalensis* whole herb (31.48 and 66.07 µg RE/mg respectivily) (Huang et al., 2008) have been reported to contain high amount of total flavonoids.

We detected a lower amount of flavonoid content in our plant extracts which might be due to the presence of a lower amount flavonols (quercetin, morin, kaempferol and rutin) and flavones luteolin in the plant extracts since this method is specific for these compounds in the presence of potassium acetate (Pękal & Pyrzynska, 2014). However, there is still the chance that they display the higher amount of rutin, luteolin and catechins when tested in the presence of $NaNO_2$ (Pękal & Pyrzynska, 2014).

We observed that our plant extracts demonstrated antimicrobial, cytotoxic and anticancer activities. Though present in a small amount, flavonoids have a part to play whatever be the degree of their activities. Other in-depth studies are needed to further confirm their presence, and to isolate and elucidate them to perform *in vitro* and *in vivo* studies.

5.4 Antioxidant Activity

An organism's metabolism fights against oxidative effects with its own antioxidant defence systems. Elimination and neutralisation of reactive oxygen species is handled by both enzymatic and non-enzymatic antioxidant mechanisms. Natural antioxidants have a diversity of biochemical activities, some of which include the inhibition of reactive oxygen species generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential (Finkel & Holbrook, 2000). Many antioxidant compounds are known to have anticancer effects (Dragsted et al., 1993; Johnson et al., 1994). Plants rich in secondary metabolites, including phenolics, flavonoids and carotenoids, have antioxidant activity due to their redox properties and chemical structures.

We examined the antioxidant activity of the plant extracts by their ability to scavenge DPPH free radicals and presented it as IC_{50} value which is the concentration of the extract necessary

to inhibit 50% of the free radicals. Thus, an extract with the lower IC_{50} value possess the higher capacity to neutralize the free radicals. DPPH is an organic free radical that does not disintegrate in water, methanol, or ethanol. The odd electron in the DPPH radical is responsible for its deep purple colour and the miximum absorbance at 517nm (Kumarasamy et al., 2007). DPPH can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule (Soares et al., 1997). It results in discoloration from purple to yellow due to the decreasing quantity of DPPH radicals which indicates the radical scavenging nature of the extract (Guo et al., 2007; Molyneux, 2004).

DPPH assay is a quick, reliable and reproducible method widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of plant extracts (Mosquera et al., 2007). The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components (Fukumoto & Mazza, 2000; Shimada et al., 1992).

Plant extracts displayed a dose dependent antioxidant activity with their IC_{50} values ranging from 4.54 µg/ml to 195.55 µg/ml. *T. dumosa* (4.54 ± 0.18 µg/ml) exhibited the highest antioxidant activity as shown by the lowest IC_{50} value – lower than that observed in ascorbic acid (4.57 ± 00 µg/ml) – followed by *P. emblica* (6.64 ± 0.71 µg/ml), *G. wallichianum* (7.24 ± 0.48 µg/ml), *T. bellerica* (12.08 ± 0.76 µg/ml), and *S. elata* (14.20 ± 0.94 µg/ml). *R. scleratus* (195.55 ± 2.45 µg/ml) showed the highest IC_{50} value with the lowest antioxidant activity.

Methanol extract of *G. wallichianum* root (6.9 µg/ml) in our study has been found to possess higher scavenging activity (IC_{50}) than that previously reported in ethanol, *n*-butanol, and water extracts of *G. wallichianum* whole plant (19.05, 24.133 and 25.35 µg/mL) (Ismail et al., 2009). Methanol extract of *P. emblica* bark (7.14 µg/ml) in our study has been found to possess higher scavenging activity (IC_{50}) than that previously reported in water extract of *P. emblica* fruits (51.3 µg/ml) (Charoenteeraboon et al., 2010) and lower scavenging activity than that previously reported in 70% methanol extract of *P. emblica* fruits (1.43 µg/ml) (Hazra et al., 2010). DPPH radical scavenging activity (EC_{50}) of methanolic extracts of *P. emblica* fruit from six regions in China have been reported to vary from 11.23 to 45.44 µg/mL (Liu et al., 2008). Methanol extract of *T. bellerica* fruit (11.37 µg/ml) in our study has been found to possess lower scavenging activity (IC_{50}) than that previously reported in 70% methanol extract of *T. belerica* fruit (IC_{50} 1.45µg/ml) (Hazra et al., 2010). Methanolic extract of *H. javanica* whole plant (144.39 µg/ml) in our study has been found to possess higher scavenging activity (IC_{50}) than that previously reported in ethanol extract of *H. nepalensis* whole plant (314.51 µg/mL) and lower scavenging activity than that previously reported in water extract

of *H. nepalensis* whole plant (84.20 µg/mL) (Huang et al., 2008). DPPH radical scavenging activity (% inhibition at a given concentration) has been reported for *n*-hexane (84.6% at 1000 µg/ml), chloroform (77.9% at 500 µg/ml), ethyl acetate (80.9% at 30 µg/ml), *n*-butanol (80.8% at 60 µg/ml), and remaining aqueous (77.1% at 1000 µg/ml) fractions of whole plant of *R. sceleratus* (Shahid et al., 2015).

We identified a number of antioxidant compounds by GC-MS analyses. Phytochemical investigations also showed the presence of phenolic and flavonoid compounds. These compounds are known to display antioxidant activities due to their redox properties (Rice-Evans et al., 1995; Rice-Evans et al., 1996; Soobrattee et al., 2005). This indicates that our plants can have great implication in isolation of therapeutic agents to prevent oxidative stress related degenerative diseases.

5.4.1 Correlation of TPC and TFC with Antioxidant Activity

Several studies have reported a positive correlation between total phenolic content and antioxidant activity in plants (Bouayed et al., 2007; Djeridane et al., 2006; Kim et al., 2003; Lim & Quah, 2007; Tawaha et al., 2007). There are also studies which report that there is no positive relationship (Hesam et al., 2012; Rafat et al., 2010). A study on methanolic extracts of *P. emblica* fruit from six regions in China has presented good correlations of antioxidant activity with total phenolic content and total flavonoid content (Liu et al., 2008). Such correlations have also been reported from fruit extracts of *P. emblica* and *T. belerica* (Hazra et al., 2010).

In our study, we observed a significant correlation between the antioxidant activity and the total phenolic content while comparing the TPC and the IC_{50} of DPPH RSA of twelve medicinal plants ($r = -0.9684$, $p<0.0001$). However, we could not establish a good correlation between antioxidant activity and total flavonoid content. Our results suggest the that total phenolic contents of these plants highly attributed their antioxidant activities.

5.5 Antimicrobial Activity

In recent years, multiple drug resistant pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases, making it a growing problem worldwide. In addition to this problem, antibiotics are sometimes associated with adverse effects on host including hypersensitivity, immune suppression and allergic reactions (Khanahmadi et al., 2010). Therefore, there is a need to develop alternative antimicrobial drugs from various sources such as medicinal plants for the treatment of infections.

We analysed antimicrobial effects of plant extracts against six different microbial strains *viz*. *Staphylococcus aureus,* methicillin resistant *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella* Typhi*, S. cerevisiae* and *Pichia sp.* by agar well diffusion method. This method is widely used to examine the antimicrobial effects of plant extracts. Plant extracts are introduced into the wells in the agar plate whose agar surface is already swabbed with uniform microbial inoculum. The antimicrobial compounds diffuse during incubation to inhibit the growth of microbial strains showing the zone of inhibition on the agar surface. The size of inhibition zone is proportional to the activity shown by the extracts.

We observed that maximal inhibition of *S. aureus* and MRSA both were exerted by *T. bellerica*. Maximum inhibition of *K. pneumoniae* and *S.* Typhi were shown by *L. involuta* and *T. dumosa*. However, *L. involuta* was found ineffective against any of the gram positive bacteria. Antimicrobial activity of *S. lucida* was observed only against MRSA while *S. elata* and *T. bellerica* inhibited all the tested microorganisms. It was found that inhibition of at least three of the six microbes were exerted by ten of the twelve medicinal plants. Eight of the extracts inhibited at least four of the total organisms. *G. wallichianum* was ineffective against *K. pneumoniae* but it inhibited other five organisms. *R. scleratus* could not inhibit any of the bacteria. *S. lucida* did not inhibit any of the fungi. Maximum inhibition of *S. cerevisiae* and *Pichia sp.* were exerted by *H. javanica* and *A. rivularis*, respectively.

Methanol extract of *H. javanica* whole plant in our study showed antimicrobial activity against *Staphylococcus aureus* (8mm) similar to that previously reported from methanol extracts of *H. javanica* leaf (81 and 162 mg/mL, 30µl) against *S. aureus* (7.30 and 8.50 mm) (Mandal et al., 2016). Antibacterial activity of methanol extract of *P. emblica* bark against *Staphylococcus aureus* (13 mm) in our study has been found to be supported by previous studies in methanol extract of leaves and fruits of *P. emblica* against *S. aureus* (31.00 and 35.66 mm) (Javale & Sabnis, 2010), methanol extract of fruits of *P. emblica* (10mg/ml) against *Staphylococcus aureus* (18mm) (Raghu & Ravindra, 2010). Methanol extract of *P. emblica* bark in our study was found to be inactive against *Klebsiella pneumonia*. However, previous studies have reported significant activities of methanol extract of leaves and fruits of *P. emblica* against *K. pneumoniae* (30.00, and 26.17 mm) (Javale & Sabnis, 2010), and methanol extract of fruits of *P. emblica* (10mg/ml) against *Klebsiella pneumoniae* (19mm) (Raghu & Ravindra, 2010). Antibacterial activity of methanol extract of *P. emblica* bark in our study against *Staphylococcus aureus* (13 mm) has been supported by a previous report on antibacterial activity of flavonoids and saponins from *Emblica officinalis* fruit and *Emblica officinalis* leaf against *S. aureus* (Javale & Sabnis, 2010). Methanol extract of *P. emblica* bark in our study was found to be inactive against *Klebsiella pneumoniae*. Flavonoids and saponins from

Emblica officinalis leaf have also been reported to be inactive against *K. pneumoniae*, however, that from *Emblica officinalis* fruit have been reported to be active (Javale & Sabnis, 2010).

Methanol extract of *G. wallichianum* root in our study was found to be active against *Staphylococcus aureus* (10mm) which have been supported by similar studies on 70% ethanolic extract of *G. wallichianum* against *Staphylococcus aureus* (15mm) (Ahmad et al., 2003), and methanol extract of *G. wallichianum* rhizomes (1mg/ml, 100 and 200µL) against *S. aureus* (14.33 mm) (Ismail et al., 2012). Methanol extract of *G. wallichianum* root in our study showed very low activity against *Salmonella* Typhi (8mm) which is somewhat similar to the previous reports on inactivities of 70% ethanolic extract of *G. wallichianum* against *Salmonella* Typhi (Ahmad et al., 2003), and inactivity of methanol extract of *G. wallichianum* rhizomes (1mg/ml, 100 and 200µL) against *S.* Typhi (Ismail et al., 2012). Methanol extract of *G. wallichianum* leaves (1mg/ml, 100 and 200µL) has been reported to be inactive against *S. aureus* and, *S.* Typhi (Ismail et al., 2012). Methanol extract of *G. wallichianum* root in our study showed no activity against *Klebsiella pneumoniae* which is similar to the previous report on inactivity of 70% ethanolic extract of *G. wallichianum* against *Klebsiella pneumoniae* (Ahmad et al., 2003).

Antimicrobial activity of methanol extract of *T. bellerica* fruit against *Staphylococcus aureus* (16mm) in our study has been supported by the previous study on methanol extract of *T. bellirica* fruit pericarp (50 and 100mg/ml) against *S. aureus* (18 and 30mm) by paper disc diffusion (Nagendraswamy et al., 2013). Antimicrobial activity of methanol extract of *R. lepidotum* leaf and stem against *Staphylococcus aureus* (11mm) in our study has been supported by previous study on methanol extract of *R. lepidotum* leaves and twigs against *S. aureus* (13mm) by filter disc diffusion (Bhattarai et al., 2010). Antibacterial activities of methanol extract of *R. lepidotum* leaf and stem against *Staphylococcus aureus* (11mm), and methicillin resistant *Staphylococcus aureus* (11mm) in our study has been found to be supported by a report on antibacterial activity of coumarin compounds from methanolic extract of *R. lepidotum* against *Staphylococcus aureus*, and methicillin resistant *Staphylococcus aureus* (Shakeel-U-Rehman et al., 2010).

Methanol extract of *R. scleratus* whole plant was found to be inactive against *Staphylococcus aureus* and *Salmonella* Typhi in our study, however on a previous report, chloroform, ethyl acetate, *n*-butanol and aqueous fractions of whole plant of *R. sceleratus* have been reported to inhibit *S. aureus* (12.0, 10.8, 11.2, and 10.5 µg/200µl), and *S.* Typhi (10.4, 10.8, 10.4, and 11.3 µg/200µL) by 96-well microplate assay (Shahid et al., 2015).

There are studies which report the inhibitory activities of some of these medicinal plants against some other fungal strains such as *G. wallichianum* against *Trichophyton Schoenleinii*, *Pseudallescheria Boydii*, *Candida albicans*, *Aspergilus niger, Microsporum canis*, and *Fusarium solani* (Ahmad et al., 2003; Ismail et al., 2012), *R. sceleratus* against *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporum gypseum,* and *Microsporum fulvum* (Sharma et al., 2012), and *H. javanica* against *Candida albicans* (Sood & Yadav, 2014).

We found our plant extracts to be more effective against the gram-positive bacteria than to the gram-negative. It might be described based on the fact that gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide outer membrane which render the cell wall impermeable to lipophilic solutes including the plant extracts. In addition, gram-negative bacteria also have inherent overexpressed or multiple efflux pumps that prevent the intracellular accumulation of antibacterial agents. Gram-positive bacteria, on the other hand, have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Burt, 2004; Qadan et al., 2005; Rameshkumar et al., 2007; Stefanello et al., 2008).

It can be said of the plants that they are what they contain. Antimicrobial activities shown by the plants must have been contributed by the compounds they possess. We identified a variety of antimicrobial compounds by GC-MS analyses. Phytochemical investigations also showed the presence of phenolic and flavonoid contents. Phenolics can inhibit hydrolytic enzymes or other interactions that inactivate microbial adhesins, cell envelope transport proteins and non-specific interactions with carbohydrates (Pyla et al., 2010). Flavonoids can form complex with extracellular and soluble proteins and with bacterial cell walls or disrupt microbial membranes (Cowan, 1999). Other compounds have similar inhibitory actions. Tannins can bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006). Saponins can cause leakage of proteins and certain enzymes from the cell (Zablotowicz et al., 1996). Steroids may associate with membrane lipid and cause leakages from liposomes (Epand et al., 2007).

The results clearly show that our plant extracts can find potential therapeutic uses in isolation of broad-spectrum antimicrobial compounds to fight the war against drug resistant microbial pathogens. The presumed safety of the plant derived drugs signifies their great market potential.

5.6 Brine Shrimp Cytotoxicity

Toxicity of pharmaceutical compounds possess a great risk on public health. Brine shrimp lethality has been used as a very useful tool for testing general toxicity (Almeida et al., 2002). It is used in determination of bioactivity of plant products (Lhullier et al., 2006; McLaughlin et al., 1991; Meyer et al., 1982; Stefanello et al., 2006), and synthetic compounds (Almeida et al., 2002). It has found an application in detection and isolation of bioactive compounds from plant extracts (Mackeen et al., 2000; Meyer et al., 1982; Solis et al., 1993; Krishnaraju et al., 2005; Ogunnusi & Dosumu, 2008). It has been known to have a good correlation with cytotoxic, antitumor and pesticidal activities (McLaughlin et al., 1998; Pelka et al., 2000).

Standard brine shrimp lethality bioassay stipulates that an LC_{50} value < 1000 µg/ml is considered bioactive in toxicity evaluation of plant extracts (Clarkson et al., 2004). Based on this benchmark, we found that nine of the twelve medicinal plants exhibited significant and dose dependent toxicity to brine shrimps in which *A. rivularis* (58.11 µg/ml), *L. involuta* (91.72 µg/ml) and *T. bellerica* (94.65 µg/ml) presented high toxicity with LC_{50} value less than 100 µg/ml while *G. wallichianum* (135.90 µg/ml), *H. javanica* (195.68 µg/ml), *C. aciculatus* (252.82 µg/ml), *S. lucida* (292.19 µg/ml), *P. emblica* (306.27 µg/ml) and *S. elata* (400.35 µg/ml) exerted moderate toxicity with LC_{50} value less than 500 µg/ml. *T. dumosa* (9523.10 µg/ml), *R. scleratus* (2069.84 µg/ml), and *R. lepidotum* (1607.17 µg/ml) identified themselves as non-toxic showing the LC_{50} values higher 1000 µg/ml.

Cytotoxicity (LC_{50}) of methanol extract *G. wallichianum* root (136 µg/ml) in our study has been found to be higher than that previously reported in methanol and 70% ethanol extracts of *G. wallichianum* rhizome (>1000 µg/ml) (Ismail et al., 2012; Ahmad et al., 2003), and ethyl acetate fraction of *G. wallichianum* rhizome (LC_{50} 333.256 µg/ml) (Ismail et al., 2012). Cytotoxicity of methanolic extract of *P. emblica* bark (306 µg/ml) in our study has been found to be lower than that reported in ethanolic extract of *P. emblica* fruit (60 µg/ml) (Hossen et al., 2014). Cytotoxicity of methanol extract of *R. lepidotum* leaf and stem (1607 µg/ml) in our study has been found to be lower than that reported in methanol extract of *R. lepidotum* leaves and twigs (111.59 µg/ml) (Bhattarai et al., 2010).

We already know that brine shrimp lethality shows a good correlation with insecticidal, cytotoxic, and anticancer properties. Higher lethality shown by our plant extracts may suggest that they contain a number of bioactive compounds with insecticidal and anticancer properties. Higher cytotoxicity shown by the plants may also pose a risk on human health. Their suitability for human consumption needs to be ascertained by several in-depth researches.

5.7 Anticancer Activities

Cancer is one of the most important diseases of the 21st century. The incidence of cancer is increasing during the last decades in most countries. In spite of numerous efforts to combat this disease, world medicine is facing with the lack of perfect cure for it. Many chemotherapeutic drugs are presently placed in a predicament of reduced therapeutic effect due to the problem of drug resistance (Peters et al., 2002). Chemotherapeutic drugs also exert toxicity to normal cells, which in turn causes the unpleasant side effects to the patients.

In the last decades, there has been a great emphasis towards the researches on complementary and alternative medicine for cancer management (Guevara et al., 1999). Several studies have been conducted on medicinal herbs to find new antitumor compounds. It has been reported that about 3000 plants with anticancer properties have been used for anticancer drugs (Hartwell, 1971; Pandey, 2002).

Numerous compounds are reported from plants with anticancer properties such as phenolics, flavonoids, alkaloids, phenylpropanoids, and terpenoids (Kintzios, 2006; Park et al., 2008). Plants contain almost unlimited capacity to generate compounds that fascinates researchers in the quest for novel chemotherapeutics (Reed & Pellecchia, 2005). The persistent search for new anticancer compounds in medicinal plants is a releastic and promising strategy for its prevention (Hu et al., 2009).

We determined the antiproliferative activities of plant extracts against HeLa and MDCK cell lines by MTT assay. This assay is based on the conversion of yellow MTT tetrazolium salt to purple insoluble formazan crystals. Dehydrogenase enzymes from mitochondria and endoplasmic reticulum in viable cells are involved in the reaction (Fotakis & Timbrell, 2006; Slater et al., 1963). Cells which have undergone death due to toxic damage cannot transform MTT into formazan. So, the amount of formazan produced is directly proportional to the number of viable cells.

We observed that the methanol extracts of twelve medicinal plants demonstrated a dose dependant growth inhibition of HeLa and MDCK cell lines. HeLa cell line was most effectively inhibited (EC_{50}) when exposed to *S. lucida* (52.22 µg/ml) followed by *T. dumosa* (122.3 µg/ml), *C. aciculatus* (177.6 µg/ml), *R. scleratus* (249.4 µg/ml), *S. elata* (304 µg/ml), *A. rivularis* (345.6 µg/ml), and *R. lepidotum* (389.7 µg/ml) while it was least affected by the presence of *T. bellerica* (1048 µg/ml). MDCK cell line was susceptible to the highest inhibition when exposed to *L. involuta* (240 µg/ml) followed by *T. dumosa* (305 µg/ml), *A. rivularis* (407.7 µg/ml), *S. elata* (751.1 µg/ml), and *P. emblica* (925.3 µg/ml) while it was less affected by the presence

of other extracts with their EC_{50} values higher than 1000 µg/ml. Ten of the twelve medicinal plants including *S. lucida, C. aciculatus, R. scleratus, S. elata, R. lepidotum, G. wallichianum,* and *H. javanica* exhibited higher inhibition against HeLa cell line in compared to the MDCK cell line to which only *L. involuta* and *P. emblica* showed higher inhibition. This suggests the usefulness of these plants in cancer chemoprevention.

In our study, methanol extract of leaf and stem of *R. lepidotum* was found to be effective (EC_{50}) against HeLa cell line (389.7 µg/ml) but not much effective against MDCK cell line (4448 µg/ml). In a similar study, inhibitory effects of *R. lepidotum* aerial parts or leaves and twigs have been reported against MDCK cell line (CC_{50} of >100 µg/ml) (Rajbhandari et al., 2009), and HeLa cell line (Bhattarai et al., 2010) by using MTT assay. Methanol extracts of *P. emblica* bark in our study was found to show lower inhibitory effects against HeLa cell line (EC_{50} 991.6 µg/ml) using MTT assay than that previously reported from water extracts of *P. emblica* (LC_{50} of 87.5 µg/mL) using Sulforhodamine B assay (Ngamkitidechakul et al., 2010). These plants have also been reported to show activities against other cell lines such as *H. nepalensis* against human hepatoma 3B cell line (Huang et al., 2008), *R. lepidotum* against Vero cell line (Rajbhandari et al., 2009), and *P. emblica* against A549, HepG2, MDA-MB-231, SKOV3, and SW620 human cancer cell lines (Ngamkitidechakul et al., 2010).

Compounds isolated from these plants have been reported to exert inhibitory effects against different cancer cell lines such as triterpenes and triterpene saponins from *H. nepalensis* against HL-60, SMMC-7721, A-549, MCF-7 and SW480 (Ma et al., 2016), flavonoid named aciculatin from *C. aciculatus* against HCT116 (Lai et al., 2012), apigenin derivatives and other compounds from *Chrysopogon aciculatis* against MCF-7, H460, HT-29, and CEM (Shen et al., 2012), saponins and other compounds from *A. rivularis* against HL-60, HepG2, A549 and HeLa (Wang et al., 2014).

We identified a number of anticancer compounds by GC-MS analyses. We also demonstrated that phenolic compounds possessed by plants attributed their antioxidant activities which in turn may unveil anticancer effects. Further in-depth research may help unravel the compounds responsible for anticancer activities shown by plants.

5.8 GC-MS analysis

The GC-MS analysis of twelve medicinal plants afforded the identification of a wide range fatty acids, esters, sugars, steroidal, phenolic, and heterocyclic compounds with bioactivities reported in most of them by different researchers.

1,2,3-Benzenetriol (Syn. Pyrogallol) was identified from *G. wallichianum* (57.73%), *P. emblica* (57.39%), *S. elata* (25.12%), and *T. bellerica* (14.43%). It is a polyphenol compound and a hydrolysable tannin (Khanbabaee & van Ree, 2001) obtained through ingestion of tea and coffee (Müller et al., 2006) and degradation of gallic acid in colon (Yasuda et al., 2000). It exhibits anticancer activity against MCF-7, PC-3, LNCaP, DU145 and HCT-116 cell lines (Chew et al., 2014). It has been reported as an antioxidant which increases H_2O_2 resistance associated with a reduction in intracellular oxidation and protein carbonylation (Mendes et al., 2015). It also acts as a superoxie generator which has been reported to enhance iNOS gene expression (Kuo et al., 2000).

9,12-Octadecadienoic acid (*Z,Z*)- was identified from *H. javanica* (29.21%), *A. rivularis* (1.89%), and *R. scleratus* (1.34%). It is a linoleic acid ester. It has been reported to exhibit anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary activities (Maruthupandian & Mohan, 2011; Parthipan et al., 2015; Rajeswari et al., 2012).

2-Propanone, 1,3-dihydroxy- was identified from *C. aciculatus* (29.07%). It is an aliphatic ketone. It has been reported to be used in artificial respiration, and found to possess antioxidant and analgesic activities (Sivakumar & Gajalakshmi, 2014).

Diethyl phthalate was identified from *R. scleratus* (19.41%), *S. elata* (18.14%), *T. bellerica* (16.72%), *A. rivularis* (7.35%), *S. lucida* (1.9%), *L. involuta* (1.11%), *C. aciculatus* (0.87%), *P. emblica* (0.75%), and *R. lepidotum* (0.59%). It has been reported to be used for the preparation of about 67 consumer formulations including bath preparations (oils, tablets, and salts), eye shadow, toilet waters, perfumes, other fragrance preparations, skin care preparations and also as a component in insecticide sprays, mosquito repellents and camphor substitute (Jayaraman et al., 2011). It has been reported to possess antifouling, antimicrobial (Diaz & Thilaga, 2016), acetylcholinesterase and neurotoxic activities (Velanganni et al., 2011). It has also been found to reduce growth rate, food consumption and increase organ weights in human (Hauser & Calafat, 2005).

2-Thiazolamine, 5-nitro- (Syn. Thiazole, 2-amino-5-nitro-) was identified from *R. scleratus* (18.4%) in this study. It has been found to be evaluated as a hypoxic radiosensitizer using bacteria (Rockwell et al., 1982). It has been found to be effective as antihistomonad in turkeys and against trichomoniasis in pigeons (O'Neil, 2006).

n-Hexadecanoic acid (Syn. palmitic acid) was identified from *H. javanica* (15.53%), *C. aciculatus* (2.97%), *L. involuta* (2.78%), *R. lepidotum* (2.64%), and *P. emblica* (0.65%). It is a fatty acid reported to exhibit antioxidant, hypocholesterolemic, antiinflammatory, antibacterial (Konovalova et al., 2013; Sermakkani & Thangapandian, 2012), nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic and 5-alpha reductase inhibitor activities (Parthipan et al., 2015; Rajeswari et al., 2012).

Phenol, 4,4'-methylenebis[2,6-dimethyl- was identified from *T. bellerica* (10.94%). It is an antioxidant compound which has also been reported to be used in fuel, polymers, and as an additive in petroleum-based lubricants (Revathi et al., 2013).

4-O-Methylmannose was identified from *T. dumosa* (10.18%). This methylated sugar has been reported to possess antibacterial activity (Kumar & Bhaskar, 2012).

Mome inositol was identified from *T. dumosa* (10%). It has been reported to display antialopecic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic (Kumar et al., 2012) and antiproliferative activities (Neda et al., 2013).

1-Naphthalenepropanol, *α*-ethenyldecahydro-*α*,5,5,8a-tetramethyl-2-methylene-, [1S-[1*α*(R*),4a*β*,8a*α*]]- (Syn. 13-Epimanool) was identified from *T. dumosa* (9.55%). This labdane type diterpene finds its use as rosin or sizing agents (Salem et al., 2015).

4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol was identified from *S. lucida* (9.35%), *C. aciculatus* (1.28%), and *R. lepidotum* (0.54%). This phenolic compound possess antimicrobial, antioxidant, antiinflammatory and analgesic activities (Gopalakrishnan & Vadivel, 2011).

A furan aldehyde named 2-Furancarboxaldehyde, 5-(hydroxymethyl)- is a useful product found in the fossil fuel resources (Andreia et al., 2011). It was identified it from *L. involuta* (9%), *C. aciculatus* (8.65%), and *A. rivularis* (1.25%) in this study. It has been reported to exhibit antifungal, antibacterial (Oskoueian et al., 2011), anti-inflammatory (Brustugun et al., 2005; Li & Lu, 2005; Xu et al., 2007), clastogenic, uterotonic (Gopalakrishnan & Udayakumar, 2014) and preservative properties (Gopalakrishnan & Vadivel, 2011; Lilhore & Pande, 2016). It has been found to display protective effects against CCl_4-induced injury to the liver and vascular endothelium (Graff & Pollack, 2005) and cytotoxicity against BGC823 cell line (Guo L et al., 2016).

Phenol, 2-methoxy-4-(2-propenyl)-, acetate (Syn. eugenol acetate or eugenyl acetate) was identified from *T. dumosa* (8.89%). It has been reported to show antioxidant and antibacterial

activity (Vanin et al., 2014). It has been known to be toxic and repellent to red imported fire ants *Solenopsis invicta* (Kafle & Shih, 2013).

Benzoic acid was identified from *T. dumosa* (8.58%). It is an aromatic carboxylic acid which has been reported to be used in perfumes, flavorings and as an anti-fungal agent (Rajeswari & Rani, 2014-15).

Verrucarol is a sesquiterpene portion of several macrocyclic dilactones which was identified from *R. lepidotum* (8.4%) in this study. It has been reported as a mycotoxin (Johanning et al., 2002; Tuomi et al., 2000) with potent anticancer activity (Kraus & Frazier, 1980). Certain derivatives of verrucarol are reported to inhibit viral infection (Coohon et al., 1980; Snider & Amin, 1978).

1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid (Syn. Quinic acid) is a phenolic acid which was identified in this study from *T. dumosa* (7.96%), and *G. wallichianum* (3.5%). It is used in the synthesis of anti-influenza (anti-swine flu) medication and shows anti-aging effects. It is also used in treatment of prostate Cancer (Inbathamizh & Padmini, 2013; Pero & Lund, 2009). It exhibits anti-inflammatory activities (Kumar et al., 2015; Zeng et al., 2009). It is reported to exert a potent antibacterial and antifungal activities against typical microbial strains affecting the human respiratory or urinary tract, including *Candida* species and also exhibits anti-PI-3, anti-herpes effects and cytotoxicity against MDBK cells (Ozçelik et al., 2011). It induces antioxidant activity enhancing the synthesis of tryptophan and nicotinamide (Pero et al., 2009).

2H-1-Benzopyran, 7-methoxy-2,2-dimethyl- (syn. Precocene I) was identified from *R. lepidotum* (7.42%). It is a naturally occuring chromonoid (Bowers et al., 1976) known to display insect antijuvenile hormonal activity (Bowers, 1985). It has been reported to exhibit insecticidal activities against *Drosophila melanogaster* (Wilson et al., 1983), *Aedes albopictus* (Liu & Liu, 2014), *Liposcelis bostrychophila* (Lu et al., 2014), and a malathion-resistant *Oryzaephilus surinamensis* (Saleem & Wilkins, 1984). It has also been known to inhibit mycotoxin 3-acetyldeoxynivalenol in Fusarium graminearum (Yaguchi et al., 2009).

Cyclopentane, 1-acetyl-1,2-epoxy- is a steroid that was identified from *L. involuta* (7.13%), and *C. aciculatus* (4.01%) in this study. It has been found as a precursor for cyclopentane monoterpenoid synthesis (Prabu et al., 2013).

cis-Vaccenic acid is a monounsaturated and non-essential *ω*-7 fatty acid which was identified from *T. dumosa* (6.97%), and *G. wallichianum* (2.25%) in this study. It has been reported to exhibit anti-inflammatory and antioxidant activities (Anyasor et al., 2014). It has been found

to inhibit the growth of *Bacillus subtilis* (Laser, 1951). It has been reported to suppress VCAM-1 and ICAM-1 expression (Abbasia et al., 2015) and exhibit hypolipidaemic and antihypertensive activities (Bhattacharya et al., 2014). It has been found to reduce the risk of myocardial infarction (Djoussé et al., 2012) and heart failure with antecedent coronary heart disease (Djoussé et al., 2014). However, it has been positively associated with reduced kidney function (Block et al., 2012).

Benzenemethanol, 2,5-dimethoxy-, acetate was identified from *S. lucida* (6.88%). It has been reported to possess antibacterial activity (Jebastella & Reginald, 2015).

Decanoic acid, octyl ester (Syn. octyl decanoate) was identified from *A. rivularis* (5.93%). It has also been reported from honey bees such as *Apis mellifera* queens (Bernasconi et al., 1999) and *Trigona silvestriana* workers (Johnson et al., 1985).

A sugar named *β*-D-Glucopyranose, 1,6-anhydro- (Syn. levoglucosan) is a pyrolysis product of cellulose and is one of the major organic components in the biomass combustion of particulate matter. It was identified from *P. emblica* (5.49%), *C. aciculatus* (4.53%), and *A. rivularis* (3.46%) in this study. It has been frequently used as an environmental tracer for biomass burning (Fraser & Lakshmanan, 2000; Simoneit et al., 1999).

2-Butenal, 2-methyl-, (E)- (Syn. tiglic aldehyde) was detected from *P. emblica* (5.47%). This hemiterpene molecule displays properties such as fuel and energy storage, cell signaling, and membrane integrity (HMDB, 2016; Yannai, 2004).

α-D-Glucopyranoside, O-*α*-D-glucopyranosyl-(1.fwdarw.3)-*β*-D-fructofuranosyl (Syn. Melezitose) was identified from *R. lepidotum* (5.27%). It is a trisaccharide molecule that has been obtained from plant exudations or honey made from such exudations, and also incorporated by some mushrooms (Barros et al., 2008).

1-[3-(1-Adamantyl)-1-methylpropylidene]thiosemicarbazide was found in *R. lepidotum* (5.04%). It has been reported to possess antimicrobial activity (Kumaravel et al., 2016).

γ-Sitosterol was identified from S. elata (4.8%), and *S. lucida* (3.15%). It has been suggested to exert hypolipidemic (Balamurugan et al., 2015), antihyperglycemic (Balamurugan et al., 2011), anti-diabetic, anti-angeogenic, anticancer, antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral properties (Venkata et al., 2012). It shows anticancer activity against MCF-7 and A549 cells exhibiting growth inhibition, cell cycle arrest and apoptosis through c-Myc suppression (Sundarraj et al., 2012).

D-Allose is a rare sugar that was identified from *T. dumosa* (4.56%), *G. wallichianum* (3.15%), *S. elata* (2.98%), and *R. scleratus* (0.77%) in this study. It has been reported to possess antioxidant (Murata et al., 2003), immunosuppressive (Hossain et al., 2000) and anti-inflammatory activities (Gao et al., 2011). It has also been reported to exhibit anticancer effects against human ovarian (Sui et al., 2005a), prostate (Naha et al., 2008), liver (Sui et al., 2005b), leukemia (Hirata et al., 2009), and head and neck (Mitani et al., 2009) cancer cell lines.

1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- is a glycerol/nitrogen compound which was identified from *A. rivularis* (4.21%), and *S. elata* (1.2%) in this study. It has been reported to have anticataract, antiear-wax, antiketotic, antineuralgic, arrhythmigenic, emollient, hyperglycemic activities (Kumar et al., 2012). It exhibits antimicrobial activities (Gunasekarana et al., 2013), and thus used as a bacteriostat in disinfectants (Popendorf et al., 1995).

Benzaldehyde, 2-hydroxy-6-methyl- (Syn. 6-Methylsalicylaldehyde or 2,6-Cresotaldehyde) is an aromatic aldehyde detected from *L. involuta* (4%). It has been reported to exhibit antimicrobial (Rajeswari et al., 2013), anticancer, antimutagenic, antitumor and immunostimulant activities (Balasubramanian et al., 2014).

Sucrose is a non recucing sugar formed by combination of glucose and fructose. It was identified from *C. aciculatus* (3.97%). It has been reported that diets very high in sucrose decrease insulin sensitivity (Thresher et al., 2000), induce hypertriglyceridemia and hyperinsulinemia (Daly et al., 1997). Dietary sucrose has also been found as a promotor of colon tumor (Caderni et al., 1997; Caderni et al., 1996; Kristiansen et al., 1996; Luceri et al., 1996; Stamp et al., 1993).

3,7,11,15-Tetramethyl-2-hexadecen-1-ol was identified from *H. javanica* (3.96%), *R. scleratus* (2.43%), *A. rivularis* (1.01%), *R. lepidotum* (0.79%), *S. elata* (0.79%), *P. emblica* (0.75%), and *T. dumosa* (0.58%). It is a terpene alcohol which has been reported to posses antimicrobial, anti-inflammatory (Rajeswari et al., 2012), anticancer and diuretic effects (Gopinath et al., 2013).

1,2-Benzenediol (Syn. catechol) was identified from *L. involuta* (3.87%), and *R. lepidotum* (0.96%). It is a phenolic compound reported to possess antioxidant, fungicidal, antibacterial, pesticidal (Saravanan et al., 2014), antiseptic (Cowan, 1999), anti-complementary (Chen, 2011) and anti-inflammatory activities (Park & Lee, 2012). It has been reported to inhibit NO production, tyrosinase activity and melanin production (Park & Lee, 2012). It is a powerful

reducing agents with potential application against oxidative stress (Kalt & Cock, 2014). It shows anticancer activity against breast cancer (Manorenjitha et al., 2013).

1,2-Benzenediol, 3-methoxy- was identified from *G. wallichianum* (3.81%), and *R. lepidotum* (0.55%). It is an aromatic compound which has been reported to exhibit a potent anti-complementary activity (Chen, 2011).

9-Octadecynoic acid (Syn. Stearolic acid) is the acetylenic analogue oleic acid identified from *L. involuta* (3.81%), and *C. aciculatus* (1.62%). It has been implicated as a hypotensive compound (Teres et al., 2008). It has also been reported as a DNA binding agent which binds to DNA and weakly inhibits DNA polymerase activity (Berry et al., 1991).

Pentadecanoic acid is a lauric acid identified from *T. dumosa* (3.78%), *A. rivularis* (3.65%), *G. wallichianum* (3.48%), and *R. scleratus* 2.99%). It has been reported to have antioxidant activity (Lawal et al., 2015).

Phthalic acid, di-(1-hexen-5-yl) ester is an organic acid ester identified from *S. elata* (3.76%) which possess antifungal properties (Sridevi et al., 2014).

Octadecane, 2,2,4,15,17,17-hexamethyl-7,12-bis(3,5,5-trimethylhexyl)- was identified from *L. involuta* (3.75%). It has been reported to exert anticancer activities (Babar et al., 2016; Camerman et al., 1978; Corbett et al., 1982).

2-Methoxy-4-vinylphenol was identified from *C. aciculatus* (3.74%), *R. lepidotum* (0.91%), and *H. javanica* (0.33%). It is a phenolic compound used as a flavouring agent (Jeong et al., 2011) which has been reported to possess antimicrobial, antioxidant and analgesic effects (Gopalakrishnan & Vadivel, 2011; Rajeswari & Rani, 2014). It exhibits strong anti-inflammatory activity through the elimination of NF-κB and MAPK activation (Jeong et al., 2011). It has been reported to arrest abnormal cell cycle progression by preventing the hyperphosphorylation of retinoblastoma protein in NIH 3T3 cells (Jeong & Jeong, 2010).

Stigmast-5-en-3-ol, (3*β*)- (Syn. *β*-Sitosterol) was identified from *T. dumosa* (3.66%), and *G. wallichianum* (2.94%). It has been reported to exert phosphorylation of extracellular-signal regulating kinase and p38 mitogen-activated protein kinase (Moon et al., 2007) and promote apoptosis by increasing FAS levels and caspase-8 activity (Awad et al., 2007). It has been found to modulate antioxidant enzyme (Vivancos & Moreno, 2005), decrease free radical generation (Moreno, 2003), and inhibit colon cancer (Baskar et al., 2010; Jayaprakash et al., 2007) and prostate cancer (Awad et al., 2005). It exhibits analgesic, anti-inflammatory (Dighe et al., 2016) and cholesterol lowering effect (Wang & Ng, 1999). It has also been

reported to possess antimicrobial, antiarthritic, antiasthma, diuretic (Mujeeb et al., 2014), antidiabetic, antiangeogenic, antidiarrhoeal and antiviral activities (De Oliveira et al., 2014).

2-Deoxy-D-galactose was identified from *L. involuta* (3.41%). It has been reported as a substrate for the Galactose Transport System of *Escherichia coli* (Henderson & Giddens, 1977) which has been found to inhibit tumors in mice (Laszlo et al., 1960) and prevent accumulation of toxic phosphorylated metabolites of 2-deoxygalactose (Nagelkerke & Postma, 1978).

9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)- is an unsaturated fatty acid identified from *R. scleratus* (3.08%), and *A. rivularis* (1.64%). It has been reported to exhibit antimicrobial, antialopecic, antianaphylactic, antiarteriosclerotic, antileukotriene, antimenorrhagic, antiprostatic, carcinogenic, antigranular, metastatic, immunomodulatory (Mickymaray et al., 2016) as well as nematicide and antieczemic activities (Kavitha et al., 2014). It has also been reported to display antiandrogenic (5-Alpha-reductase-inhibitiory), antiacne, antiarthritic, anticoronary, antihistaminic, antiinflammatory, cancer preventive, hepatoprotective, hypocholesterolemic and insectifuge activities (Kavitha et al., 2014; Mickymaray et al., 2016). It has also been reported to be applicable as drugs for genital or sexual disorders and in contraceptives (Rawal & Sonawani, 2016).

Cholest-5-en-3-ol, 24-propylidene-, (3*β*) (Syn. 24-Propylidenecholest-5-en-3*β*-ol) was identified from *S. lucida* (3.06%). It has been reported to exhibit antibacterial and antioxidant activities (Thanigaivel et al., 2015).

9,12-Octadecadienoic acid (*Z*,*Z*)-, 2,3-dihydroxypropyl ester was identified from *R. scleratus* (3.04%). It has been reported to exhibit antipyretic, anticonvulsant, antiseptic, analgesic (Edewor et al., 2016), hypocholesterolemic, antieczemic, nematicide, hepatoprotective, antiarthritic, antiacner and antihistaminic activities (Gnanavel & Saral, 2013).

Maytansine was identified from *L. involuta* (2.86%), and *C. aciculatus* (1.34%). It is an ansa macrolide structure attached to a chlorinated benzene ring which has been found to suppress microtubule dynamics (Lopus et al., 2010) and exhibit antimitotic effect (Bhattacharyya & Wolff, 1977; Mandelbaum-Shavit et al., 1976; Remillard et al., 1975). It has been reported to be effective against breast cancer, lung carcinoma and murine melanocarcinoma solid tumors and has an antileukemic activity against lymphocytic leukemia (Cassady et al., 2004; Lopus et al., 2009). It inhibits microtubule assembly and kills cancer cells, but has severe side effects (Cassady et al., 2004). It shows systemic toxicity such as neurotoxicity and gastrointestinal tract adverse reaction (Haddley, 2013; Thery et al., 2014).

Phytol was identified from *H. javanica* (2.75%), *L. involuta* (2.68%), *A. rivularis* (3.19%), *R. scleratus* (1.27%), *S. elata* (0.52%), *C. aciculatus* (0.44%), and *R. lepidotum* (0.41%). It is an acyclic monounsaturated diterpene alcohol known to be the precursor for synthetic forms of vitamin E (Qiu et al., 2014) and vitamin K1 (Daines et al., 2003). It has been found in chlorophyll and tocopherols and has been known to be a strong immunomodulator but it exerts strong toxicity (Kagoura & Morohashi, 1999; Lim et al., 2006). It has been reported to exhibit antischistosomal (de Moraes et al., 2014), antinociceptive, antioxidant (Santos et al., 2013), antimicrobial, diuretic (Jananie et al., 2011; Sudha et al., 2013), antiinflammatory (Silva et al., 2014), antiallergic (Ryu et al., 2011) and anticancer activities (Guo et al., 2014; Islam et al., 2015; Kim et al., 2015; Pejin et al., 2014).

We identified a number of bioactive compounds with proven bioactivities. The presence of these and other bioactive compounds either alone or in combination are responsible for medicinal properties of these plants. Further isolation and elucidation of safe therapeutic phytocompounds may help address the problem of drug resistance and toxicity. Novel phytopharmaceuticals can bring infinite possibilities in modern medicine.

6 Summary and Conclusion

Preliminary study was conducted on bioactive compounds and biological activities of twelve medicinal plants with some history of use as traditional medicine around the world. The results from total phenolic content, total flavonoid content, GC-MS analysis, radical scavenging activity, antimicrobial activity, brine shrimp lethality, and cancer cell inhibition advocated that the bioactive compounds afforded by plants display one or more of the biological activities either individually or synergistically.

The methanolic extracts of *T. dumosa, P. emblica, G. wallichianum, T. bellerica, S. elata, S. lucida,* and *R. lepidotum* showed a good antioxidant activity with IC_{50} value less than 50 µg/ml. Phenolic compounds were attributed to be the major contributor of antioxidant activity as suggested by a significant correlation between TPC and IC_{50} of DPPH RSA. These plants and probably others could be the good source of natural antioxidants. The higher antimicrobial activities against *S. aureus* and MRSA both were displayed by *T. bellerica, P. emblica, R. lepidotum, T. dumosa, G. wallichianum,* and *S. elata. S.* Typhi was inhibited maximally by *T. dumosa* followed by *S. elata, C. aciculatus,* and *T. bellerica*. Minimum number of plants showed activity against *K. pneumoniae* with higher effect exerted by *L. involuta* followed by *C. aciculatus*. Ten of the twelve plant extracts inhibited at least three of the six organisms tested while eight extracts inhibited at least four organisms. *G. wallichianum* demonstrated effectiveness against five of the organisms tested except *K. pneumoniae. S. elata* and *T. bellerica* were active against all the tested microorganisms. Plants inhibited gram positive bacteria more effectively. Nine of the twelve medicinal plants showed significant toxicity to brine shrimps with LC_{50} value less than 500 µg/ml including the three *viz*. *A. rivularis, L. involuta,* and *T. bellerica* showing high toxicity with LC_{50} less than 100 µg/ml. Maximum growth inhibition on HeLa was shown by S. lucida followed by *T. dumosa, C. aciculatus, R. scleratus, S. elata, A. rivularis, R. lepidotum,* and *L. involuta* while that on MDCK was exerted by *L. involuta* followed by *T. dumosa,* and *A. rivularis* with EC_{50} value less than 500 µg/ml. Higher number of plants were effective against HeLa as compared to MDCK. The major bioactive compounds identified from GC-MS analysis of plant extracts have been found to have previously been reported to exert different biological activities including antioxidant, antimicrobial, and anticancer.

Multidrug resistant microbial pathgens are on rise. Cancer treatment still remains a big challenge. The lack of efficient treatment in addition to the side effects resulting from current therapies need to be effectively addressed. At this stage, our study offers a scientific basis for traditional use of medicinal plants. Plants showed the rich presence of bioactive compounds.

The significant inhibition of *S. aureus* and MRSA, multidrug resistant *S.* Typhi and *K. pneumoniae* by some of the plant extracts suggest their use in isolation of compounds with antimicrobial activity and as the substitute for antibiotics. The significant toxicity of some of the medicinal plants signifies their potential use as the source of anticancer drugs and at the same time demands their careful analysis before use in medical formulations. Hence, the medicinal plants could be used as an important source of therapeutic components in pharmaceutical and medical industries. For this reason, further work need to be conducted to isolate, identify and characterize bioactive compounds and test them rigorously *in vitro* and *in vivo* and assess their safety which helps discover novel drugs.

7 Recommendation

Further investigations are required for:

- isolation, identification, and characterization of the bioactive compounds from different solvent fractions
- observation of biological activities of isolated compounds either alone or in combination with other compounds *in vivo* and *in vitro*
- examination of therapeutic mechanisms of plant extracts and their compounds with the focus to explore their specific cellular and molecular targets

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Appendices

List of Reagents and Culture media

1. Preparation of 1 M Na_2CO_3 – 100 ml
10.599 g of Na_2CO_3 (Merk specialities Pvt. Ltd., Mumbai, India) was carefully weighed and then dissolved in distilled water, and the volume was adjusted to 100 ml at the end.

2. Preparation of glacial acetic acid (20%) – 200
40 ml of the commercially supplied glacial acetic acid (Thermo Fisher Scientific, India) was taken and mixed with ethanol. Finally, the volume was adjusted to 200 ml by the addition of ethanol.

3. Preparation of aluminium chloride (10%) – 100 ml
10 g of the commercially supplied aluminium chloride (Merk Specialities Pvt. Ltd., Mumbai, India) was weighed and dissolved in water. Finally, the volume was maintained to 100 ml.

4. Preparation of 1M potassium acetate (CH_3COOK) – 100 ml
Weigh 9.814 g of the potassium acetate Merk Specialities Pvt. Ltd., Mumbai, India) and dissolve on water. Finally maintain the volume to 100 ml by the addition of water.

5. Preparation of 0.2 Mm DPPH solution – 100 ml
1,1-diphenyl-2-picrylhydrazyl (DPPH) has the molecular weight of 394.32 g/mol. Thus, 100 ml of 0.2 mM solution of DPPH is prepared by weighing the 7.886 mg of the DPPH carefully and dissolving it on ethanol and finally maintaining the volume to 100 ml by addition of ethanol.

6. Preparation of the Folin-Ciocalteu pheol reagent (1:10 dilution)
6 ml of the commercially supplied Folin – Ciocalteu phenol reagent (Merk Specialities Pvt. Ltd., Mumbai, India) was taken and mix it with 54 ml of the distilled water to prepare the 60 ml of 1:10 dilution of Folin – Ciocalteu phenol reagent.

7. Composition of nutrient agar media
The composition of nutrient agar media (Hi Media Laboratories Pvt. Ltd., Mumbai, India) is as follows:

Components	gram/L
Peptic digest of animal tissue	5.0
Beef extract	1.5
Yeast extract	1.5
Sodium chloride	5.0
Agar	15.0
Ph	7.4±0.2

8. Composition of Mueller Hinton Agar (MHA)

The composition of Mueller Hinton Agar (MHA) media (Hi Media Laboratories Pvt. Ltd., Mumbai, India) is as follows:

Components	gram/L
Beef infusion form	300
Casein hydrolysate	17.5
Starch	1.56
Agar	17
Final pH	7.3±0.2

9. Composition of Potato Dextrose Agar (PDA)

The composition of PDA broth (Hi Media Laboratories Pvt. Ltd., Mumbai, India) is as follows:

Components	gram/L
Potato	200
Agar	2
Dextrose/Glucose	2

10. Composition of artificial sea water

SN	Salt	Molecular wt (g/mol)	Amount in gram/L
1	NaCl	58.44	23.50
2	Na_2SO_4	142.04	4.00
3	KCl	74.55	0.68
4	H_3BO_3	61.83	0.026
5	$MgCl_2.6H2O$	203.33	10.78
6	$CaCl_2$	110.98	1.47
7	$NaHCO_3$	84.00	0.196
8	Na_2EDTA	372.24	0.0003

pH = 8.0±0.2

11. General Classification of brine shrimp cytotoxicity

LC_{50} Values (µg/ml)	Classification
<1	Extremely toxic
1-100	Highly toxic
100-500	medium toxic
500-1000	low toxic
>1000	non-toxic

Photographs

Medicinal Plants

a) *Loxogramme involuta*

b) *Hydrocotyle javanica* Thunb

c) *Phyllanthus emblica* Linnaeus

d) *Chrysopogon aciculatus* (Retzius) Trinius

Plate 1 Some medicinal plants (a) *Loxogramme involuta*, (b) *Hydrocotyle javanica* Thunb, (c) *Phyllanthus emblica* Linnaeus, and (d) *Chrysopogon aciculatus* (Retzius) Trinius

a) *Rhododendron lepidotum* Wallich ex G. Don

b) *Geranium wallichianum* D. Don ex Sweet

c) *Tsuga dumosa* (D. Don) Eichler

d) *Anemone rivularis* Buchanan-Hamilton ex De Candolle

Plate 2 Some medicinal plants (a) *Rhododendron lepidotum* Wallich ex G. Don, (b) *Geranium wallichianum* D. Don ex Sweet, (c) *Tsuga dumosa* (D. Don) Eichler, and (d) *Anemone rivularis* Buchanan-Hamilton ex De Candolle

a) *Scurrula elata* (Edgeworth) Danser

b) *Terminalia bellirica* (Gaertner) Roxburgh

c) *Ranunculus scleratus* Linnaeus

d) *Symplocos lucida* (Thunberg) Siebold & Zuccarini

Plate 3 Some medicinal plants (a) *Scurrula elata* (Edgeworth) Danser, (b) *Terminalia bellirica* (Gaertner) Roxburgh, (c) *Ranunculus scleratus* Linnaeus, and (d) *Symplocos lucida* (Thunberg) Siebold & Zuccarini

Methanol extraction

Methanol extraction

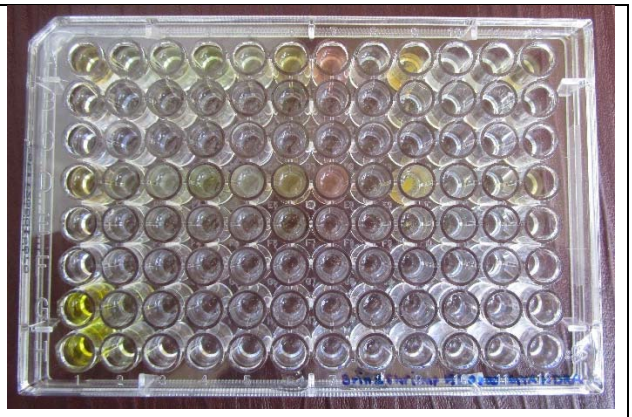
Research team members with principal investigators

The project on methanol extraction for bioprospecting of underutilised medicinal plants of Nepal

GC-MS analysis

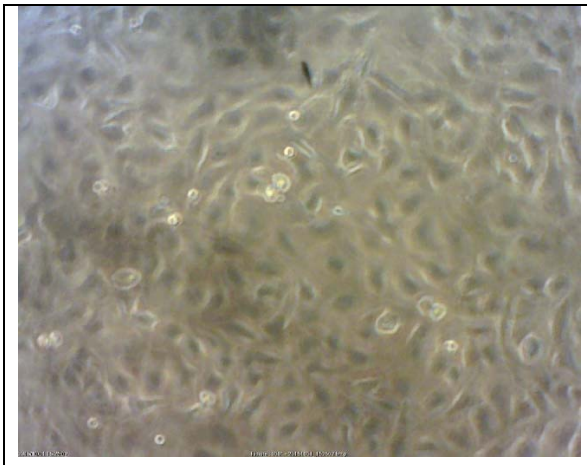
GC-MS analysis: the training and the experiment

Brine Shrimp Lethality

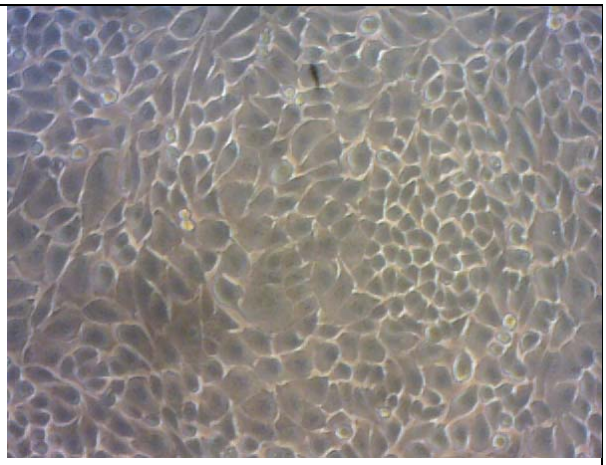


Brine shrimp lethality bioassay: death count through stereoscope

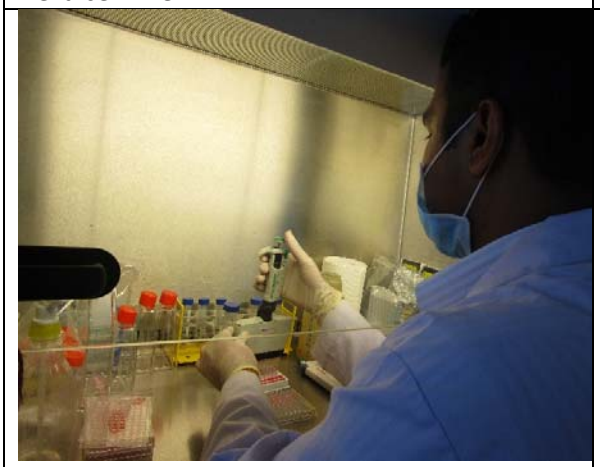
MTT Assay



HeLa cell line



MDCK cell line



Cell seeding in 96-well tissue culture plate

Examination of effects of extracts on HeLa cells

	
Formation of formazan crystals	Dissolution of formazan salts

MTT cell proliferation assay: cell seeding to formazan dissolution