“EVALUATION OF IN VITRO ANTIDIABETIC, ANTIOXIDANT, CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF SOME NEPALESE MEDICINAL PLANTS EXTRACTS”

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This is to certify that the research work entitled **"EVALUATION OF IN VITRO ANTIDIABETIC, ANTIOXIDANT, CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF SOME NEPALESE MEDICINAL PLANTS EXTRACTS"** has been carried out by **Ms. Archana Chataut** under our supervision. This thesis work was performed for the partial fulfillment of the Master of Science in Biotechnology under the course code BT 621. The result presented here is her original findings. We hereby, recommend this thesis for final evaluation.

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TO WHOM IT MAY CONCERN

Ms. Archana Chataut who enrolled in Master of Science in Central Department of Biotechnology, Tribhuvan University, Kathmandu, Nepal, conducted successfully a part of her M.Sc. thesis work entitled **"EVALUATION OF IN VITRO ANTIDIABETIC, ANTIOXIDANT, CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF SOME NEPALESE MEDICINAL PLANTS EXTRACTS"** in my laboratory for the partial fulfillment of her academic program. The thesis was mutually supervised by me and Prof. Dr. Rajani Malla.

As a supervisor, I wish her for successful submission of her thesis.

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CERTIFICATE OF EVALUATION

This is to certify that this thesis entitled **"EVALUATION OF IN VITRO ANTIDIABETIC, ANTIOXIDANT, CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF SOME NEPALESE MEDICINAL PLANTS EXTRACTS"** presented to evaluation committee by Ms. Archana Chataut is found satisfactory for the partial fulfillment of Master of Science in Biotechnology.

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Thank you!

Archana Chataut

ABBREVIATIONS

Abs:	Absorbance
ABTS:	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
$AlCl_3$:	Aluminum Chloride
$CaCl_2$:	Calcium chloride
CV:	Coefficient of variation
DMSO:	Dimethyl sulfoxide
DPPH:	2,2-Diphenyl-1-picrylhydrazyl
EDTA:	Ethylene diamine tetra acetic Acid
ELISA:	Enzyme linked immune sorbent assay
GAE/g:	Gallic acid Equivalent per Gram
GAE:	Gallic acid equivalent
H_2O_2:	Hydrogen peroxide
H_3BO_3:	Boric acid
IC_{50}:	Half maximal Inhibitory concentration
KCl:	Potassium chloride
LB:	Luria Bertani
LC_{50}:	Lethal concentration 50
LF:	Laminar flow
$MgCl_2$:	Magnesium Chloride
MHA:	Mueller Hilton Agar
NA:	Nutrient Agar
Na_2SO_4:	Sodium sulfate
NaCl:	Sodium Chloride
$NaHCO_3$:	Sodium bicarbonate
QE:	Quercetin equivalent
ROS:	Reactive Oxygen Species
RSA:	Radical Scavenging Activity
Spp:	Species
TFC:	Total flavonoid contents
TPC:	Total phenolic contents
UV:	Ultraviolet
Vs:	Versus

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ABSTRACT

Medicinal plants have been recognized to treat simple to life-threatening diseases in different societies of Nepal since long time. Hexane, ethylacetate and aqueous extract of *Phyllanthus emblica*, *Zanthoxylum armatum*, *Azadirachta indica*, *Ephedra gerardiana*, *Terminalia bellirica* and *Stereum ostrea* were taken to evaluate the antibacterial, cytotoxicity, antioxidant and antidiabetic activities.

Antibacterial test was done by agar well diffusion method and zone of inhibition (ZOI) was measured. The plants extract was found active against Gram-positive *Staphylococcus aureus* with the largest Zone of Inhibition (ZOI) as 1.77cm for ethylacetate extract of *P. emblica*. These extracts didn't show effect on all of the tested Gram-negative *Klebsiella penumoneae*, *Escherichia coli* and *Salmonella typhii* bacteria. Brine shrimp lethality bioassay was performed for cytotoxicity assay. The highest LC_{50} (217.04 µg/ml) was showed by all 3 extracts of *S. ostrea*; hexane and ethylacetate extract of *Z. armatum*; water and hexane extract of *E. gerardiana*. DPPH free radical scavenging test was done for antioxidant activity. Ethylacetate extracts of *S. ostrea* (14.76 µg/ml), aqueous and hexane extract of *A. indica* (16.06 µg/ml, 18.03 µg/ml) showed potent antioxidant activity. Ethylacetate extract of *S. ostrea* contains 4.39 mg/g GAE TPC and 102.5 mg QE/ g TFC. Similarly, aqueous and hexane extract of *A. indica* contain TPC and TFC as 9.61 mg/g GAE, 44.1 mg QE/ g, 3.05 mg/g GAE, 60.9 mg QE/ g respectively. Alpha amylase inhibition assay was performed to test antidiabetic activity of plant extract taking Acarbose as standard. Ethyl acetate extract of *S. ostrea* had peak percentage inhibition i.e.99.89% with IC_{50} of 788.25µg/ml followed by same extract of *P. emblica*. whereas, hexane extract of *E. gerardiana* had the lowest inhibition i.e. 72.71% with IC_{50} of 138.67 µg/ml.

The results recommended that plant extracts can be used against gram positive bacteria as they showed good inhibition on them. Furthermore, *Stereum ostrea* and *Phyllanthus emblica* can be consumed as diet as they reduce amylase in significant way thus might control diabetes. Although, the plant extracts were not cytotoxic to brine shrimp but further study in need to know the exact mechanism and bioactivities of plant extracts.

Key words: **Biological activities, In-vitro, Medical plants, *Terminalia bellirica***

1 INTRODUCTION

1.1 Background

Plants are dynamic component of the world's biodiversity and essential natural resource for human well-being. The plant kingdom represents a massive reservoir of biologically active compounds with various chemical structures and diseases preventing properties called phytochemicals (Peteros et al, 2010). These phytochemicals, often secondary metabolites, which are present in small amount in plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others (Chhetri et al, 2008). A large proportion of world population in the developing countries depends on traditional system of medicine for varieties of diseases. The type of plant and its medical applications depend on the region where they are found and practice introduced by local/ indigenous people. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population (Jayachandran et al, 2010; Maroyi, 2016). Phytochemical can be derived from barks, leaves, flowers, fruits, seeds, roots. Knowledge of the chemical constituents of plant is desirable because such information will be valuable for the synthesis of complex chemical substances. Medicinal plants are the "back bone" of herbal remedy. The traditional medicine related to the treatment of both human and animal diseases with plant-derived preparations is considered a valuable knowledge. The potential of higher plants as sources for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Prabhu et al, 2010). Furthermore, Elujoba (1997) noted that a plant become a medicinal plant only when its biological activity has been ethno botanically reported or scientifically established. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002).

Nepal is well known for its enriched biodiversity and of course, herbal plants affluent. Solely on Nepal's Alpine zone, more than ten thousand medicinal plants have been covered. Most of aromatic and medicinal plants have been documented for their utilization in healing human disorders i.e. up to 1792 to 2331 in number. Local people have practiced such plants for livelihood, conventional therapies and home remedies since ancient times (Baral and Kurmi, 2006). Several findings unveiled that the plants are the robust sources of drugs. Hence, medicinal plants are a pillar in both traditional and modern medical interventions, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hancock,2005). Antimicrobial activities are found in several medicinal plant compounds and used medicinally as a source of potent drugs (Uniyal et al, 2006). Since ancient time, plants have been used as

a major source of drugs (Newman et al, 2003). Modern medicines are also based on indigenous knowledge and ethno-pharmacological practices which have become back bone for noble drug discovery (Devkota, 2014).

1.2 Plants as a source of antimicrobial

Antibiotics are certainly one of the most crucial therapeutic discoveries of the 20th century. On the other hand, only one third of the infectious illnesses have been cured from these synthetic medicines. Because of widespread indiscriminate and misuse of antibiotics, antibiotic resistance has raised in the recent years. One of the ways to decrease the resistance to antibiotics is by utilizing antibiotic resistance inhibitors obtained from plants. Plants generate many compounds to defend themselves against the pathogens (Guimaraes et al, 2020). Development of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drug of plant origin. Medicinal plants having antimicrobial properties are progressively identified from different parts of the world. Antimicrobials therefore may have a significant clinical value in treatment of resistant microbial strain (Manikandan et al, 2013). Compounds for example alkaloids, flavonoids, tannins and phenolic compounds are most commonly found in medicinal plants. The phenolic and 5 polyphenolics are one of the largest groups of secondary metabolites that have exhibited antimicrobial activity (James et al, 2018). Polyphenols inhibit the growth of microorganisms, especially bacteria. Polyphenols also influence protein biosynthesis, change metabolic processes in bacteria cells and inhibit ATP and DNA synthesis (Efenberger-Szmechtyk, 2020).

1.3 Plants as a source of cytotoxicity

The killing ability of produced chemicals, naturally occurring toxins or immune-mediator cells is known as cytotoxicity (Izadiyan et al, 2018). Commonly, plants are primary source of antimutagens as well as natural toxic agents (Plewa and Wagner, 1993) and also contain cytotoxic and genotoxic substances. Current research has shown that many plants used as food or in traditional medicine have mutagenic effects and cytotoxic and genotoxic effects in vitro and in vivo tests (Higashimoto et al, 1993). Sustainable use from long time may presume that herbs used in traditional medicine may have low toxicity. However, in some case; cause adverse effects or have the potential to interact with other medications (Zink and Chaffin, 1998); besides this, there is little evidence on the possible risk to health of such herbs (Basaran et al, 1996). In vitro and in vivo investigations demonstrate that many plants used as food or in traditional medicine exhibits mutagenic, cytotoxic and genotoxic effects (Zink and Chaffin, 1998).

1.4 Plants as a source of antioxidant

Substances that can preserve cells from unstable molecules that can which are harmful such as reactive oxygen species (ROS) and free radicals are termed as antioxidants. These unstable molecules are responsible for many health problems such as cancer (Thyagarajan et al, 2018) cardiovascular diseases (Leopold, 2015), heart diseases (Chen and Alpert, 2016), inflammation (Arulselvan et al, 2016) and gastric problems. Antioxidant is a molecule accomplished of reducing or preventing the oxidation of other molecules. The chemical reaction that can transfers electrons from a substance to an oxidizing agent and produce free radicals, which start chain reactions that damage cells such reaction is called oxidation. These chain reactions were stopped by antioxidants to remove free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Therefore, antioxidants are reducing agents as thiols, ascorbic acid or polyphenols and widely used as dietary components to maintain good health and prevent from diseases as cancer and coronary heart disease (Sies, 1997). Many of the plants natural products can be used as medicinal plant and used for therapy, which is now become a topic of concern for integrated cancer management (Kamble and Gacche, 2019). Phenolic and flavonoids compound of medicinal plant are supposed as protective dietary ingredients have become an increasingly important area of human nutrition research. (Liu, 2004). antioxidant activity of medicinal plants is mainly due to the presence of Phenolic and Flavonoid compound (El-toumy, 2011; Kim et al, 2003 and Lu, 2004). These phenolic and flavonoid compounds combine with vitamins and enzymes to acts as antioxidant inside the body. These compounds also act as antidiabetic and cytotoxic (Saxena et al, 2012 and Seifu et al, 2012).

1.5 Plants as a source of antidiabetic

Diabetes mellitus is a metabolic condition, which is increasing gradually. Insulin is a key player to control carbohydrate, fat and protein metabolism. Insulin deficiency may affect the above important metabolisms. The enzyme alpha amylase and alpha glucosidase may be responsible for the breakdown of carbohydrates into glucose (Abhijit et al, 2014).

The substrate binds to the active site of enzyme to form enzyme-substrate complex and then the enzyme hydrolyze a bond of a substrate molecule to form products. Finally, the products leave the active site of the enzyme. In the case of the alpha-amylase mode of action, the substrate is usually starch which is hydrolyzed into oligosaccharide of starch as products while in case of the alpha glucosidase mode of action, the substrate is usually oligosaccharides which are hydrolyzed into monosaccharides as products (Nguyen and Le, 2012)

Medicinal plants have been widely used as pharmacological medicines and constitute a rich source for antidiabetic drug discovery (Zhao et al, 2019). Medicinal plants are used to treat various diseases (Daimari et al, 2019). Presence of various phytoconstituents such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides had made plant products as antidiabetic substance (Afrisham et al, 2015, Kooti et al, 2015). These phytochemicals were noted with no side effects for the treatment of diabetes around the world (Gupta and De, 2012). Most tests have demonstrated the benefits of medicinal plants containing hypoglycemic properties in diabetes management. The antidiabetic activity of herbs depends upon variety of mechanisms. The mechanism of action of herbal antidiabetic could be grouped as: Adrenomimeticism, pancreatic potassium channel blocking, cAMP (2nd messenger) stimulation, Inhibition in renal glucose reabsorption (Rani et al, 2020).

1.6 Current studies

Tradition medicine are used by many countries to treat or cure many diseases. Natural products extracted from plants are more cost effective therapies that are reasonable to the population (Jayachandran et al, 2010 and Maroyi, 2016). Plant products can be derived from barks, leaves, flowers, fruits, seeds, roots. Knowledge of the chemical constituents of plant is desirable because such information will be valued for the synthesis of complex chemical substances. In recent years, phyto-chemicals possessing have been in demand due to their potential use in the remedy of various enduring and contagious diseases. Synthetic drugs also have lost their effectiveness due to the development of resistant strains, mostly through the appearance of resistance genes (Davies, 1994 and Harbottle et al, 2006). Since the medicinal plant extract will show remarkable benefits due to having a variety of bioactive compounds and thus can be used in the phytotherapy.

1.7 Hypothesis

1.7.1 Alternative Hypothesis, H_1:

Plants can be used in the phytotherapy or medicinal purpose to treat people which will not have side effect

1.7.2 Null Hypothesis, H_0:

Plants having significant side effect cannot be used in the phytotherapy or medicinal purposes to treat the people.

1.8 Objectives

1.8.1 General

- To evaluate in vitro antidiabetic, antioxidant, cytotoxic and antibacterial activities of some Nepalese medicinal plants extracts

1.8.2 Specific

- Determination of the antibacterial property of plant extracts
- Determination of total phenolic contents of plant extracts
- Determination of total flavonoid content of plant extract
- Determination of antioxidative property of plant extracts
- Determination of cytotoxicity of plant extract
- Determination of antidiabetic activity of plant extract

1.9 Rationale

Nepal is a small country in the Himalayan mountain range, located between India and Tibet. The country has many rural parts where western pharmaceuticals or pre-packaged Indian Ayurveda medicines are not available. The use of traditional medicine is widespread in these rural areas, with much of the population relying on it. Himalayan country Nepal is rich in medicinal, endemic and poisonous plants. These plants might have a number of bioactivity such as anti-bacterial, antidiabetic, antioxidant, anti-cancer etc. However, the information based on research work is limited in this area. Nepalese medicinal plants often show potent antioxidant, antidiabetic, anticancer activity and can be used for the management of various ailments. The need of this study is to evaluate the different plants to investigate antibacterial, antioxidant and antidiabetic activity. This study also provides guidelines to extracts active constituents present in plants having antibacterial, antioxidant and antidiabetic properties, which can be further utilized in the allopathic system of treatments.

2 LITERATURE REVIEW

a) *Azadirachta indica*

Azadirachta indica is tall evergreen tree of 15-20m height, distributed up to 100-900 m range from east to west. It. It contains phytochemicals like terpenoids, azadirachtin, and palmitic, stearic, linoleic, and arachidonic acids. (Siddiqui et al, 1993; Husain et al, 1992).

Antibacterial activity of *Azadirachta indica* leaves extracts of methanol, Hexane, ethylacetate, petroleum ether, acetone, benzene, chloroform had been studied against various non-pathogenic gram positive and gram negatives bacteria. Because of this crucial role of *A. indica* in antibacterial activity, further studies have been carried out and found that methanolic and acetone extracts were more effective compared to that of aqueous extract, also Gram-positive bacterial strains were found more sensitive than the Gram-negative ones for each extracts (Dhami et al, 2019). According to Sahrawat and Sharma (2018) ethanolic extract of *A*. *indica* (Neem leaves) showed promising antibacterial activity against *P. aeruginosa* with zone of inhibition of 11mm. Similarly, Kumari et al (2018) studied antibacterial properties of seed oil of *A. indica* against *E. coli, Bacillus subtilis, Rhizobium meliloti* and *P. aeruginosa* and found ZOI of 18mm and 17 mm for *E. coli* and *P. aeruginosa* respectively but Toppo et al (2018) recorded ZOI of 13mm for *E. coli* and 12mm for *S. aureus* in chloroform extract of neem. Manandhar et al (2019) also found antibacterial activity with ZOI of 0.8mm against *S. aureus*. A new study was conducted to find MIC for the bark extract of *A. indica*, which showed zone of clearance against *E. faecalis*, *P. aeruginosa*, and *P. mirabilis* at all concentrations but the bark extract did not show significant effect at a low concentration of 500 µg/mL against *S. aureus*, higher concentrations of 1000 and 2000 µg/mL showed zone of inhibition against *S. aureus* (Singaravelu et al, 2019). Malar et al (2020) performed antibacterial assay of methanolic extracts of *A. indica* against four different organism *K. pneumonia, S. aureus, P. aeruginosa* and *E. coli* and found the ZOI of 14mm, 12mm, 9mm and 6 mm respectively but Adhikari et al (2020) recorded the highest ZOI against *S. aureus* (14±2) for chloroform neem extract.

Kumar et al (2018) found dose-dependent antioxidant capacity of *A. indica* leaves as the mean values of total phenolic content and flavonoids are 70 mg GAE/g & 119 mg QE/g respectively. Another study conducted by Manandhar et al (2019) determined IC_{50} value of methanol extract to be 109.6±7.2 µg/mL through antioxidant activity study with DPPH. Similarly, Adhikari et al (2020) recorded the DPPH free radical scavenging activity of methanol extract of neem and found 84.85±0.04% followed by chloroform extract (24.53±0.003%), and extract in cow urine (21.8±0.012%). The aqueous extract of neem showed most potent antidiabetic with IC_{50} of 9.51mg/ml (Kazeem et al, 2013). *Azardirachta indica*-yogurt may have the potential to serve as enhanced functional yogurt

with anti-diabetic and anti-hypertension activities as Maximum inhibition of α-amylase (47.4 ± 5.8%) in 7 days (Shori and Baba, 2013).

b) Phyllanthus emblica

Phyllanthus emblica is small to medium in size, reaching 1–8 m in height belongs to the family Phyllanthaceae. It has finely pubescent branchlets, the leaves are simple, pinnate, sub-sessile and light green. The flowers are greenish-yellow, spherical, light greenish-yellow fruit, with six vertical stripes or furrows on it.

The plant genus *Phyllanthus* (Euphorbiaceae) is broadly dispersed in most of tropical and subtropical countries. Raghu and Ravindra (2010) also stated a significant antimicrobial activity for *P. emblica*, where the MIC exhibited against the gram positive and negative microbes ranges between 0.261 to 0.342 cm. Similarly, Dhale and Mogle (2011) found inhibition for chloroform extract as 9mm, 8mm, 13mm and 10mm for *E. coli, P. aeruginosa, S. aureus* and *B. subtilis* respectively of amla leaf. Similarly, he also noticed ZOI of 10mm ,11mm, 17mm and 14mm for same species respectively for fruit extract. Sharma (2017) recorded ZOI of 9mm,13mm, 10mm and 10mm for *S. aureus, B subtilis, S typhii* and *E. coli* for methanol extract of *P. emblica*. Al-Gbouri, and Hamzah (2018) performed antibacterial activity of ethanol extract of *P. emblica* against *E. coli, S. aureus* and *P. aeruginosa* and found ZOI as 27mm, 25mm, and 15mm at 20mg/ml. The methanol leaf extract of *P. emblica* at concentration of 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml showed inhibition zone of 22mm, 24mm, 27mm and 29mm respectively against *S. aureus* (Mahato and Sharma, 2018). In the antimicrobial study, it was observed that, the extract of *Phyllanthus emblica* from Bangladesh showed high activity against all gram- positive bacteria.

Dinesh et al (2016) recorded IC50 value of 85.92 µg/mL for the seed extract of *P*. *emblica* with total phenol and flavonoid content 48.242 and 12.72 mg/mL respectively. Similarly, Chaphalkar et al (2017) determined total phenolic, flavonoid, and tannin content and in vitro antioxidant activities by using H_2O_2 scavenging and ABTS decolorization assays and found that *P. emblica* was rich in total phenols (mg GAE/g) and total flavonoids (mg quercetin hydrate/g). Nampoothiri et al (2011) evaluated the antioxidant potential of different solvent extracts; Hexane (HE), ethyl acetate (EA), methanol (ME), 70% methanol (MW) and Water (WA)) of *P. emblica* by flow cytometry using DCFH-DA as probe and showed that Methanol extract act as potent α-amylase inhibitor. Similarly, *P. emblica* fruit (73.9%) and *P. debilis* whole plant (53%) also showed significant amylase inhibitory effects at 1mg/ml. Similarly, IC_{50} of methanol extract *P. emblica* fruit from Srilanka was found to be 397.67µg/ml (Poongunran et al, 2015). Again, new study done by Singh and Kaur (2015) found that the ethanol extract of *P. emblica* leaves exhibited 61.12% inhibition against alpha-amylase at pH 7 and temperature 40˚C. The chloroform soluble fraction of the crude methanolic extract of the ripe fruits of *P. emblica* containing alkaloids was subjected to brine shrimp lethality bioassay for observing cytotoxic activity and found the chloroform

soluble fraction of the methanol extract as strong cytotoxicity having a LC_{50} of 10.257±0.770 µg mL^{-1} (Rahman et al, 2009). Similarly, Krishnaraju et al (2005) found LC_{50} of 58 µg/mL for ethanol extract. The LC_{50} value of green synthesized *P. emblica* capped Pallidum nanoparticles and the *P. emblica* seed extract were found to be less toxic for *Artemia salina* with a value of 1.00 µg/mL and 1.25 µg/mL (Dinesh et al,2017).

c) Zanthoxylum armatum

Zanthoxylum armatum belong to family Rutaceae. It is commonly called Timur in Nepali. It is prickly shrub or small tree up to 6 m tall growing on shrubberies. It is known as timur in Nepali and belongs to Rutaceae family. It grows throughout Nepal in between 1500-2400m altitude. There are increasing scientific interest in medicinal plants as source of novel agents to fight infectious diseases (Subedi et al, 2020).

The leaf oil and seed oil of *Z. armatum* showed variation in ZOI. Negi et al (2012) recorded the Essential oil of *Z. armatum* exhibited moderate antibacterial activity. Negi et al (2012) also found that the gram-positive bacteria are more sensitivity to the essential oil than gram negative bacteria. Another study conducted by Mehmood et al (2013) recorded ZOI of 1.8cm, 1.6cm, 1.8cm, 2.0cm and 1.4cm by leaf oil extract for *B. cereus, S. aureus, S. typhimurium*, *E. coli* and *P. aeruginosa*. Similarly, he also noticed ZOI of 0.9cm, 1.2cm, 2.1cm, 2.2cm and 1.8cm for oil seed extract respectively. Again Barkatullah et al (2013) recorded, essential oil of leaves of *Z. armatum* showed maximum inhibition against *M. leutus* followed by *B. subtilis* with percent zone of inhibition 28.45% and 20.45%, respectively. The ZOI also differs according to the solvent type used. Nooreen et al (2017) recorded Hexane and ethyl acetate fraction exhibited moderate antibacterial efficacy (MIC: 250–1000 µg/mL) against selected pathogenic microbes while Ombuin displayed broad spectrum antibacterial effect with MIC ranges from 125 to 500 µg/mL . Dahal et al (2017) studied the antibacterial activity of Timur (*Zanthoxylum armatum*) through agar well diffusion method and found potent antibacterial against *P. aeruginosa*. Sakha et al (2018) collected *Z. armatum* (Timur) from hilly regions of Nepal and showed good result against *E. coli* with zone of inhibition 10mm.

Kumar et al (2016) recorded the ethanol extract of stem bark of *Z. armatum* showed antioxidant activities. Similarly, Dahal et al (2017) studied antibacterial and antioxidant activities of four common Nepalese kitchen spices and found *Zanthoxylum* (500 µg) extract with 88.73%±5.64% inhibition. Phuyal et al (2019) determined the presence of alkaloids, sterols, phenolics, lignins coumarins, terpenoids and flavonoids have been identified from leaves, fruits, stem, bark and seed that played important role in determining antioxidant. Again Phuyal et al (2020) recorded the highest TPC value was 226.3 ± 1.14 mg GAE/g in wild fruits, and the lowest was 137.72 ± 4.21 mg GAE/g in cultivated seeds. Similarly, the highest TFC value was 135.17 ± 2.02 mg QE/g in cultivated fruits, and the lowest was 76.58 ± 4.18 mg QE/g in cultivated seeds.

The median inhibitory concentration (IC_{50}) of ethanol extract of *Z. armatum* from china was (1.185 ± 0.132) mg/ml (Zhang et al, 2015). Again new study done by, Rynjah et al (2018) found that the IC_{50} of aqueous leaf extract was 7.40 mg/ml while Acarbose 4.42 mg/ml.

Similarly, Alam et al (2018) found the methanol extracts of leaves and barks with maximum α-glucosidase inhibition, 96.61 ± 2.13% and 93.58 ± 2.31% respectively. Nyongesa (2019) found that species of *Zanthoxylum* from Brazil, the half maximal inhibitory concentration (IC_{50}) values for the leaf and stem extracts was 25.9 and 61.5 µg/mL. Similarly, Jhamta and Kaur (2020) showed, IC_{50} for methanol leaf extract of *Z. armatum* was 89.37±4.68 µg/ml which is higher than standard Acarbose. Barkatullah et al (2013) also found significant cytotoxicity of *Z. armatum* oil. Methanol extract of *Z. armatum* showed satisfactory phenolic and flavonoid content which support the cytotoxicity with LC_{50} value 102.30 ± 0.81 µg/mL (Karmakar et al, 2015).

d) Terminalia bellirica

Terminalia bellirica (Barro) belongs to Combretaceae family. Distributed at the range of 200-1000 m, from east to west.it is a large deciduous tree, 20 40 m tall. Leaves clustered towards the end of the branches. The herb is rich in vitamin C (Anonymous, I, 1988). Leaves contain lawsone (Asolkar et al, 1992). A native species on Bangladesh, Bhutan, Cambodia, China, Indonesia, Laos, Malaysia, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam. The acetone extracts of *T. bellir*ica were highly potent against S. *typhimurium, E. aerogenes, S. aureus*, and *E. coli*. The methanol extract of *T. bellirica* (up to 50%) was also antibacterial to all test pathogens except for *P. vulgaris* (Tambekar et al, 2007). The synthesized silver nanoparticles of *T. bellirica* showed potential antibacterial and antibiofilm activity against bacterial pathogens like *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus* (Patil et al, 2017). Aqueous and methanol extracts of *T. bellirica* displayed antibacterial activity (MIC 0.25–4 mg/mL) against 16 tested microorganisms (Dharmaratne et al, 2018). The extract of *T. bellirica* was more active against *S. aureus* than *T. chebula* extract but against *E. coli* both extracts showed almost same zone of inhibition (Sharma et al, 2018). James et al (2018) found the presence of flavonoids in *T. bellirica*, which showed a strong α-amylase inhibition while comparable to the standard Acarbose. similarly, Gupta et al (2020) measured antidiabetic assay by in vitro α-amylase inhibitory activity and in vivo serum biochemical assays in alloxan-induced diabetic rats and found the Ethyl acetate extract comparatively better α-amylase inhibitory activity (IC_{50} 43.5 µg/ml) as compared to aqueous extract (IC_{50} 74.8 µg/ml). Cytotoxicity results depict the general trend among plants of genus *Terminalia*, which are known to contain cytotoxic compounds such as hydrolysable tannins. Saleem et al (2002) reported that a growth of cancer cell was inhibited by crude extract and the phenolics (gallic acid, ethyl gallate, luteolin and tannic acid) which supports the potent brine shrimp toxicity exhibited by extracts of T*erminalia* species. Cytotoxic potential of the methanolic extract of bark

of *T. bellirica* was assessed with the brine shrimp lethality bioassay and evaluated to be LC_{50} = 3.21 µg/ml (Ali et al, 2013).

e) Ephedra gerardiana

Ephedra gerardiana belong to family Ephedraceae and is concentrated in the range of 2400-5000m from east to west Nepal. It is tall and rigid shrub in dry and rocky soil with many densely assembled smooth, green and jointed branches with scales. It contains ephedrine and ephedroxyane (Husain et al, 1992). *Ephedra* species showed positive antimicrobial toward *S. aureus, B. subtilis, P. arugeunosa, E. coli* (Bhattarai et al, 2009). Shawarb et al (2017) evaluated antioxidant by DPPH (free radical scavenging assay) method and found 15.85 µg/mL IC_{50} value for *Ephedra* spp. Similarly, Jaradat et al (2017) found IC_{50} 15.85 µg/mL again Shawarb et al (2017) found 15.85 µg/mL IC_{50} for *Ephedra* spp.

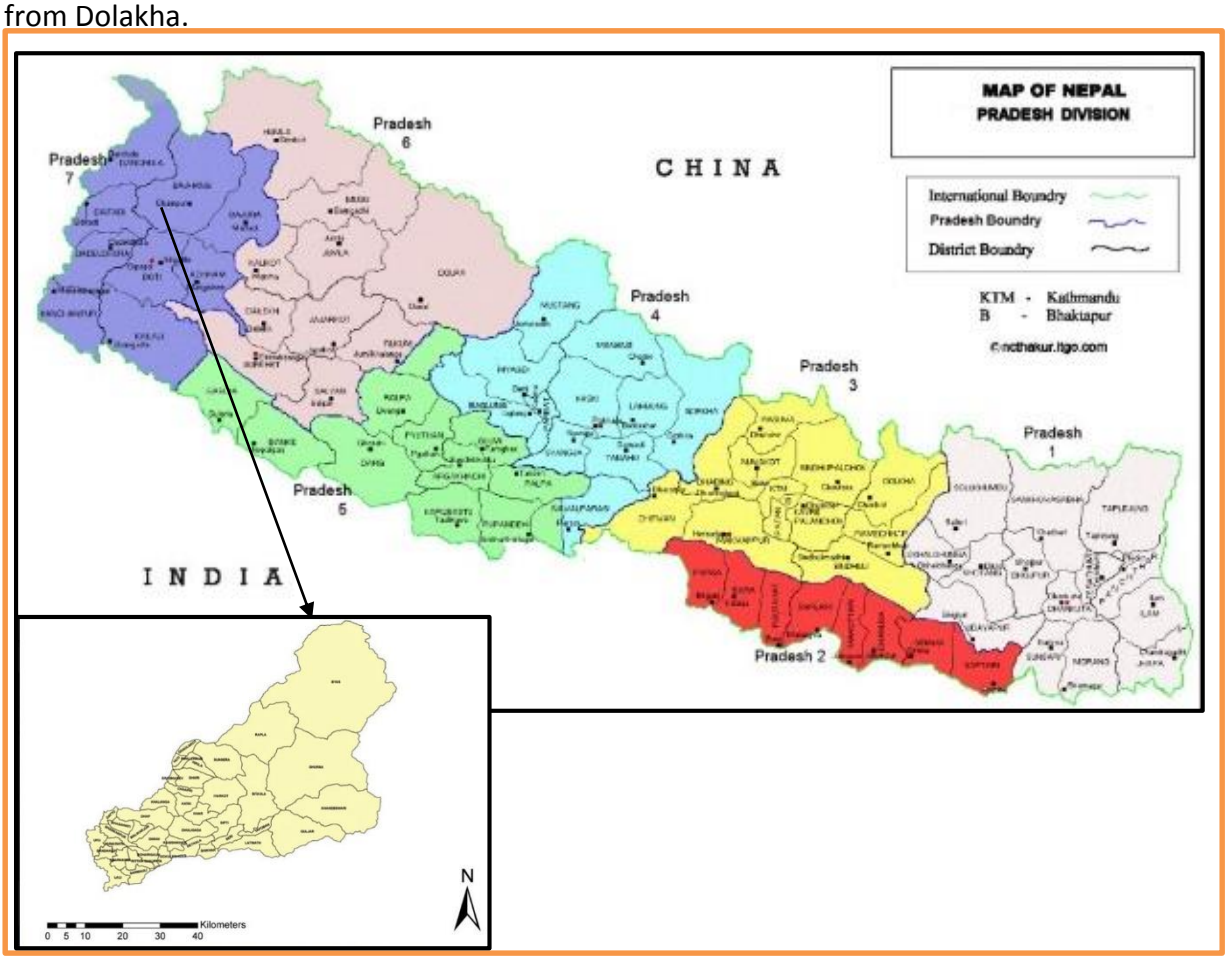
f) Stereum ostrea

Stereum ostrea belongs to basidiomycete family of fungus, also called false turkey-tail. It is a plant pathogen and a wood decay fungus. The name ostrea describes its shape with concentric circles of many colors. The fruiting body is stemless shell-like, tough that grows 1–7 cm high. It grows on tree bark and grows all year round. Praveen et al (2012) found the Crude culture filtrate highly inhibitory when compared to methanol extract, as Crude culture filtrate of *S. ostrea* showed highest and lowest percent of inhibition zone against *B. subtilis* (15.9 mm) and *K. pneumonia* (9.1mm). Similarly, Prust et al (2014) studied the antibacterial activities of acetone, ethanol and aqueous extracts of fruiting body of *S. ostrea* and recorded maximum zone of inhibition of 19.17mm, 12.67mm and 10.17mm respectively against *B. subtilis*, and minimum zone of inhibition of 11.33mm, 8.50mm and 7.33mm respectively against *P. aeruginosa*. Water and ethanol extracts were effective against both Gram positive and Gram negative bacteria, and water extract was better than ethanol extract. In water and ethanol extract, inhibition zones were 23.6 and 21.0 mm *(S. aureus)* and 26.3 and 22.3 mm (*P. aeruginosa*), respectively (Ferreira-Silva et al, 2017). Kim et al (2012) found the methanol extracts of *S. ostrea* as potent antioxidant activity in all bioassays tested. Similarly, Cayan et al (2019) recorded the methanol extract of *S. sanguinolentum* (IC_{50}: 34.26 ± 0.31 µg/mL) as higher $ABTS^{\cdot+}$ scavenging activity than α-tocopherol (IC_{50}: 38.51 ± 0.54 µg/mL) and concluded that *Stereum* species could be an antioxidant source.

3 MATERIALS AND METHODS

3.1 Study site

Six plants samples were collected from Darchula district and mushroom was collected from Dolakha.



(Source: http://ncthakur.itgo.com/map15.htm)

Figure 1: Darchula district

3.2 Laboratory

All the chemicals and reagents used during research work were of analytical grade. The entire research work was conducted at Nepal Academy of Science Technology (NAST).

3.3 Plant samples collection and identification

Plants samples were collected according to the medicinal value among the society and with potential drug formulations. Primary sample data collection was done by group discussion and others aspects with elderly local peoples and field observations. The collected samples were photographed and the herbarium was also prepared simultaneously and identification was done at NAST.

3.4 Preparation of plant materials

Collected specimens were cleaned off for removing mud, fungi and other unwanted materials by dusting off or wiping. The samples were then shade dried in room temperature for about 2 weeks to remove all its moisture contents. Dried samples were then grinded in electric mixture grinder to get the powdered form. Thus, powdered samples were then put into boxes and labeled well for further use.

3.5 Preparation of plants extracts and extracts dilution

About 13 grams of powdered sample was weighed and dissolved in 95% methanol prepared with water and filtration was done through Watmann filter paper. And then again 40ml methanol was used for washing out extracts and filtered out. The filtrate solution was then further used for Rota evaporation. The sample product (minimum) obtained in round bottom flask of Rota evaporator was poured into petriplate, it was further left for drying. Well dried plates were now used further.

Now, 20ml of distilled water was placed into dry sample plates and sample was well dissolved in it. The solution was then transferred into separating flask. Now, 40ml of hexane was added into the flask, it was well shaken in order to mix for few minute and then left for about 1-3 hour into a stand (Abu et al, 2017)

Different layers were obtained, lower heavy water extract was collected in a vessel and upper extract was collected into other vessel. The water extract was again put into the flask and 40ml of hexane was added and the same process was repeated. Finally, hexane extract was combined and kept for drying in room temperature.

Now the ethylacetate was taken with water extract and same process was repeated as with hexane (20ml water extract +40ml ethyl acetate). Hexane, ethylacetate and water extract was also put in a separate beaker and left for drying and finally transfer to plate.

In this way, extraction for single extract was done with 3 different solvents (hexane, ethyl acetate and water) on the basis of polarity.

3.6 Determination of Antibacterial activity

Nutrient Agar (NA) plates and NA broth were prepared for the antibacterial tests. About 28 grams of NA powder was carefully weighed and transferred in a conical flask. The content was dissolved in water completely in a conical flask and the final volume was maintained to 1000ml followed by boiling for uniform mixing. The media was sterilized by autoclaving at 15lbs pressure at 121˚C for 15 minutes. This media was allowed to cool for 45-50˚C, the media was then poured in Petri plates and labeled. About 30ml of media was poured in sterile condition using laminar air flow hood. All the plates were left for media solidification. For the preparation of NA broth, screw tight bottles were filled with media and autoclaved.

Luria Bertani (LB) broth was prepared to culture and sub-culture the microorganism prior to antimicrobial tests. About 25gram media powder was weighed and transferred to the conical flask. The content was then dissolved in distilled water and final volume was maintained in 1000ml distilled water and allowed to autoclave. Finally, media was cooled in laminar air flow and dispensed in sterile dry culture tubes. Similarly, 38 grams of the powder was weighed and the final volume was maintained up to 1000ml for Mueller Hinton Agar (MHA). The media was sterilized by autoclaving and mixed carefully before pouring onto sterile petriplate in the laminar hood in an aseptic condition.

The individual pure culture of *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa (P. aeruginosa)*, *Klebsiella pneumoniae* (*K. pneumoniae)*, *Staphylococcus aureus (S. aureus)* was obtained from NAST. Standard bacterial culture inoculums were streaked on NA culture plate to the LB tubes by inoculating loop. The tubes were labeled well and incubated at 37˚c for about 30 min. The turbidity was compared with the blank without culture. The standard was used for the swabbing on the MHA plates to test the anti-microbial effects of the plants extracts.

Media plates and broth tubes were brought to the Laminar Flow (LF) for Antibacterial test. Plates were exposed with Ultraviolet (UV) radiations in order to reduce the chances of the contamination during storage and left for 15 min of exposure. The NA culture plates were taken to room temperature. Now a loop full of culture was taken and dipped into the tube broth and it was left for incubation at 37˚C after that, sterile cotton swab was dipped into the culture tube and was swabbed on the entire surface of MHA plate by carpet culture method and left it for drying. Then make a hole with the borer of size 6mm in plates. positive Streptomycin negative DMSO about 50µl and plant extract were loaded on each hole and left for drying for few minutes in airflow. After 30 min, it was put into an incubator at 37˚C for 24 hours and on next day zone of inhibition was measured.

3.7 Phytochemical analysis for antioxidant

The phenolic content was obtained by mixing 30 µl of different extracts of plant samples with 150µl of FC reagents (1:10 diluted) and then 120µl of 7.5% sodium carbonate was added. The mixture was incubated for 30 min in dark at room temperature. Absorbance of sample measured at765nm against the blank DMSO using ELISA plate method. A calibration curve was constructed using Gallic acid solutions as standard and total phenolic content was expressed in terms of milligrams of Gallic acid per gram of dry weight and standard graph.

The flavonoids content was obtained by mixing 100µl of different extracts of plant samples with 100µl of 2% $AlCl_3$, it was then incubated at room temperature for an hour. The absorbance of the reaction mixture was measured at 450nm against the blank. A

calibration curve was constructed using quercetin solution as standard and results were expressed as equivalents (mg quercetin /gm dried extract).

DPPH assay was used for determining the anti-oxidants activity. The plant extracts were assessed on the basis of the radical scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical scavenging activity. Different concentration of plant extract and standard was prepared in DMSO. 100µl of 0.1mM DPPH was mixed with 100µl of different concentration of extracts; it was then incubated for 30 min in dark at room temperature. The absorbance was taken at 517nm against the blank DMSO and control.

Radical Scavenging Assay (RSA) =[(control-sample)/control] *100%

Then the standard graph was plotted.

The IC_{50} value of different species was compared. The species having the lowest IC_{50} value was considered as to have the best antioxidant property.

3.8 Preparation of Brine Shrimp Bioassay for cytotoxicity

Brine shrimp bioassay is rapid, inexpensive and screening technique to evaluate the toxicity of plant materials using brine larvae *(Artemia salina*). The process was carried out by following standard protocol which involves introducing the newly hatched Brine shrimp Nauplii (*Artemia salina*) to each extract (Amadi et al*,* 2018). The method determines the Lethal concentration (LC_{50}) values (µg/ml) of crude extracts. Plant extracts of Inhibitory Concentration (IC_{50}) values less than 1000 ppm is considered as pharmacologically active. Artificial sea water needed for entire bioassay was freshly prepared by dissolving the following chemicals in distilled water.

Table 1: Composition of artificial sea water

Composition	**Gm per liter**
NaCl	23.50
Na_2So_4	4.00
KCl	0.68
H_3Bo_3	0.02
$MgCl_2.2H_2O$	10.68
$CaCl_2.\ 2H_2O$	1.48
$NaHCO_3$	0.197
Na_2EDTA	0.0003

A pinch of brine shrimp eggs was sprinkled on the beaker filled with artificial sea water and illuminated with table lamp 60 W for 24 hours by adjusting the temperature at 30 ˚c for hatching of the shrimps.

Protocol

1. Sea salt water was prepared
2. Samples were made ready and left it to evaporate for 24 hours
3. A beaker with sea water was taken over which a pinch of eggs was sprinkled and leave it for 24 hours until those eggs hatched out
4. Count the fish up to 10 and put into the measuring cylinder, volume makeup by sea water up to 5ml and then transferred it to the sample vials
5. It was left for 24 hours and numbers of survivors were counted with the help of pipette. Lethal concentration dose required to kill 50% of the shrimps is termed as LC_{50} value. It was determined as follows;

   Here, n is number of replicates (n= 3), x is the log of the concentration of the solution in µg/ml and y is the Probit for an average survivor of all replicates. Thus, logx value for three replicates were log10, log100, log1000 respectively.

   Then we have,

   $$\alpha = (\sum xy - \beta \sum x)/n \quad \ldots\ldots (1)$$

   $$\beta = (\sum xy - \sum x \sum y/n)/(\sum x^2 - (\sum x)^2/n) \quad \ldots\ldots (2)$$

   Now, from Probit regression,

   $$Y = \alpha + \beta x \quad \ldots\ldots (3)$$

   $$X = (Y - \alpha)/\beta \quad \ldots\ldots (4)$$

   Where Y is constant having value 5 for calculating LC_{50} value.

   Thus, LC_{50} =antilog X…… (5)

   In this, brine shrimp bioassay of hexane, ethyl acetate and aqueous extract of different samples were carried out.

3.9 Determination of Antidiabetic assay

In order to determine the antidiabetic potential of plants, α-amylase inhibition assay was carried out using the standard protocol with slight modification. (Jamuna et al, 2012). The undigested starch due to enzyme inhibition was detected through the blue starch iodine complex detected at 630nm.

3.9.1 Preparation of iodine solution (0.0025M)

At first 5gm potassium iodide (KI) was weighed out and 250ml 2% KI solution was prepared in 250ml volumetric flask by dissolving in distilled water. The 100ml of 2.5Mm solution of iodine was prepared by weighing out 0.064gm iodine and dissolving in 2% KI solution in 100ml volumetric flask.

3.9.2 Preparation of phosphate buffer(0.02M)

Disodium hydrogen phosphate (Na_2HPO_4) crystals (0.1174gm) were weighed out, poured in 100ml volumetric flask, dissolve and volume was made up to the label by distilled water

to make 100ml of 0.02M Na_2HPO_4 solution. Similarly, sodium dihydrogen phosphate (NaH_2PO_4) crystals (0.209 gm) were weighed out and 100 ml of 0.02M NaH2PO4 solution was prepared in distilled water. Then 50 ml of 0.02 M Na_2HPO_4 solution and 50ml of 0.02M NaH_2PO_4 solution were mixed and 0.0196gm (6.7mM) NaCl was added. Finally, the pH of the 0.02M phosphate buffer was maintained at 6.9 by adding dilute HCl and dilute NaOH.

3.9.3 Preparation of the starch solution (1% starch)

About 1gm of soluble starch was weighed and dissolved in boiling water (80ml). It was left for cooling and pH was adjusted to 7 and volume makeup was done by adding 20ml d/w.

3.9.4 Preparation of 0.1M HCl Solution

Concentrated HCl available in lab was of 11.66M.tus, 0.9ml conc. HCl was pipette out and 100ml was prepared by adding d/w in a volumetric flask.

3.9.5 Preparation of stock solution of sample extracts and Acarbose

The stock solution of concentration 1000µg/ml to dry extracts were prepared by dissolving 10mg of extracts in 10ml of DMSO. Serial dilution of thus obtained stock solution was done to prepared 4 different concentrations i.e. 20,40,80,160 µg/ml of each extracts. Finally, this solution was prepared in different Eppendorf tubes and stored for the future use. Following the same protocol above, Acarbose solution of different concentration was also prepared and stored.

3.9.6 Measurement of Anti-diabetic activity

About 100µl of substrate (starch solution)was pre-incubated with 50µl of varying concentrations (20,40,80,160µg/ml) of plant extracts and Acarbose separately at 37˚C for 5minutes.thereafter 50µl of α-amylase solution was added to each of them and then again incubated for 15minutes at 37˚C after incubation, the enzymatic reaction was quenched with 200µl of HCl(0.1M) then, 250µl of iodine reagent was added and the absorbance was measured at 630nm.The assay was carried out in triplicates% of enzyme inhibition was calculated by using the following formula.

$$\% \text{ Inhibition} = [1-(Abs_2-Abs_1/Abs_4-Abs_3)] *100$$

Where,

Abs_1=absorbance of an incubated mixture containing plant extract, starch, and amylase.

Abs_2=absorbance of an incubated mixture containing starch and amylase.

Abs_3=absorbance of an incubated mixture containing starch and amylase

Abs_4=absorbance of an incubated solution containing starch only.

The procedure can be made more clear from following schematic representation.

for Abs_1

- 100µl starch + 50µl plant extract/ Acarbose (Incubated at 37˚C for 5 minutes)
- Addition of 50µlof amylase enzyme (Incubated at 37˚C for 15 minutes)
- Termination of reaction by 200µl of HCl (0.1M)
- 250µl Iodine reagent (2.5mM)
- Absorbance at 630nm

for Abs_2

- 100µl starch+50µl plant extract/ Acarbose (Incubated at 37˚C for 5 minutes)
- Addition of 50µlof H_2O (Incubated at 37˚C for 15 minutes)
- Addition of 200µl of H_2O
- 250µl Iodine reagent (2.5mM)
- Absorbance at 630nm

for Abs_3

- 100µl starch+50µl of DMSO (Incubated at 37˚C for 5 minutes)
- Addition of 50µl of amylase enzyme (Incubated at 37˚C for 15 minutes)
- Termination of reaction by 200µl of HCl (0.1M)
- 250µl Iodine reagent (2.5mM)
- Absorbance at 630nm

for Abs_4

- 100µl starch + 50µl DMSO (Incubated at 37˚C for 5 minutes)
- Addition of 50µlof H_2O (Incubated at 37˚C for 15 minutes)
- Addition of 200µl of H_2O
- 250µl Iodine reagent (2.5mM)
- Absorbance at 630nm

The standard graph was plotted by taking the concentration on X-axis and % inhibition on the Y-axis. Based on this graph, the IC_{50} value of each sample was calculated and the vales of the different species were compared. The species having lowest IC_{50} is considered to have the best inhibition property i.e. anti-diabetic property.

4 RESULTS AND DISCUSSION

Total 6 plants species were collected from Darchula and Kathmandu. Percentage extract of these plants in three different solvents showed variation in yield percentage (Table 2). The height yield of extract was found in the water extract (8.46%) followed by ethylacetate (3.69%) and Hexane (1.32%) extract. Among hexane extract percentage yield of *P. emblica* was found to be highest and lowest is of *E. gerardiana* but among water extract yield of *E. gerardiana* was highest and lowest is of *S. ostrea.* Similarly, among Ethylacetate extract yield of *P. emblica* was highest and lowest is of *S. ostrea.*

Table 2: Percentage yield of plants dry extract in three different solvents

S.N.	Name of plant	Hexane extract(%)	Water extract(%)	Ethylacetate extract (%)
1	*Phyllanthus emblica* (Amla)	1.28	7.11	3.69
2	*Terminalia bellirica* (Barro)	1.32	1.43	3.62
3	*Zanthoxylam armatum* (Timur)	0.79	5.14	1.49
4	*Azadirachta indica* (Neem)	0.71	2.42	2.21
5	*Stereum ostrea* (Mushroom)	0.34	1.24	1.12
6	*Ephedra gerardiana* (Ephedra)	0.23	8.46	1.15

4.1 Antibacterial activities of plant extract

A total of 18 extracts from 6 plants species with three different solvents were tested for antibacterial activity against the 4 clinical bacterial species. Gram positive; *Staphylococcus aureus* and three Gram negative; *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhii* bacterial strains were used for antibacterial test. Among 18 extracts examined for Gram Positive S*. aureus*, 33.3% of ethyl acetate extract, 16.6% of water extract and 27.78% of hexane extract showed antibacterial test and remaining 22.22% extract were negative. Whereas, these extract didn't show antibacterial test for *E. coli, S. typhii* and *K. pneumonea.* Gram positive bacteria showed comparatively high spectrum activity and high zone of inhibition than Gram negative bacteria with these plant extracts. This is supported by previous reports that, the antibacterial activity as Gram positive or Gram negative, generally expected that greater number would be active against Gram positive than Gram negative bacteria (Chand and Nair, 2007). Plant extracts are more active against Gram positive than Gram negative bacteria (Rabe and Van Staden 1997, Parekh and Chanda, 2007). These difference may have attributed to the fact that, the cell wall in Gram positive bacteria are of single layered whereas that of Gram negative bacteria are multilayered (Yao et al, 1995). So the passage of the active compound through the Gram negative cell wall may be inhibited thus the microorganisms show variable sensitivity to chemical substances related to different resistant level between strains (Cetin and Gurler,1989).

Antibiotic drug streptomycin was taken as positive control and DMSO was used as negative control against 4 bacterial strain of each extract. Plant extracts of three solvents exhibited antibacterial activity towards Gram Positive *S. aureus*. However, these plants extract exhibit different zone of inhibition for different bacterial strains. The strongest antibacterial activity (50µl load) was observed for the ethylacetate extract of *P. emblica* against *S. aureus* with ZOI 1.77cm, followed by water and hexane extract of its against same bacteria with ZOI of 1.63cm and 1.5cm respectively (Table 3) but Dhale and Mogle (2011) found inhibition for chloroform extract as 9mm for *S. aureus*. A mixture of triterpene glycosides and six antibacterial compounds were isolated from ethyl acetate extract of the stem bark of *Phyllanthus emblica* (Khoa et al, 2020) which might had showed ethylacetate extract of *P. emblica* as strongest antibacterial extract. Sharma (2017) also recorded ZOI 10mm for methanol extract. This variation is due to organic nature of chloroform and methanol. These organic solvents can easily dissolve phenolic and flavonoid contents. Al-Gbouri, and Hamzah (2018) performed antibacterial activity of ethanol extract of *P. emblica* against *E. coli*, *S. aureus* and found ZOI as 2.7cm, 2.5cm at 20mg/ml, these high ZOI than present finding may be due to organic nature of solvent as methanol and hexane. Plants are rich sources of important phytoconstituents which has been proved from various previous studies. Each constituent has its own effect against microorganisms; tannins and flavonoids were known to possess antimicrobial potential against bacteria and fungi (Adegoke et al, 2009 and Dinesh et al, 2016).

4.1.1 Zone of inhibition of plant extract for *Staphylococcus aureus*

Gram positive, *S. aureus* showed variation in Zone of inhibition for different plant extract in 3 different solvents with 1.87cm for positive control in streptomycin antibiotic (Table 4). All three solvent extracts of *P. emblica*, *E. gerardiana and T. bellirica* showed zone of inhibition. Similarly, Hexane and ethylacetate extract both of *Z. armatum, S. ostrea* exhibit zone of inhibition. *Azadirachta indica* extracts used in this study had shown an antibacterial effect on *S. aureus* only, while the *E. coli*, *K. pneumonea* and *S. typhii* were not affected by any of the used extracts. This might be due to the fact that *E. coli* can alter their genetic makeup with astonishing rapidity. In general, gram negative bacteria show resistance to antibiotics because of their cell wall. Resistant bacteria change their cell walls slightly, so the antibiotics cannot attach or they produce enzymes to disable the antibiotics, so the *E. coli* might have done the same and consequently, *A. indica* (neem) extracts did not show any effect on it. Present finding are different to those obtained during a study carried out by Gajendrasinh et al (2012) whereby *E.coli* was the most susceptible bacterium to aqueous and ethanol extracts of *Azadirachta indica* but matched with Dhami et al (2019) who found methanolic and acetone extracts of *A. indica* were more effective against the bacteria compared to that of aqueous extract, he also found the Gram-positive bacterial strains were found more sensitive than the Gram-negative

one among all extracts (Dhami et al, 2019). Similarly, Kirtikar and Basu (1975) said that the active ingredients are slightly soluble in water and freely soluble in organic solvents such as alcohols, these ingredients include: azardirachtin, 1-maliantriol, salannin, nimbin, nimbdin and others.

Zanthoxylam armatum showed inhibition in hexane and ethylacetate extract with 0.9 and 1.5 cm respectively as aqueous extract didn't show any inhibition but Ibrar et al (2012) didn't found ZOI in hexane extract as he recorded 11.67mm and 17mm for *E. coli* respectively. Similarly, Guleria et al (2013) also demonstrate *Z. armatum* could be used as a resource antimicrobial compounds which may find applications in food and pesticide industries. presence of sterols, triterpenes, volatile oils, coumarins, alkaloids, flavonoids, flavonic glycosides, saponins and tannins (Joshi and Gyawali, 2012) has made it antibacterial, cytotoxic, antioxidant activity (Mukhtar and Kalsi, 2018). All three extract of *Terminalia bellirica* showed inhibition against *S. aureus* as Panda (2020) recorded presence of Saponin, Phenolic compounds, Flavonoids, Tannins like phytochemicals in hexane, aqueous and ethylacetate extract.

Table 3: Zone of inhibition (Diameter in cm) of plant extract (20 mg/ml) for *Staphylococcus aureus*

Plant extracts	*Staphylococcus aureus*			
	Hexane	Water	Ethylacetate	Streptomycin
Phyllanthus emblica	1.5	1.63	1.77	1.87
Terminalia bellirica	1.13	1.37	1.6	1.87
Zanthoxylam armatum	0.9	-	1.5	1.87
Azadirachta indica	-	-	0.97	1.87
Stereum ostrea	0.8	-	1.6	1.87
Ephedra gerardiana	1	1.3	1.47	1.87

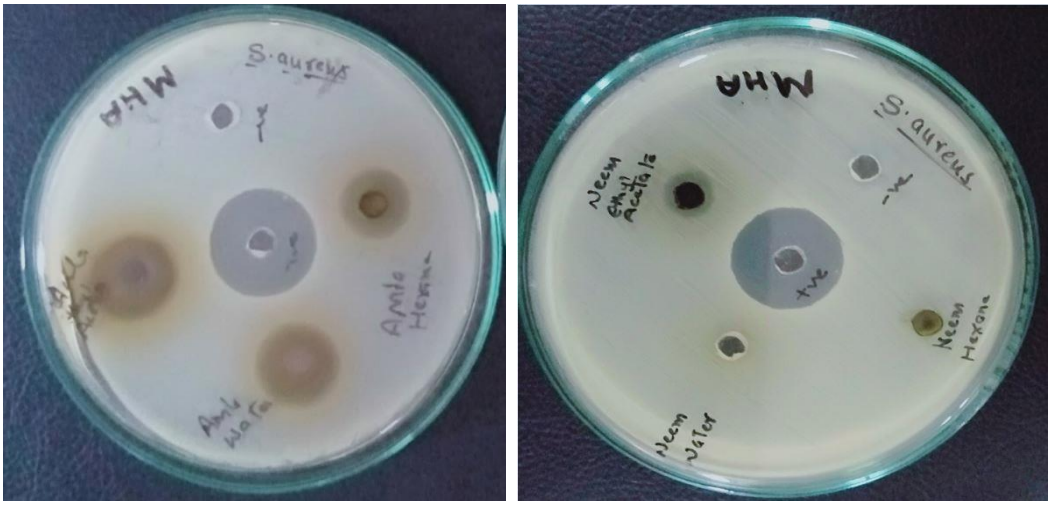


Figure 2: Zone of inhibition by *P. emblica* (Amla) in all extract and *A. indica(*Neem*)* in ethylacetate extract

4.1.2 Zone of inhibition of different plants extract for *Escherichia coli*

Plant extract didn't show inhibition zone for *Escherichia coli*

Table 4: Zone of inhibition (Diameter in cm) of plant extract (20 mg/ml) for *E. coli*

Plant extracts	*Staphylococcus aureus*			
	Hexane	Water	Ethylacetate	Streptomycin
Phyllanthus emblica	-	-	-	1.87
Terminalia bellirica	-		-	1.87
Zanthoxylam armatum	-	-	-	1.87
Azadirachta indica	-	-	-	1.87
Stereum ostrea	-	-	-	1.87
Ephedra gerardiana	-	-	-	1.87

Figure 3: Zone of inhibition by *K. penumoneae*, *S. aureus, E. coli* and *S. typhii* in *A. indica(*Neem*)* extracts.

4.1.3 Zone of inhibition of different plants extract for *Klebsiella penumoneae*

Plant extracts didn't show antibacterial action against *K. penumoneae*

Table 5: Zone of inhibition (Diameter in cm) of plant extract (20 mg/ml) for *K. penumoneae*

Plant extracts	*K. penumoneae*			
	Hexane	Water	Ethylacetate	Streptomycin
Phyllanthus emblica	-	-	-	1.87
Terminalia bellirica	-	-	-	1.87
Zanthoxylam armatum	-	-	-	1.87
Azadirachta indica	-	-	-	1.87
Stereum ostrea	-	-	-	1.87
Ephedra gerardiana	-	-	-	1.87

4.1.4 Zone of inhibition of different plants extract for *Salmonella typhii*

Plant extracts didn't show antibacterial action against *S. typhii*

Table 6: Zone of inhibition (Diameter in cm) of plant extract (20 mg/ml) for *S. typhii*

Plant extracts	*S. typhii*			
	Hexane	Water	Ethylacetate	Streptomycin
Phyllanthus emblica	-	-	-	1.87
Terminalia bellirica	-	-	-	1.87
Zanthoxylam armatum	-	-	-	1.87
Azadirachta indica	-	-	-	1.87
Stereum ostrea	-	-	-	1.87
Ephedra gerardiana	-	-	-	1.87

4.2 Cytotoxicity of plant extracts on Brine shrimp

The maximum mortalities of the brine shrimp took place at the concentration of 1000µg/ml. Highest LC_{50} was 217.04 µg/ml which is showed by all 3 extract of *S. ostrea*; Hexane and ethylacetate extract of *Z. armatum*; water and hexane extract of *E. gerardiana*. The Basidiomycetes *Stereum* genus has been a source of such bioactive compounds as it content sesquiterpenoids, polyketides, vibralactone, triterpenoids, sterols, carboxylic acids and saccharides and most of them showed cytotoxic effect (Tian et al, 2020) thus it showed lethality in all solvents. Hexane extract of *A. indica* showed the least (118.08 µg/ml) LC_{50} thus found *to* be most biologically active due to high toxicity toward the brine shrimp. Similarly, in brine-shrimp bioassay, ethyl acetate extract of *Z. armatum* showed proper bioactivity lethality (Joshi and Gyawali, 2012) matched with present finding (Table 4). Amongst the extracts, the aqueous extracts of *T. bellirica* was most active at higher dose of 500microgram/ml (Panda, 2020) also concede with present finding as there is dose dependent lethality. Rahman et al (2008) found LC_{50} of 3.62 ± 1.35

µg/ml for *T. bellirica* and Ved et al (2010) found LC_{50} of 107 µg/ml for *Terminalia* species in ethanol solvent which differed from present finding as lowest LC_{50} of 191.08 µg/ml observed for ethylacetate extract.

Table 7: Cytotoxicity for ethylacetate, hexane and aqueous extracts of medicinal plants of Nepal, 2020

S.N	Sample plants	Ethylacetate LC_{50} (µg/ml)	Aqueous LC_{50} (µg/ml)	Hexane LC_{50} (µg/ml)
1	*Phyllanthus emblica*	197.01	197.01	206.83
2	*Terminalia bellirica*	191.08	206.83	206.83
3	*Azadirachta indica*	133.63	206.83	118.08
4	*Zanthoxylam armatum*	217.04	197.01	217.04
5	*Ephedra gerardiana*	206.83	217.04	217.04
6	*Stereum ostrea*	217.04	217.04	217.04

4.3 Antioxidative property of plant extracts

The DPPH free radical assay of standard ascorbic acid showed the linear regression increase in the percentage free radical scavenging assay (RSA%) against the concentration gradients. Inhibitory concentration (IC_{50}) of ascorbic acid was found to be 57.75µg/ml with 97.84% (R^2= 0.9784) confidence interval.

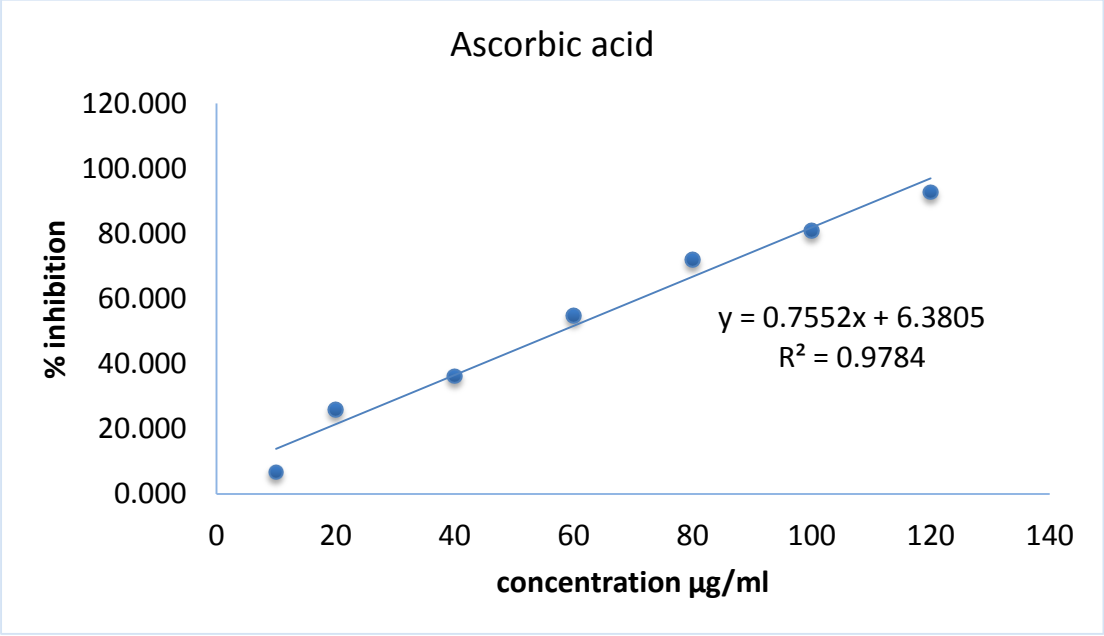


Figure 4: Free Radical Scavenging Assay (RSA %) percentage v_s concentration curve for Ascorbic acid

The IC_{50} value ranged from 14.76 to 76.92 µg/ml for ethylacetate extract, 16.06 to 67.44 µg/ml for aqueous and 18.03 to 70.38 µg/ml for hexane extracts of different 18 extracts (Table 5). The lower IC_{50} value indicated better antioxidants. Inhibitory concentration (IC_{50}) of all 3 extracts of *P. emblica* was highest i.e. 76.92 µg/ml for ethylacetate, 67.44 µg/ml water and 70.38 µg/ml hexane extract. Charoenteeraboon et al (2010) found IC_{50} of 51.3 µg/ml for aqueous extract which is low than present finding but Ibrahim et al (2017) recorded IC_{50} value of 263.53 mg/mL and 143.56 mg/mL for hexane and ethyl acetate fractions respectively of *Phyllanthus* spp which is high as compared to present finding.

Ethylacetate extract of *S. ostrea* has lowest (14.76 µg/ml) $IC_{50,}$ followed by aqueous and hexane extract *A. indica*. Thus, ethylacetate extract of *S. ostrea* was found to be better antioxidant followed by aqueous and hexane extract of *A. indica*.

Akata (2020) determined antioxidant using Rel assay kit and found TAS value for ethanol extract to be 4.464mMol/L. Methanol extract of *Stereum spp.* Showed higher $ABTS^{\cdot+}$ scavenging activity i.e. (IC_{50}: 34.26 ± 0.31 µg/mL), which suggest that *Stereum* species could be an antioxidant source in pharmaceutic, food, and cosmetics industries. (Cayan et al, 2019).

Table 8: Inhibitory concentration (IC_{50} µg/ml) of ethylacetate, aqueous and hexane extracts of different plant species

S.N	Sample plants	Ethylacetate	Aqueous	Hexane
		IC_{50} (µg/ml)	IC_{50} (µg/ml)	IC_{50} (µg/ml)
1	*Phyllanthus emblica*	76.92	67.44	70.38
2	*Terminalia bellirica*	60.39	60.79	49.18
3	*Azadirachta indica*	20.63	16.06	18.03
4	*Zanthoxylam armatum*	26.62	32.87	30.38
5	*Ephedra gerardiana*	55.97	64.51	27.89
6	*Stereum ostrea*	14.76	37.84	31.12

4.3.1 Total phenolic content (TPC) of plants extract

The standard equation generated for TPC was Y= 0.0089X + 0.0342 and R^2= 0.9835.

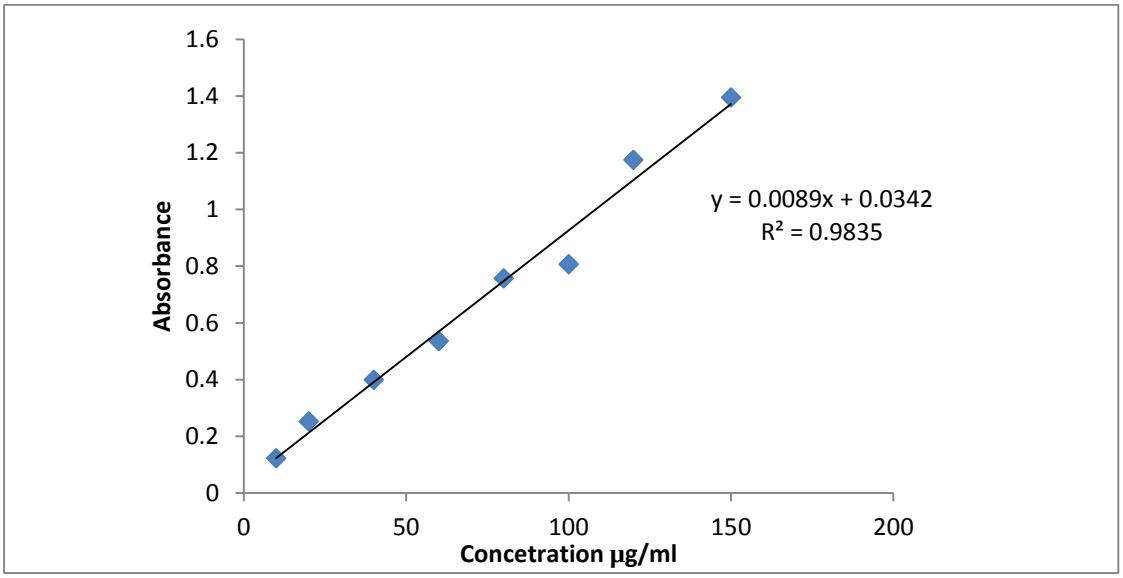


Figure 5: Standard graph of Gallic acid for total phenolic content

The phenolic content of *T. bellirica* was found to be highest among 6 plants samples for all 3 solvents types and lowest of *A. indica* for both Hexane and ethylacetate extract; *S. ostrea* for water extract (Figure 2).

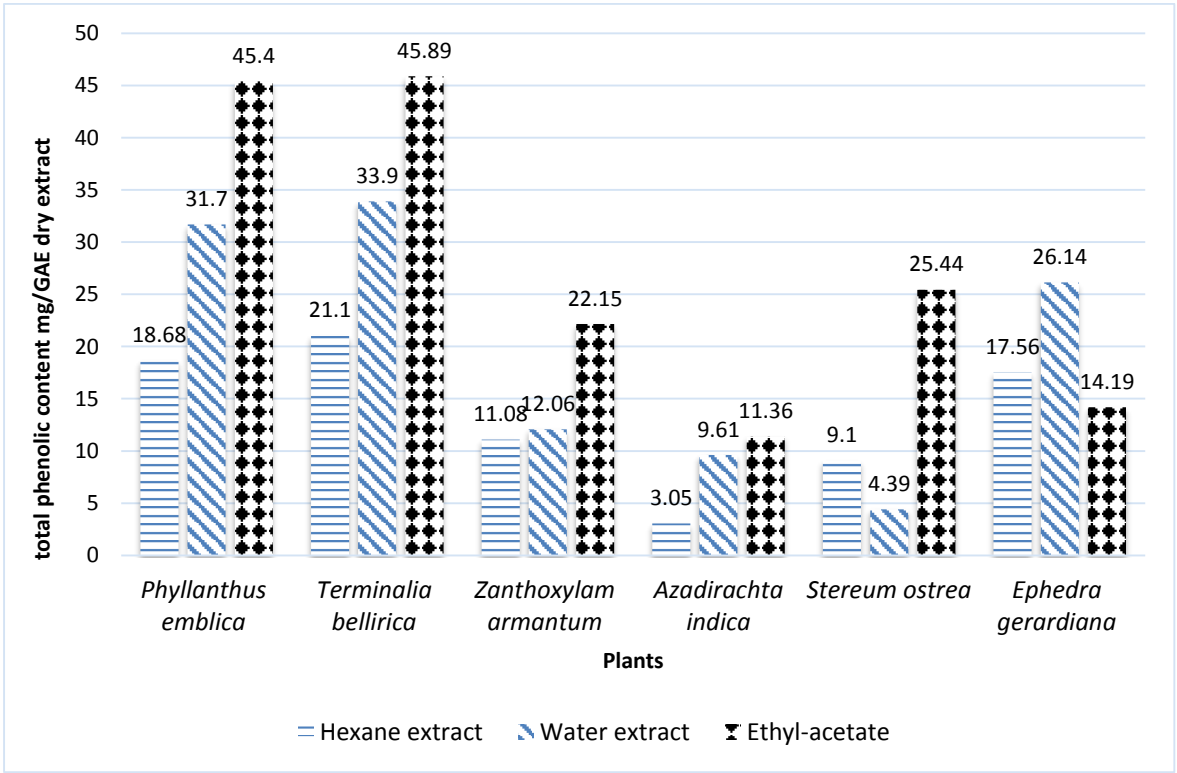


Figure 6: Total Phenolic content for different plant extract in three different solvents

4.3.2 Total Flavonoid Content (TFC) of plants extract

The standard equation generated for TFC was Y= 0.0101X + 0.9861 and R^2= 0.9861

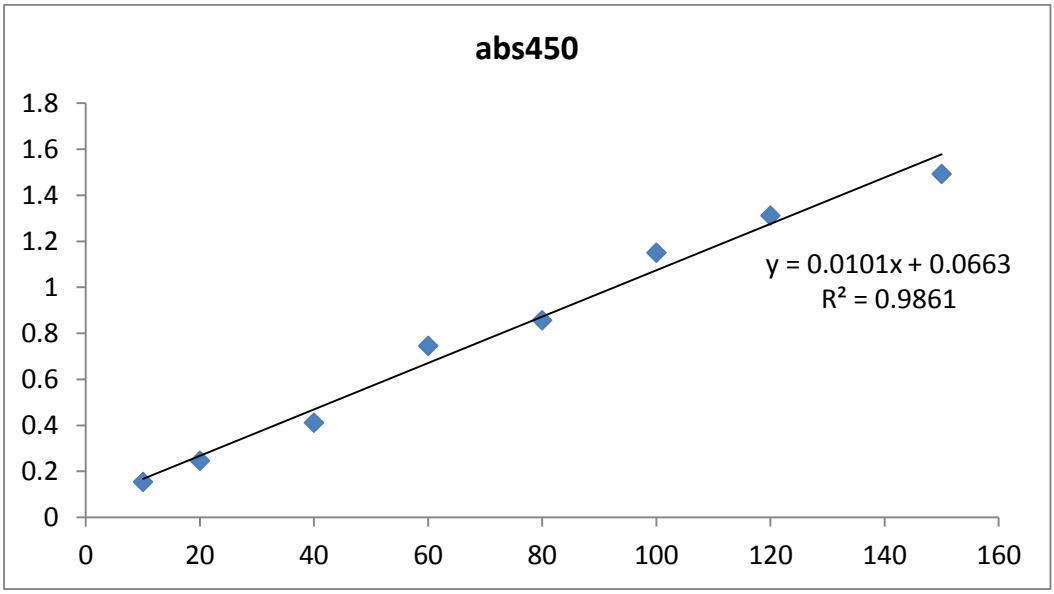


Figure 7: standard equation for total flavonoid content (TFC) generated for plant extracts

The flavonoid content (TFC) of hexane extract for *Z. armatum* was found to be highest and lowest for *E*. *gerardiana*. Similarly, the TFC of water extract was found highest for *A. indica* and lowest for *P. emblica.* Likewise, TFC of ethyl acetate extract for *A. indica* was found highest and that for *T. bellirica* to be lowest (Figure 4). The flavonoids are under the group of polyphenols so it was mostly considered that phenolic values are more than the flavonoids (Figure 6 and Figure 8) but it is not the compulsion as in some cases we can find flavonoids are more than phenols. It may be influenced by nature of solvent, methods, reagents, pH temperature, extraction time, particle size, etc. All phenolic could not be estimated by single extraction method due to complexity of compounds (Katsube et al, 2004). The contents of Phenolic compounds measured by HPLC after enzymatic hydrolysis, were found to be different from those measured by Folin-Ciocalteu method (FC method). The amount of polyphenol was dependent on extraction method the samples for HPLC when subjected to enzymatic hydrolysis which in contrast to methanol extraction resulted in specific disruption of linkages and deglycosylation of phenolic compound

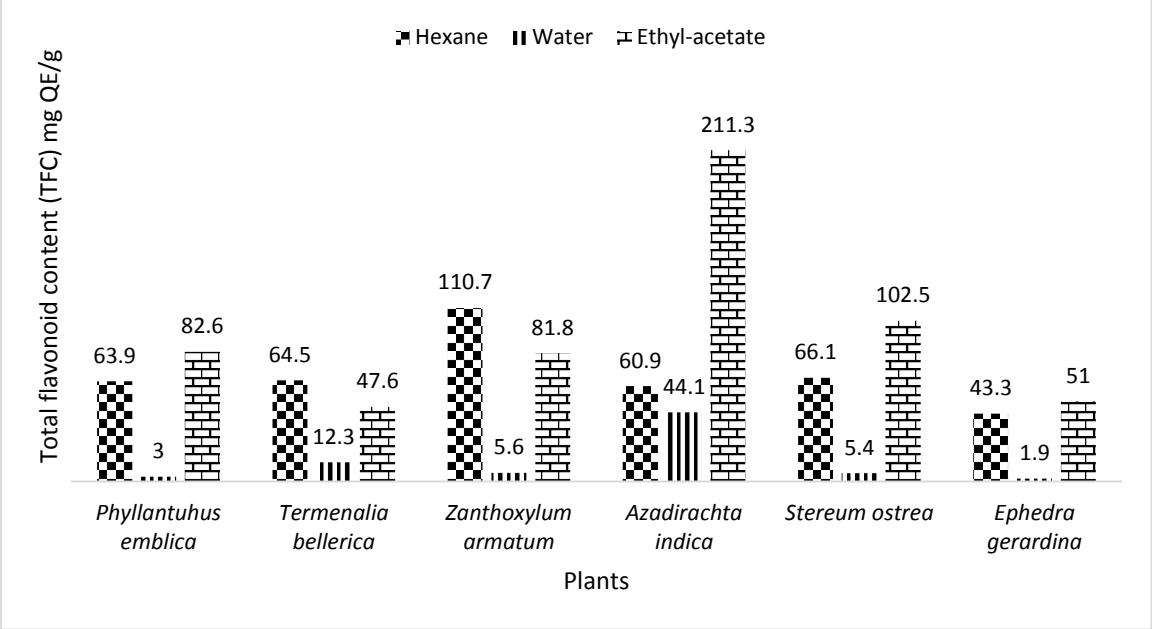


Figure 8: Total flavonoid contents (TFC) of various plant extract in three different solvents

4.4 Alpha amylase inhibition activity or anti-diabetic activity of plants extracts

Antidiabetic activity of different extracts of the plants were calculated using Acarbose as standard and IC_{50} was found to be 86.79 µg/ml. The percentage inhibition of enzyme increases with respect to the concentration of Acarbose as it ranges from 64% to 83%. i.e. concentration dependent increase in the percentage inhibition.

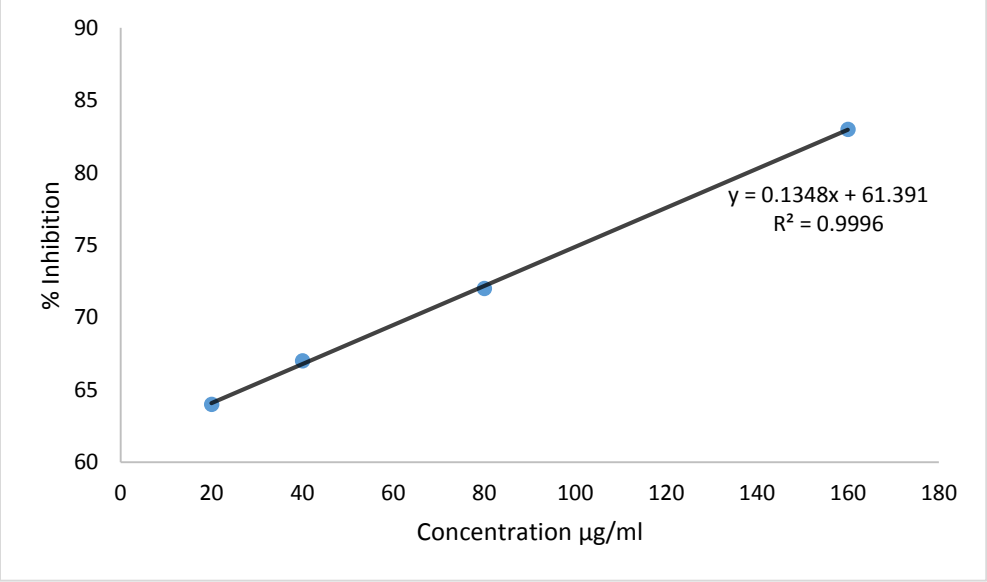


Figure 9: Alpha-amylase inhibition percentage of Acarbose in various concentration

Acarbose, the standard drug had % inhibition of 83% at 160µg/ml. Inhibitory concentration(IC_{50}) ranges from 138.67 µg/ml to 1344.36 µg/ml. The lowest inhibition was of Hexane extract of *E. gerardiana* and highest of Aqueous extract of *Z. armatum*.

previous study also showed *Z. armatum* as good alpha amylase inhibitor. Rynjah et al (2018) found that the IC_{50} of aqueous leaf extract was 7.40 mg/ml while Acarbose, 4.42 mg/ml. Inhibitory concentration (IC_{50}) of ethanol extract of *Z. armatum* from china was found to be 1.185 ± 0.13 mg/ml (Zhang et al, 2015). During present study all 3 extracts of *Z. armatum* showed highest IC_{50}. Ethylacetate and hexane extract of *E. gerardiana* has lowest IC_{50} (Table 6). Similarly, aqueous extract of T. bellirica showed lowest Inhibition. The lowest IC_{50} showed higher anti-diabetic properties thus, hexane extract of *E. gerardiana* was found to be best anti-diabetic followed by aqueous extract of *T. bellirica*.

Table 9: Comparison of α-amylase inhibitory activities (IC_{50} of three extracts of 6 different medicinal plants.

S.N	Sample plants	Ethyl acetate IC_{50} (µg/ml)	Aqueous extract IC_{50} (µg/ml)	Hexane extract IC_{50} (µg/ml)
1	*P. emblica* (Amla)	810.84	655.55	235.29
2	*T. bellirica* (Barro)	650.05	180.66	627.11
3	*A. indica* (Neem)	844.78	679.29	164.50
4	*Z. armatum* (Timur)	1157.94	1344.36	1150.40
5	*E. gerardiana* (Ephedra)	655.96	595.70	138.67
6	*S. ostrea* (Mushroom)	788.25	1086.94	489.85

Table 10: Alpha (α)-amylase inhibition % by different concentration of plant extract in three solvents.

Concentration µg/ml	Hexane extract						Ethylacetate extract						Water extract					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
20	79.64	83.8	93.82	73.45	72.71	86.14	91.36	85.07	87.53	90.83	86.14	92.64	89.02	81.02	90.09	90.09	87.21	93.07
40	87.1	87.1	94.99	86.35	77.93	91.36	93.07	87.53	88.7	92.75	86.67	93.5	93.39	84.65	91.79	92.11	87.85	93.6
80	94.88	89.55	95.74	87.21	88.59	95.1	96.59	88.91	89.87	94.78	90.94	97.65	94.88	87.53	92.64	95.2	88.91	95.74
160	99.36	92.22	99.25	98.51	95.2	97.97	98.61	93.07	92.22	97.87	93.71	99.89	98.61	92.11	94.67	98.51	95.42	98.4

A= *P. emblica*, B= *T. bellirica*, C= *A. indica*, D= *Z. armatum*, E= *E. gerardiana*, F= *S. ostrea*

Among 18 extract of the 6 different plants, ethyl acetate extract of *S. ostrea* had highest %inhibition i.e.99.89% with IC_{50} of 788.25µg/ml followed by same extract of *P. emblica* (Table 7) whereas, hexane extract of *E. gerardiana* had lowest inhibition i.e. 72.71% with IC_{50} of 138.67 µg/ml. The alpha amylase inhibitory action was due to the availability of tannins and terpenoids as they have ability to bind carbohydrates and proteins (Khan et al, 2014 and Poongunran et al, 2015) beside these Phenolic and flavonoid contents also effectively inhibit alpha-amylase activity based on the ability to form quinone with the 4-oxo-pyrane structure of the enzyme through the hydroxyl group at C-3 and C-4 of ring B (Sim et al, 2010). Inhibitory action of extracts increases with increased concentration or dose dependent. Similarly, in present study within Hexane and water extract *P. emblica* showed high inhibition whereas in ethylacetate *S. ostrea* showed high inhibition i.e. 99.36 %, 98.61% and 99.89% respectively. Similarly, *P. emblica* fruit (73.9%) and whole plant (53%) also showed significant amylase inhibitory effects at 1mg/ml (Poongunran et al 2015). Again, new study done by Singh and Kaur (2015) found that the ethanol extract of *P. emblica* leaves exhibited 61.12% inhibition against alpha-amylase at pH 7 and temperature 40˚C. The lowest inhibition within ethylacetate and aqueous extract during present study was showed by *T. bellirica* i.e. 85.07%, 81.02% respectively whereas, within hexane extract it was showed by *E. gerardiana* i.e. 72.71%.

5 SUMMARY

Six medicinal plants were used for the study of which four (*Phyllanthus emblica*, *Zanthoxylum armatum*, *Terminalia bellirica*, *Azadirachta indica*) were collected from Darchula and two samples (*Ephedra gerardiana* and *Stereum ostrea*) were provided by NAST, Khumaltar lab.

Plant extraction were done in ethylacetate, hexane and water in which maximum high yield of extract was found in the water extract (8.46%) followed by ethylacetate (3.69%) and hexane (1.32%) extract.

Antibacterial activity against the 4 clinical bacterial species. Gram positive; *S. aureus* and three Gram negative; *E. coli*, *K. pneumonia*, *S. typhii* bacterial strains were used for antibacterial test. Among 18 extracts studied 33.3% of ethylacetate extract, 16.6% of water extract and 27.78% of hexane extract indicated antibacterial test for Gram Positive S*. aureus* and remaining 22.22% extract were negative. However, these extract didn't display antibacterial test for *E. coli, S. typhii* and *K. pneumonea.*

Cytotoxicity of plant extract on brine shrimp was performed by brine shrimp bioassay and LC_{50} was also calculated. Maximum mortalities of the brine shrimp took place at the concentration of 1000µg/ml. Highest LC_{50} was 217.04 µg/ml which is showed by all 3 extract of *S. ostrea*; Hexane and ethylacetate extract of *Z. armatum*; water and hexane extract of *E. gerardiana*. In ethylacetate extract *A. indica* has lowest LC50 and highest is of *S. ostrea*. Whereas in aqueous both *P. emblica* and *T*. *bellirica* had lowest LC50 and highest is of *E. gerardiana* and *S. ostrea*. Similarly, in Hexane extract highest is of *Z. armatum*, *E. gerardiana* and S. ostrea and lowest is of *A. indica*.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were recorded by absorbance method in ELISA plate and calculated by using standard linear equation. The phenolic content of *T. bellirica* was found to be highest among 6 plants for all 3 solvents types and lowest of *A. indica* for both hexane and ethylacetate extract; *S. ostrea* for water extract. The flavonoid content (TFC) of hexane extract for *Z. armatum* was found to be highest and lowest for *E*. *gerardiana*. DPPH free radical scavenging assay (RSA%) against the concentration gradients was performed and IC50 was calculated. Inhibitory concentration (IC_{50}) of all 3 extracts of *P. emblica* was highest i.e. 76.92 µg/ml for ethylacetate, 67.44 µg/ml water and 70.38 µg/ml hexane extract. Whereas, ethylacetate extract of *S. ostrea* has lowest (14.76 µg/ml) $IC_{50,}$ followed by aqueous and hexane extract *A. indica.*

Antidiabetic activity was done by alpha amylase inhibition assay and recorded highest %inhibition (99.89%) in ethylacetate extract of *S. ostrea* with IC_{50} of 788.25µg/ml followed by same extract of *P. emblica* whereas, hexane extract of *E. gerardiana* had lowest inhibition i.e. 72.71% with IC_{50} of 138.67 µg/ml

6 CONCLUSION

In alpha amylase inhibition assay, highest %inhibition (99.89%) was shown by ethylacetate extract of *S. ostrea* with IC_{50} of 788.25µg/ml followed by same extract of *P. emblica.* Hexane extract of *E. gerardiana* had lowest inhibition i.e. 72.71% with IC_{50} of 138.67 µg/ml. DPPH assay shows ethylacetate extract of *S. ostrea* has lowest inhibitory concentration (IC_{50}) of 14.76 µg/ml. In brine shrimp lethality, ethylacetate extract *A. indica* has lowest LC50. All plant extract indicated antibacterial test for Gram Positive S*. aureus* with largest ZOI of 1.77cm by ethylacetate extract of *Phyllanthus emblica*. However, these extract didn't display antibacterial test for *E. coli, S. typhii* and *K. pneumonea*.

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7 APPENDICES

Appendix 1:

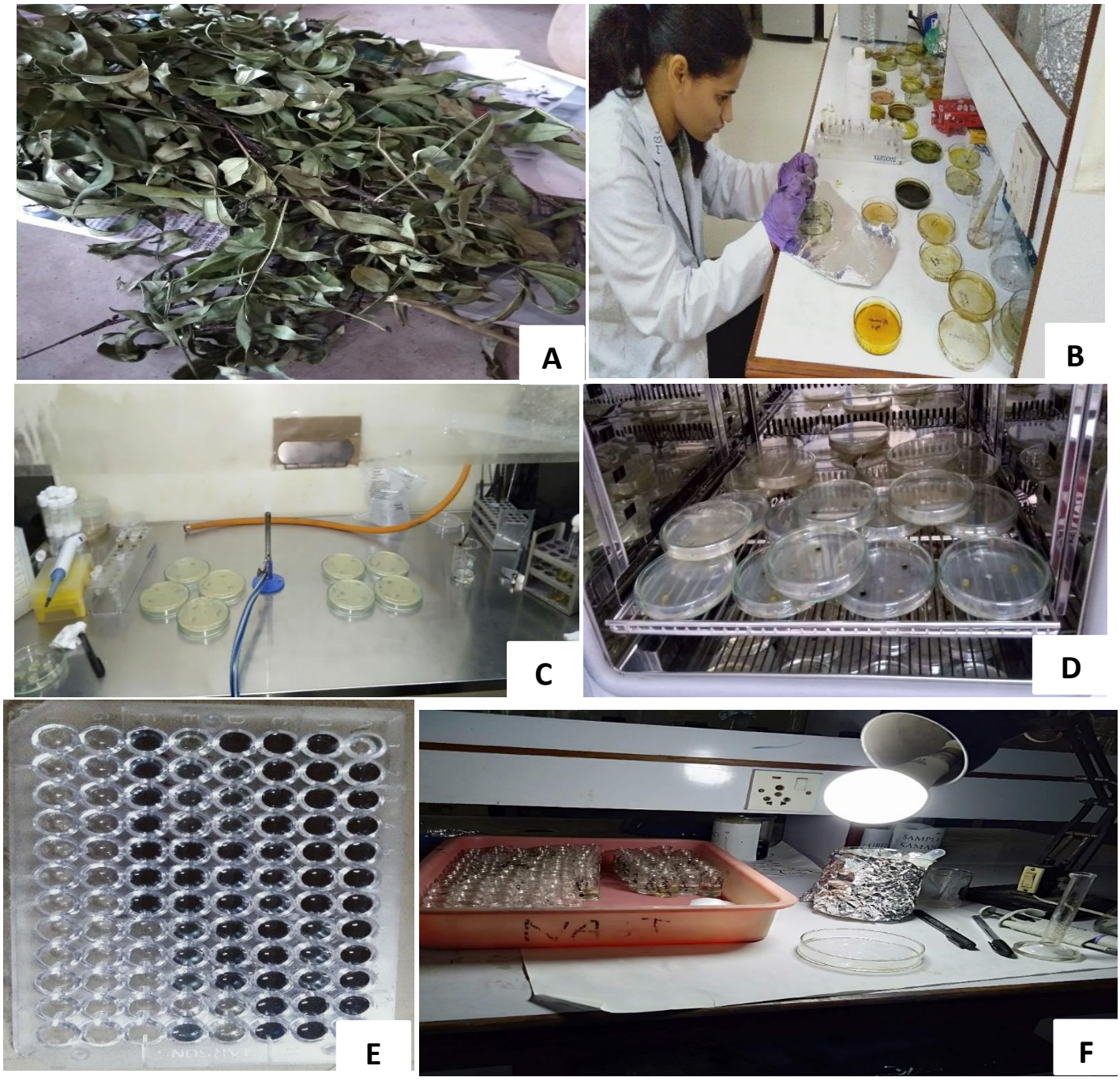

Figure 1: A: Sample extraction,B: Plant extraction, C: Laminar air flow, D:Incubator, E: ELISA plate, F: cytotoxicity

Appendix 2:

TFC of plant extract in different solvent

S.N	Name of plant	Hexane extract	Water extract	Ethylacetate
1	*Phyllanthus emblica* (Amla)	18.68	31.7	45.4
2	*Terminalia bellirica* (Barro)	21.1	33.9	45.89
3	*Zanthoxylam armatum* (Timur)	11.08	12.06	22.15
4	*Azadirachta indica* (Neem)	3.05	9.61	11.36
5	*Stereum ostrea* (Mushroom)	9.1	4.39	25.44
6	*Ephedra gerardiana* (Ephedra)	17.56	26.14	14.19

Antioxidant of standard ascorbic acid

Control	Absorbance	Blank	control (AC)	actual absorbance of sample=absorbance-blank (AS)		% RSA=(AC-AS)/AC*100
Ascorbic acid	0.645	0.063	0.624	0.582		6.731
	0.525	0.063	0.624	0.462		25.962
	0.461	0.063	0.624	0.398		36.218
	0.345	0.063	0.624	0.282		54.808
	0.238	0.063	0.624	0.175		71.955
	0.182	0.063	0.624	0.119		80.929
	0.108	0.063	0.624	0.045		92.788

Antidiabetic Ic_{50} of Hexane extracts of plants

Sample	abs4	abs3	abs2	abs1	Concentration	%inhibition	IC_{50}
Amla	2.384	1.446	3.192	3.001	20	79.64	235.29
	2.384	1.446	3.246	3.125	40	87.10	
	2.384	1.446	3.324	3.276	80	94.88	
	2.384	1.446	3.384	3.378	160	99.36	
Barro	2.384	1.446	3.147	2.995	20	83.80	627.11
	2.384	1.446	3.225	3.105	40	87.21	
	2.384	1.446	3.294	3.196	80	89.55	
	2.384	1.446	3.377	3.304	160	92.22	
Timur	2.384	1.446	2.943	2.885	20	93.82	1150.4
	2.384	1.446	3.136	3.089	40	94.99	
	2.384	1.446	3.158	3.118	80	95.74	
	2.384	1.446	3.172	3.165	160	99.25	
Neem	2.384	1.446	3.036	2.787	20	73.45	164.5
	2.384	1.446	3.136	3.008	40	86.35	
	2.384	1.446	3.238	3.118	80	87.21	

	2.384	1.446	3.278	3.264	160	98.51	
Ephedra	2.384	1.446	2.441	2.185	20	72.71	138.67
	2.384	1.446	2.58	2.373	40	77.93	
	2.384	1.446	2.65	2.543	80	88.59	
	2.384	1.446	2.762	2.717	160	95.20	
S. ostrea	2.384	1.446	2.484	2.354	20	86.14	489.854
	2.384	1.446	2.581	2.5	40	91.36	
	2.384	1.446	2.629	2.583	80	95.10	
	2.384	1.446	2.665	2.646	160	97.97	

Antidiabetic Ic50 of water extracts of plants

Sample	abs4	abs3	abs2	abs1	Concentration	%inhibition	IC50
Amla	2.384	1.446	3.204	3.101	20	89.02	655.548
	2.384	1.446	3.249	3.187	40	93.39	
	2.384	1.446	3.29	3.242	80	94.88	
	2.384	1.446	3.377	3.364	160	98.61	
Barro	2.384	1.446	2.194	2.016	20	81.02	180.66
	2.384	1.446	2.256	2.112	40	84.65	
	2.384	1.446	2.366	2.249	80	87.53	
	2.384	1.446	2.385	2.311	160	92.11	
Timur	2.384	1.446	3.127	3.034	20	90.09	1344.36
	2.384	1.446	3.198	3.121	40	91.79	
	2.384	1.446	3.39	3.321	80	92.64	
	2.384	1.446	3.43	3.38	160	94.67	
Neem	2.384	1.446	2.978	2.885	20	90.09	679.29
	2.384	1.446	3.252	3.178	40	92.11	
	2.384	1.446	3.269	3.224	80	95.20	
	2.384	1.446	3.329	3.315	160	98.51	
Ephedra	2.384	1.446	2.542	2.422	20	87.21	595.7
	2.384	1.446	2.554	2.44	40	87.85	
	2.384	1.446	2.579	2.475	80	88.91	
	2.384	1.446	2.641	2.598	160	95.42	
Stereum	2.384	1.446	2.488	2.423	20	93.07	1086.94
	2.384	1.446	2.54	2.48	40	93.60	
	2.384	1.446	2.692	2.652	80	95.74	
	2.384	1.446	2.759	2.744	160	98.40	

Antidiabetic IC_{50} of ethylacetate extracts of plants

Sample	abs4	abs3	abs2	abs1	Concentration	%inhibition	IC_{50}
Amla	2.384	1.446	3.262	3.181	20	91.36	810.84
	2.384	1.446	3.294	3.229	40	93.07	
	2.384	1.446	3.3	3.268	80	96.59	
	2.384	1.446	3.305	3.292	160	98.61	
Barro	2.384	1.446	2.234	2.094	20	85.07	650.05
	2.384	1.446	2.304	2.187	40	87.53	
	2.384	1.446	2.347	2.243	80	88.91	
	2.384	1.446	2.384	2.319	160	93.07	
Timur	2.384	1.446	3.109	2.992	20	87.53	1157.94
	2.384	1.446	3.157	3.051	40	88.70	
	2.384	1.446	3.268	3.173	80	89.87	
	2.384	1.446	3.324	3.251	160	92.22	
Neem	2.384	1.446	3.026	2.94	20	90.83	844.78
	2.384	1.446	3.196	3.128	40	92.75	
	2.384	1.446	3.247	3.198	80	94.78	
	2.384	1.446	3.261	3.241	160	97.87	
Ephedra	2.384	1.446	2.438	2.308	20	86.14	622.96
	2.384	1.446	2.49	2.365	40	86.67	
	2.384	1.446	2.57	2.485	80	90.94	
	2.384	1.446	2.653	2.594	160	93.71	
Stereum	2.384	1.446	2.548	2.479	20	92.64	788.25
	2.384	1.446	2.617	2.556	40	93.50	
	2.384	1.446	2.664	2.642	80	97.65	
	2.384	1.446	2.719	2.718	160	99.89	

Percentage inhibition of standard Acarbose for antidiabetic inhibition

standard	Concentration	Abs4	abs3	abs2	abs1	%inhibition	IC_{50}µg/ml
Acarbose	20	2.384	1.446	1.413	1.078	64	86.79
	40	2.384	1.446	1.437	1.128	67	
	80	2.384	1.446	1.459	1.192	72	
	160	2.384	1.446	1.485	1.325	83	

Cytotoxicity of Ethylacetate extract on brine shrimp

Plants	concentration (c)	x=log(c)	x2	y (survivor)	xy	Σx	Σy	Σx2	Σxy	β=(Σxy-ΣxΣy)/Σx2-(Σx)2	α=Σxy-βΣx/n	Y=5 constant	X=(Y-α)/β	lc50=antilog X
Amla	10	1	1	10	10	6	28	14	54	-44.14	106.29	5.00	2.29	197.01
	100	2	4	10	20									
	1000	3	9	8	24									
Barro	10	1	1	10	10	6	27	14	52	-43.86	105.05	5.00	2.28	191.08
	100	2	4	9	18									
	1000	3	9	8	24									
Timur	10	1	1	10	10	6	20	14	31	-42.36	95.05	5.00	2.13	133.63
	100	2	4	9	18									
	1000	3	9	1	3									
Neem	10	1	1	10	10	6	30	14	60	-44.57	109.14	5.00	2.34	217.04
	100	2	4	10	20									
	1000	3	9	10	30									
Ephedra	10	1	1	10	10	6	29	14	57	-44.36	107.71	5.00	2.32	206.83
	100	2	4	10	20									
	1000	3	9	9	27									
Stereum	10	1	1	10	10	6	30	14	60	-44.57	109.14	5.00	2.34	217.04
	100	2	4	10	20									
	1000	3	9	10	30									

Cytotoxicity of water extract on brine shrimp

Plants	concentration (c)	x=log(c)	x2	y (survivor)	xy	Σx	Σy	Σx2	Σxy	β=(Σxy-ΣxΣy)/Σx2-(Σx)2	α=Σxy-βΣx/n	Y=5 constant	X=(Y-α)/β	lc50=antilog X
Amla	10	1	1	10	10	6	28	14	54	-44.14	106.29	5.00	2.29	197.01
	100	2	4	10	20									
	1000	3	9	8	24									
Barro	10	1	1	10	10	6	29	14	57	-44.36	107.71	5.00	2.32	206.83
	100	2	4	10	20									
	1000	3	9	9	27									
Timur	10	1	1	10	10	6	29	14	57	-44.36	107.71	5.00	2.32	206.83
	100	2	4	10	20									
	1000	3	9	9	27									
Neem	10	1	1	10	10	6	28	14	54	-44.14	106.29	5.00	2.29	197.01
	100	2	4	10	20									
	1000	3	9	8	24									
Ephedra	10	1	1	10	10	6	30	14	60	-44.57	109.14	5.00	2.34	217.04
	100	2	4	10	20									
	1000	3	9	10	30									
Stereum	10	1	1	10	10	6	30	14	60	-44.57	109.14	5.00	2.34	217.04
	100	2	4	10	20									
	1000	3	9	10	30									

Antioxidant of hexane extracts

PLANTS	absorbance	Blank	control (AC)	actual absorbance of sample=absorbance-blank (AS)	%sRSA=(AC-AS)/AC*100	Concentration	IC 50
AMLA	0.264	0.053	0.423	0.212	50.000	10	70.38
	0.152	0.053	0.423	0.100	76.478	20	
	0.115	0.053	0.423	0.063	85.225	40	
	0.094	0.053	0.423	0.042	90.189	80	
	0.091	0.053	0.423	0.039	90.898	160	
	0.060	0.053	0.423	0.008	98.227	320	
BARRO	0.364	0.054	0.489	0.310	36.605	10	49.18
	0.347	0.054	0.489	0.293	40.082	20	
	0.303	0.054	0.489	0.249	49.080	40	
	0.231	0.054	0.489	0.177	63.804	80	
	0.118	0.054	0.489	0.064	86.912	160	
	0.103	0.054	0.489	0.049	89.980	320	
TIMUR	0.558	0.049	0.424	0.509	-20.047	10	18.03
	0.541	0.049	0.424	0.492	-16.038	20	
	0.480	0.049	0.424	0.431	-1.651	40	
	0.342	0.049	0.424	0.293	30.896	80	
	0.286	0.049	0.424	0.237	44.104	160	
	0.218	0.049	0.424	0.169	60.142	320	
				0.000	#DIV/0!		
NEEM	0.407	0.055	0.442	0.352	20.362	10	30.38
	0.386	0.055	0.442	0.331	25.113	20	
	0.361	0.055	0.442	0.306	30.769	40	
	0.295	0.055	0.442	0.240	45.701	80	
	0.260	0.055	0.442	0.205	53.620	160	
	0.251	0.055	0.442	0.196	55.656	320	

				0.000	#DIV/0!		
EPHEDRA	0.532	0.052	0.390	0.480	-23.077	10	27.89
	0.492	0.052	0.390	0.440	-12.821	20	
	0.375	0.052	0.390	0.323	17.179	40	
	0.268	0.052	0.390	0.216	44.615	80	
	0.116	0.052	0.390	0.064	83.590	160	
	0.091	0.052	0.390	0.039	90.000	320	
				0.000	#DIV/0!		
Stereum	0.500	0.055	0.397	0.445	-12.091	10	31.12
	0.393	0.055	0.397	0.338	14.861	20	
	0.271	0.055	0.397	0.216	45.592	40	
	0.206	0.055	0.397	0.151	61.965	80	
	0.194	0.055	0.397	0.139	64.987	160	
	0.135	0.055	0.397	0.080	79.849	320	
				0.000	#DIV/0!		

Antioxidant of water extract

PLANTS	absorbance	Blank	control (AC)	actual absorbance of sample=absorbance-blank (AS)	%sRSA=(AC-AS)/AC*100	Concentration	IC 50
AMLA	0.248	0.053	0.423	0.195	53.901	10	67.44
	0.147	0.053	0.423	0.094	77.778	20	
	0.142	0.053	0.423	0.089	78.960	40	
	0.134	0.053	0.423	0.081	80.851	80	
	0.129	0.053	0.423	0.076	82.033	160	
	0.116	0.053	0.423	0.063	85.106	320	
				0.000	#DIV/0!		
BARRO	0.280	0.054	0.489	0.226	53.783	10	60.79
	0.248	0.054	0.489	0.194	60.327	20	
	0.230	0.054	0.489	0.176	64.008	40	
	0.221	0.054	0.489	0.167	65.849	80	
	0.205	0.054	0.489	0.151	69.121	160	
	0.183	0.054	0.489	0.129	73.620	320	
				0.000	#DIV/0!		
TIMUR	0.543	0.049	0.424	0.494	-16.509	10	32.87
	0.499	0.049	0.424	0.450	-6.132	20	
	0.436	0.049	0.424	0.387	8.726	40	
	0.358	0.049	0.424	0.309	27.123	80	
	0.311	0.049	0.424	0.262	38.208	160	
	0.242	0.049	0.424	0.193	54.481	320	
				0.000	#DIV/0!		
NEEM	0.399	0.055	0.442	0.344	22.172	10	16.06
	0.378	0.055	0.442	0.323	26.923	20	
	0.348	0.055	0.442	0.293	33.710	40	
	0.285	0.055	0.442	0.230	47.964	80	

	0.252	0.055	0.442	0.197	55.430	160	
	0.229	0.055	0.442	0.174	60.633	320	
				0.000	#DIV/0!		
EPHEDRA	0.249	0.052	0.390	0.197	49.487	10	64.51
	0.220	0.052	0.390	0.168	56.923	20	
	0.197	0.052	0.390	0.145	62.821	40	
	0.077	0.052	0.390	0.025	93.590	80	
	0.064	0.052	0.390	0.012	96.923	160	
	0.032	0.052	0.390	-0.020	105.128	320	
				0.000	#DIV/0!		
Stereum	0.359	0.055	0.397	0.304	23.426	10	37.84
	0.324	0.055	0.397	0.269	32.242	20	
	0.273	0.055	0.397	0.218	45.088	40	
	0.249	0.055	0.397	0.194	51.134	80	
	0.202	0.055	0.397	0.147	62.972	160	
	0.185	0.055	0.397	0.130	67.254	320	
				0.000	#DIV/0!		

Antioxidant of ethylacetate extract

Plants	absorbance	Blank	control (AC)	actual absorbance of sample=absorbance-blank (AS)	%sRSA=(AC-AS)/AC*100	Concentration	IC 50
AMLA	0.162	0.053	0.423	0.109	74.232	10	76.92
	0.156	0.053	0.423	0.103	75.650	20	
	0.149	0.053	0.423	0.096	77.305	40	
	0.142	0.053	0.423	0.089	78.960	80	
	0.125	0.053	0.423	0.072	82.979	160	
	0.118	0.053	0.423	0.065	84.634	320	
				0.000	#DIV/0!		
BARRO	0.264	0.054	0.489	0.210	57.055	10	60.39
	0.257	0.054	0.489	0.203	58.487	20	
	0.242	0.054	0.489	0.188	61.554	40	
	0.232	0.054	0.489	0.178	63.599	80	
	0.220	0.054	0.489	0.166	66.053	160	
	0.203	0.054	0.489	0.149	69.530	320	
				0.000	#DIV/0!		
TIMUR	0.573	0.049	0.424	0.524	-23.585	10	26.62
	0.557	0.049	0.424	0.508	-19.811	20	
	0.446	0.049	0.424	0.397	6.368	40	
	0.346	0.049	0.424	0.297	29.953	80	
	0.297	0.049	0.424	0.248	41.509	160	
	0.229	0.049	0.424	0.180	57.547	320	
				0.000	#DIV/0!		
NEEM	0.454	0.055	0.442	0.399	9.729	10	20.63
	0.405	0.055	0.442	0.350	20.814	20	
	0.355	0.055	0.442	0.300	32.127	40	

	0.291	0.055	0.442	0.236	46.606	80	
	0.261	0.055	0.442	0.206	53.394	160	
	0.226	0.055	0.442	0.171	61.312	320	
				0.000	#DIV/0!		
EPHEDRA	0.300	0.052	0.390	0.248	36.410	10	55.97
	0.222	0.052	0.390	0.170	56.410	20	
	0.181	0.052	0.390	0.129	66.923	40	
	0.162	0.052	0.390	0.110	71.795	80	
	0.144	0.052	0.390	0.092	76.410	160	
	0.098	0.052	0.390	0.046	88.205	320	
				0.000	#DIV/0!		
Stereum	0.443	0.055	0.397	0.388	2.267	10	14.76
	0.418	0.055	0.397	0.363	8.564	20	
	0.387	0.055	0.397	0.332	16.373	40	
	0.352	0.055	0.397	0.297	25.189	80	
	0.293	0.055	0.397	0.238	40.050	160	
	0.248	0.055	0.397	0.193	51.385	320	