ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF SOME INVASIVE ALIEN SPECIES OF NEPAL

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The Central Department of Botany, Tribhuvan University

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Submitted by

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RECOMMENDATION

This is to recommend that the dissertation work entitled **"Antimicrobial Activities and Phytochemical Screening of Some Invasive Alien Species of Nepal"** has been carried out by Ms. Ritu Kumari Das for the partial fulfillment of the requirements for the degree of Master's of Science in Botany. This is her original work and has been carried out under my supervision and guidance. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions. So I recommend that the thesis be accepted for the degree of Master's of Science in Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

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LETTER OF APPROVAL

This dissertation certificate submitted by Ms. Ritu Kumari Das entitled **"Antimicrobial Activities and Phytochemical Screening of Some Invasive Alien Species of Nepal"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for the degree of Master's of the Science in Botany.

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ABSTRACT

In the present study, the antimicrobial activities and phytochemical screening of three Invasive Alien Species were studied. The crude extracts of the alien species (*Ageratina adenophora, Ipomoea carnea* ssp. *fistulosa* and *Xanthium strumarium*) were prepared by using distilled water and methanol as solvents. The antimicrobial activity against six pathogenic bacteria, three gram negative: *Klebsiella pneumoniae* (ATCC 15380), *Proteus mirabilis* (ATCC 49132), *Escherichia coli* (ATCC 25922) and three gram positive: *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25932) and five phytopathogenic fungi: *Alternaria brassicae, Botrytis cinerea, Fusarium oxysporum, Phytophthora capsici* and *Sclerotium rolfsii* of different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml) of selected plant extracts was studied. The highest (16.48%) crude extract was obtained in *A. adenophora* in distilled water and the lowest (6.2%) in methanolic extract of *X. strumarium*. The phytochemical screening depicted the presence of terpenoids, saponins, cardiac glycosides, flavonoids, reducing compounds, tannins, phlabotannins and alkaloids. Seven replicates were used for each tested microbes.

The antibacterial activity of extracts was determined by disc diffusion method and zone of inhibition (ZOI) was measured. Gram negative bacteria was found more resistant than gram positive bacteria. The most susceptible bacteria was *S. aureus* while the most resistant bacteria was *E. coli*. Methanolic extract was found more effective than distilled water.

The antifungal activity of extracts was determined by poisoned food technique and linear mycelium growth reduction (LMGR) percentage was calculated. The distilled water extracts inhibited the growth of fungal mycelium while methanolic extracts completely inhibited (100%) the growth of some selected fungi at higher concentrations. *Fusarium oxysporum* was the most susceptible fungus while *A. brassicae* and *B. cinerea* were the most resistant fungi. Between bacteria and fungi, bacteria was found more susceptible than fungi against both distilled water and methanol extracts of selected IAS.

Key words: IAS, Antimicrobial property, ZOI, LMGR, Phytochemical Screening.

ACRONYMS

AARC	Alllopathy Advantage against Resident Species
ATCC	American Type Culture Collection
DW	Distilled water
et al.	others
fig.	Figure
G-	Gram negative
G+	Gram positive
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
IAS	Invasive Alien Species
J.	Journal
LMG	Linear mycelium growth
LMGR	Linear mycelium growth reduction
lbs	Pounds
MIC	Minimum inhibitory concentration
ml	Milliliter
NA	Nutrient Agar
NB	Nutrient Broth
NH_4OH	Ammonium hydroxide
NRs	Nepalese Rupees
NWH	Novel Weapon Hypothesis
Ppt	Precipitate
SD	Standard deviation
sp.	Species
ssp.	Sub species
viz	namely
ZOI	Zone of Inhibition

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1. INTRODUCTION

1.1 Background

A biological species introduced in an ecosystem other than its natural home is called alien species. It is also called as non-native, non-indigenous, exotic and foreign. Many alien species are known to have become aggressive and rapidly colonized displacing the native species by predation, parasitism or by competition for space and nutrients. They spread unmanageably, propagate relatively rapidly and outnumber all other native species in their own ecosystems conditions. This is mainly because of their capacity to thrive upon foreign soil and climatic conditions. They are termed "Invasive Alien Species" (IAS) (Tiwari *et al.* 2005). An invasive species defined by IUCN (2000) as "Species which becomes established in natural or semi-natural eco-systems or habitat, is an agent of cause, and threatens native biological diversity" (IUCN 2000).

The term 'invasive species' denotes plants and animals that: (i) have been introduced into ecosystems where they are not native by either intentional or unintentional human activity, (ii) have established self-reproducing populations, and (iii) have caused significant changes in pre-existing natural or artificial ecosystems (Richardson 1998). Alien species may be of three types: casual, naturalized and invasive. Casual aliens are those which survive occasionally outside cultivation. Naturalised alien when they become able to maintain population without support from human and invasive alien when they start to expand over the target region. Furthermore, if introduced plants produce reproductive offspring in areas distant from the sites of introduction (approximate scales:>100m over < 50 years for taxa spreading by seeds and other propagules; > 6m/3 years for taxa spreading by roots, rhizomes, stolons or creeping stems) are called naturalized plants (Richardson *et al.* 2000). In general, disturbance, nutrient enrichment, slow recovery rate of resident vegetation, and fragmentation of successionally advanced communities promote plant invasions (Rejmánek 1989; Hobbs and Huenneke 1992).

1.2 Dispersion of IAS

Plants must be able to disperse into new habitats in response to changing environments, whether the changes are anthropogenic or due to cyclic climatic changes (Jablonski 1991). These include intentional trade in living organisms, as well as the unintentional movement of organisms by tourists, on products, in shipping containers, and associated means of transport. People are largely responsible for moving eggs, seeds, spores, vegetative parts, and whole organisms from one place to another, especially through modern global transport and travel (McNeely 2001). Nepal has a long history of introduction of non-native species, especially species proven to be productive elsewhere and offering potential economic benefits to the country. *Tamarindus indica* (tamarind), originally from Africa, is believed to have been first introduced into Turkey in 126 B.C.-220 A.D. (Yan *et al.* 2001), spreading gradually toward China along the 'Silk Road'; by now it has been thoroughly naturalized in Nepal. The origin of IAS in Nepal dates back more than 50 years. Their richness and abundance have been increasing over the years. The country is suitable for flora and fauna with a wide range of life histories and origin due to its high climatic and physiographic diversity (MoFSC 2013).

1.3 Problems caused by IAS

In short, invasive plants have negative effects on biodiversity (i.e., the rich genetic resource of flora, fauna, and microbes) at the ecosystem level and the community and population levels. Examples of how invasive plants threaten the health of natural areas are: 1) replacement of diverse systems with single stands of nonnative species, 2) changes in soil chemistry, land form processes, fire regime, and hydrology, 3) competition with endangered species, and 4) competition with and displacement of native fauna (Niemiera and Holle 2009). Vigorous "r-selected" coloniser species with fast reproductive rates and good dispersal ability are very dangerous. Such species include many grasses, climbers, coloniser shrubs and trees with wind dispersed seeds. Thought humans depend heavily on non-native species for food, shelter, medicine, ecosystem services, aesthetic enjoyment and cultural identity these species also poses a threat to native biodiversity and rural livelihoods. From an economic standpoint,

invasive species can reduce livestock forage quality and quantity, jeopardize animal and human health (Ricciardi *et al.* 2000), increase the threat of fire or flooding, interfere with recreational activities, or lower land value. IAS have many negative impacts on human economic interests. Weeds reduce crop yields, increase control costs, and decrease water supply by degrading catchment areas and freshwater ecosystems (McNeely 2001). Invasive alien species cost millions of US dollars annually in terms of lost revenue and expenditure on control measures. While the actual costs of IAS are unknown, they are believed to be substantial. The global economic costs of IAS are estimated by IUCN to be about US$400 000 million annually (UNEP 2003); IUCN also finds that IAS threaten the success of current and planned World Bank projects to the value of more than US$13 000 million (UNEP 2004).

1.4 Phytochemical properties of IAS

Being primary producers, plants are vulnerable to primary consumers and pathogens (fungi, viruses, bacteria) and they have evolved a large arsenal of mechanical and particularly chemical means for protection. The most widespread chemical defense agents are referred as ‘plant secondary metabolites’ (PSMs). PSMs are not essential for maintaining life but they incur costs and are crucial for survival and reproduction; such allelochemicals are thus not constituents of each and every plant cell (Harborne 1993; Gordon-Weeks and Pickett 2009). Secondary metabolites (also called specialized metabolites) is a term for pathways and small molecule products of metabolism that are not absolutely required for the survival of the organism, many of which are antibiotics and pigments.

Besides changing physical environmental conditions, the invasive species also release certain secondary metabolites (i.e. allelochemical) that make the chemical environment of soil unsuitable for germination of other species (Inderjit *et al.* 2008). If the invader is resistant enough and tolerant to herbivory, then its competitive ability increases and it becomes very aggressive due to production of some defensive chemicals (Carpenter and Cappuccino 2005).

Invasive species being aggressive compete for water, light, space and nutrients, and enhance competitive ability; the invasive alien species may exhibit phytochemically unique exotics (Lockwood *et al.* 2001). The novel weapons hypothesis is that some invasive species owe their success to the production of biochemicals that are not produced by natives in the invaded range. Thus these chemicals may have the potential to exert stronger effects on native species that lack a coevolutionary-based tolerance than on coevolved competitors in the native range. Such novel biochemical effects could apply to competing plants, soil biota and generalist herbivores (Thorpe *et al*. 2009).

1.5 Effect of microorganisms

Microbial diseases rank as number one cause for almost half of the deaths in underdeveloped and tropical countries. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developed countries. Infectious diseases are world's leading causes of premature death, killing almost 50,000 people every day (Anonymous 2000). Microorganisms play a major role in contamination of stored food deteriorating quantitatively and qualitatively. Fungi are momentous destroyer of foodstuffs during storage, rendering them unfit for human consumption by hindering their nutritive value and sometimes by producing mycotoxins (Agrios 2004; Singh *et al.* 2010). More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases (Strange and Scott 2005). Plant diseases are caused by pathogens such as fungi, bacteria, nematodes and viruses. Compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. Morbidity and mortality due to diarrhea continues to be a major problem in many developing countries, especially amongst children. Food-borne illness to the individual is caused by eating food or drinking beverage contaminated with bacteria or by direct contact through pus. *K. pneumonia*, is a most opportunistic pathogen and second to the *E. coli* causing urinary tract infections (Raman *et al*. 2012).

1.6 Objectives

General objective:

Evaluation of antimicrobial activities and phytochemical screening of some invasive alien species (plants) of Nepal (*Ageratina adenophora, Ipomoea carnea* spp. *fistulosa* and *Xanthium strumarium*)

Specific objectives:

1. To test the phytochemical properties of selected plants by phytochemical screening.
2. Extraction of leaves for crude compounds from the above selected plants using two different solvents (Distilled water and Methanol) for testing antibacterial and antifungal properties.
3. To determine Zone of Inhibition (ZOI) for bacteria and Linear Mycelium Growth Reduction (LMGR) percentage for fungus in different 5 concentrations of the both methanolic and distilled water leaf extract of these plants.
4. Comparative evaluation of the activities of leaf extracts with antibiotics and fungicides by testing on selected bacteria and fungus respectively.

1.7 Justification of the study

Since, Invasive Alien Species (IAS) is a major problem of Nepal and causing a negative impact on the environment, especially on biodiversity of the country, either socially or economically. They compete with agricultural crops for water, light and nutrients, causing enormous losses in food production. In last few decades the rapid expansion of IAS into forest ecosystems of Nepal has posed serious threat to forest health. Biological invasion is the second cause of biodiversity loss after the deforestation (Vitousek 1990). These plant species may therefore contain active chemicals to resist microbial attack. Seeing these widespread changes, biologists, environmentalists and managers are alarmed about invasions leading to large-scale declines and extinctions of natives. Also, combating invasive plants is difficult and

costly. In the other side microbes are also damage the health of humans and plants. Most of these microbes are capable to digest synthetic drugs. The high use of synthetic drugs can create a side effect on human, environmental toxicity effects, high costs and it can add the pollution to the environment.

The reason to choose and work with IAS is that they may be such successful competitors due to resistance towards different pathogens. These plant species may therefor contain active principles to resist plant pathogen attack and the IAS are used as raw material for plant derived chemicals then there are large quantities of material readily available for use. Exploitation of these rapidly growing species can be done by preliminary phytochemical screening for making the different pharmaceutical products in one hand while proper management these plants and microbes on the other side.

If alien invasive plant species are used as raw material for plant-derived antimicrobials then there are large quantities of material readily available for use. Additionally collection may protect indigenous plants and at the same time may create economic uses and jobs based on these unwanted species.

1.8 Limitation of the study

A) Study was limited to only three Invasive alien species.
B) Two different study areas were selected.
C) Only leaves were selected for study.
D) MIC is also very important for the study of antibacterial property but due to the time limitation it was avoided.

2. LITERATURE REVIEW

2.1 Invasiveness of plants

Invasive plants are naturalized plants that produce repro-ductive offspring, often in very large numbers, at consider-able distances from parent plants and thus have the potential to spread over a considerable area (Kunwar 2003). The biological invasions are potent threat to species diversity around the world during 21st century (Dogra *et al.* 2010) and ranked as second important cause of current global biodiversity loss after habitat loss and destruction by fragmentation or disappearances, as exotic species clearly alter the composition and community structure as well as ecosystem properties and fundamental rules of existence of organisms in the invaded areas (Vitousek 1990). The major threats to Biodiversity of Nepal was human destruction and over exploitation which is now accompanied by introduction of exotic species leading habitat change and soil degradation. Biological invasion worldwide has threatened biodiversity, ecosystem dynamics, resources availability, national economy and human health (Ricciardi *et al.* 2000). Once established some alien species have the ability to dis-place or replace native plant species the problem will likely worsen with time because of climatic changes that promote species migration worldwide (Rashmi and Rajkumar 2011).

According to vacant niche hypothesis (Elton 1958) the empty places such as barren lands, roadsides, open grounds etc. are generally invaded by such weeds. The diversity of these weeds is governed by population, ecosystem dynamics, disturbances, nutrient supply and climatic factors. The biotic restrictions on them, force to skip from their previous habitat and start surviving in new habitats, helping in the process of invasion. The prolific nature and successful invasion in new habitats suggest that the invasive species are hypothesized to be equipped with novel biochemistry that repels native species or unique compounds in the native flora (Cappuccino and Arnason 2006). A number of explanations for this invasion success have been proposed (Mack *et al*. 2000), but consumer-based hypotheses predominate – the "natural enemies hypothesis" (Mack *et al*. 2000; Williams 1954) and a recent expansion of the natural enemies hypothesis, "the evolution of increased competitive ability" (Blossey and Nötzold 1995). Novel weapons, biochemical in nature, play a

part in the exotic plant invasions that are currently sweeping the world. To be precise, the definition of novel weapons here is limited to biochemicals released from invasive plants that affect native plants or the native soil biota, and with which native plants interact. Enemy Release Hypothesis is considered as the most acceptable to explain the mechanism of invasion. This hypothesis has given one of the probable reasons for this as such species are released from the stress of pests and diseases that occur in their native ranges (Keane and Crawley 2002), hence many species which are not dominant competitors in their natural ranges, but appear to competitively diminish or eradicate neighbors in non-native systems (Kim and Lee 2010).

2.2 Antimicrobial agents and its activities

Antimicrobial agent is a chemical substance that either kills microorganisms or prevents their growth (Tripathi 2003). According to Tripathi (1995) Antimicrobial agents are synthetic as well as natural obtained drugs that attenuate microorganisms. In general antimicrobial agent act in one or more of the following ways:

A) Damaging the cell membrane leading to loss of cell contents and so to cell death.
B) Inhibition cell wall formation leading to cell lysis.
C) Inhibition protein biosynthesis.
D) Inhibition nucleic acid production so preventing bacteria from reproducing (Cheesbrought 1993).

Different extracts from traditional medicinal plants have been tested to identify the sources of the therapeutic effects. As a result of some natural products have been approved as new antimicrobial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (Recio 1989).

The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan 2003). Some of the notable works done in world wide in the view to determine antimicrobial activities of plant includes:

Panthi and Chaudhary (2006) tested eighteen folklore medicinal plant species by disc diffusion method. The bacteria employed were Gram-positive (*Staphylococcus*

aureus) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Shigella boydii*). Extracts of eight plants showed encouraging result against three strains of bacteria, while other showed activity against one or two strains. Among the bacteria, *Staphylococcus aureus* was found the most susceptible bacteria and *Escherichia coli* was found the most resistance bacteria.

Eftekhar *et al*. (2007) studied antimicrobial activity of the aerial extracts (methanol, diethyl ether, petroleum benzene and an equal mixture of the three solvents) of *Xanthium brasilicum* using the disc diffusion method. Twelve bacterial strains were used which included; *Bacillus subtilis* (ATCC 465), two *Enterococcus faecail*is (ATCC 29737 and a clinical isolate), three *Staphylococcus aureus* (ATCC 25923, ATCC 29737 and a clinical isolate), three *Escherichia coli* (ATCC 25922, ATCC 10536 and a clinical isolate) and three *Pseudomonas aeruginosa* (ATCC 85327, ATCC 9027 and a clinical isolate) strains. The disc sensitivity results for crude extracts showed that methanol, MEP and water extracts contained antibacterial activity. Extracts prepared in other solvents showed poor or no antibacterial activity. The most susceptible organisms were *S. aureus, B. subtilis* followed by *P. aeruginosa* and *E. coli*. *E. faecalis* was resistant to the extracts at the concentration used.

Mahida and Mohan (2007) screened methanol extract of 23 plants parts (leaves or tubers) for their antibacterial activity against multi-drug resistant bacteria, viz. *Staphylococcus aureus*, *S. epidermis, Salmonella typhi* and *S. paratyphi*. Sensitivity test was performed by agar well diffusion method. Out of 23 plants 11 plant species (47%, zone of inhibition more than 5mm) and 10 plant species (43%) were found to be effective against *Staphylococcus aureus* and *S. epidermis*, respectively. Among different extracts, *Mangifera indica* Linn. and *Manilkara hexandra* (Roxb.) Dubard extracts were the most potent active extracts against both species of *Staphylococcus.* Methanol extract of *Rauvolfia tetraphylla* Linn. exhibited significant antibacterial activity against both *Salmonella* species. Leaf extract of *Xanthium strumarium* was found to possess potent antibacterial efficacy against both Staphylococcus species and *Salmonella paratyphi* A.

Mahesh and Satish (2008) studied the Methanol leaf extracts of *Acacia molotica, Sida cardifolia, Tinospora cardifolia, Withania somnifera and Ziziphus mauritiana* showed

significant antibacterial activity against *Bacillus subtilis, E. coli, Pseudomonus fiuorescens, Staphylococcus aureus.* Rootbar extracts of *Acacia mlotica* and *Sida cardifolia* leaf extracts showed highest antibacterial activity against *Bacillus subtilis.* Root and leaf extracts of *Sida cardifolia* recorded significant active against all the tested bacteria.

Rahman and Junaid (2008) reported antimicrobial activity of leaf extracts (Petroleum ether, carbon tetrachloride, chloroform, and ethyl acetate) of *Eupatorium triplinerve* vehl. against eleven bacteria and six phytopathogenic fungi. The *in vitro* antibacterial and antifungal activity of plant material was determined by disc diffusion method and poisoned food technique. The crude extracts showed good activity against the organisms tested. The chloroform extract exhibited the largest zone of inhibition (22 mm in diameter with 1000 µg/disc extract) against *Vibrio* and the highest inhibition of fungal radial mycelial growth (73.5% with 100 µg extract/ml medium) against *Colletotrichum corchori.*

Mamidala and Gujjeti (2013) Studied ether, chloroform, ethyl acetate and methanol extracts of *Acmella paniculata* against twelve strains of microorganisms using agar diffusion method. The extracts of different part of plant used as antimicrobial activity. The plant exhibited antimicrobial activity against more than one pathogen and chloroform, pet ether, and methanol extracts completely inhibited the growth of *Enterobactum aerogenus*. The chloroform extract was found to be active against *Enterobactum aerogenose*.

Shahid *et al.* (2013) studied the antimicrobial activity of selected plants viz. *Cycas revolute*, *Cupressus sempervirens*, *Araucaria celumnaris*, *Ricinus communis*, *Solanum nigrlam*, *Calotropis procera*, *Withania coagulans*, *Cominum cyminum*, *Foeniculum vulgare*, *Nigella sativa* and *Coriandrum satum*. Antimicrobial activity of methanol, ethanol and ethyl acetate extracts of selected plants were investigated by well diffusion method. Six strains of bacteria viz. *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Pseudomonas aeruginosa* (ATCC6643) *Escherichia coli* (ATCC15224), *Klebsiella pneumonia* (MTCC618) and *Salmonella typhimurium* (ATCC13048) were utilized as test organisms. It was revealed in this study that the antimicrobial activity of the extracts was enhanced by increase in the

concentration of the extracts. Greater inhibitory activity against *S. aureus, P. aeruginosa, B. subtilis* and *K. pneumoniae* was possessed by ethyl acetate extract of *C. sempervirens*. The methanolic extract of *Nigella sativa* exhibited maximum inhibitory activity against *S. typhimurium*. The ethanolic extract of *Araucaria columnaris* showed higher activity against *E. coli*.

2.3 Effect of synthetic drugs and products of plants

The increased prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents inefficient to control some bacterial diseases (Tanaka *et al*. 2006). In recent years, drug resistance to human pathogenic bacteria has commonly been reported from all over the world. Eventhough pharmaceutical companies produce number of new antibacterial drugs, but gradual resistance to these drugs has increased which is matter of global concern besides synthetic drugs are normally associated with side effects (hypersensitive, immune suppression etc). Use of phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Singh *et al*. 2012). Current control of phytopathogenic agents is done using synthetic fungicides. Along with increasing of the restrictions regarding their use due to adverse effects on human health and the environment (Harris *et al*. 2001), appeared the need to find alternatives to fungicides. Research is currently being carried for the development of biological methods that will work in the inhibition fungal growth, such as the use of antifungal active ingredients extracted from plants (Quiroga *et al*. 2001). Therefore, biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction and growth of plant pathogenic fungi, would be a more realistic and ecologically sound method for integrated plant disease management and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Gottlieb *et al.* 2002; Varma and Dubey 1999). In contrast, the fungicides of plant origin are easily biodegradable and they do not cause undesired side effects in living beings. A lot of work has been done in recent years for the investigation of antifungal activity/substances in plants (Barrows 2007; Sadeghi 2007). Drugs from the plants are

easily available, less expensive, safe, and efficient and rarely have side effects (Yadav and Agarwal 2011).

2.4 Phytochemical screening and antimicrobial properties of IAS

Plants contain thousands of biologically active molecules. For their investigation, it is important to have the necessary tools. These include suitable biological assays and chemical screening methods. The search for new antimicrobial agents is necessary due to the appearance of microbial resistance and occurrence of fetal opportunistic infections (Kivack *et al*. 2001). phytochemicals, particularly the secondary metabolites (such as alkaloids, anthraquinnones, cardiac glycosides, saponins, tannins, xanthones, flavolignans, terpenoids, coumarins, lignans, cinamic acids, etc) are not only studied for their health benefits but for their significant roles in the complex interactions between microbes, animals and plants in the ecosystems (Akenga *et al*. 2005; Raimundo 1999).

2.4.1 Phytochemical and antimicrobial activities of *Ageratina adenophora*

Extensive work on the chemical constituents as well as on the composition of the essential oils of *Ageratina* is reported in literatures. *A. adenophora* has been reported for its allelopathic effect (Rajbanshi and Inubushi 1998). A hepatotoxic compound (9-oxo-10, 11-dehydroagerophonrone) was identified from the crude extract of *A. adenophora*, this component promotes a chronic equine respiratory disease known as "Numinbah horse"and liver lesions (Oelrichs *et al.* 1995). Chemical composition of the alcoholic extracts from different *Ageratina* species have also been investigated reporting flavonoids, chromens, sesquiterpenic lactones, diterpenes, triterpenes, flavones and flavanones (Kurade *et al*. 2010; Rios *et al*. 2003).

2.4.2 Phytochemical and antimicrobial activities of *Ipomoea carnea* ssp. *fistulosa*

The literature survey reveals that the *Ipomoea carnea* spp. *fistulosa* possess various bioactive compounds such as glycosides, alkaloids, reducing sugars, flavonoids, fatty acid, esters, alcohol (Frey 1995) and tannins (Afifi *et al.* 1988). The leaves of this plant showed the presence of thirteen compounds which include hexadecanoic acid,

stearic acid, 1, 2 diethyl phthalate, n-octadecanol, octacosane, hexatriacontane, tetracontane, 3-diethylamino-1- propanol (Tirkey *et al*. 1988; Vaishali *et al*. 2009).

2.4.3 Phytochemical and antimicrobial activities of *Xanthium strumarium*

The principal compounds isolated from *X. strumarium* include xanthanol, isoxanthanol, hydroquinone, caffeyolquinic acids, alkaloids, and thiazinedione (Han *et al*. 2007; Ying-Tsun *et al*. 1998). All parts of plant possess sedative, diaphoretic and diuretic properties. The plant also shows its efficacy in mitigating longstanding cases of malarial fever (Sharma 2003). The genus *Xanthium* also possess, antibacterial (Talakal *et al*. 1995), antiviral (Tsankova *et al*. 1994), antimalarial (Joshi *et al*. 1997), fungicidal (Ginesta-Peris *et al*. 1994), insecticidal, (Kamboj and Kumar 2010), and cytotoxic activities against cancer cell lines (Kinghorn *et al*. 1999).

3. MATERIALS AND METHODS

3.1. Collection of Selected IAS

Given plant samples have been collected for the study from two different location of Nepal.

S.N.	Plants	Part used	Place of collection
i.	*Ageratina adenophora*	Leaves	Kirtipur,Kathmandu
ii.	*Xanthium strumarium*	Leaves	Kirtipur,Kathmandu
iii.	*Ipomoea carnea* ssp. *fistulosa*	Leaves	Jhojhi Kataiya, Dhanusha

Each plant's leaves were collected sufficiently to make them 1 Kg separately after dry weight.

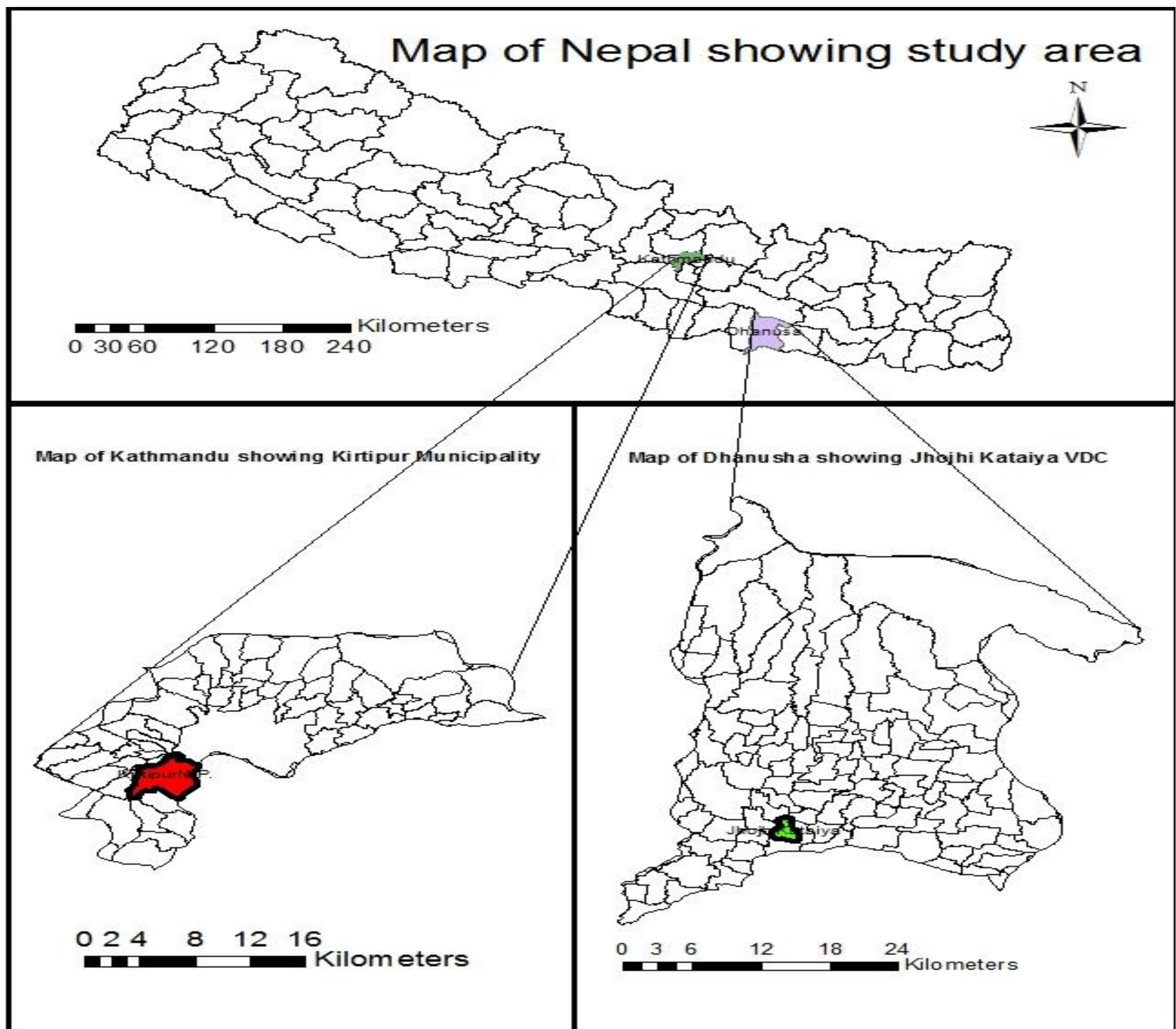
3.2. Study Area

3.2.1. Kirtipur

It is an ancient city in Nepal. It is located in Kathmandu district of Bagmati zone, 5 km south west of the Kathmandu .It is one of the five municipalities of Kathmandu. It lies 1375m above sea level and the coordinates are 27°40'55"N and 85°17'21"E. It has subtropical environment. The leaf samples of *Ageratina adenophora* and *Xanthium strumarium* were collected from different areas of Tribhuwan University.

3.2.2. Jhojhi Kataiya

Jhojhi Kataiya is a village development committee in Dhanusa District in the Janakpur Zone of south-eastern Nepal. Its coordinates are 26°41′N 85°59′E/ 26.68°N 85.98°E. It has tropical environment. The leaf sample of *Ipomoea carnea* ssp. *fistulosa* was collected from river side of this village.



3.3 Introduction of selected IAS

3.3.1 *Ageratina adinophora*

Family: Asteraceae

Common Name: Crofton weed

Local Name: Kalo banmara, Kalo haram, Kalo teta

Native Place: Central America

First Reported in Nepal: Banerji in J. Bomb. Nat. Hist. Soc. 55: 259.1958 (*Eupatorium glandulosum)*

It has recently drawn worldwide attention for its ever increasing potential weed mostly colonizing cultivated lands, cardamom based agroforestry systems, open

degraded lands, jhoom fallow and natural forest especially within the altitudinal range between 1000 - 8000 ft (Singh and Singh 2013).

3.3.2 *Ipooea carnea* ssp. *fistulosa*

Family: Convolvulaceae

Common Name: Shrubby morning glory

Local Name: Sanai phul, Besaram, Thethar, Hehara

Native place: South America

First Reported in Nepal: Yamazaki in Fl. East. Himal: 264.1966 (*Ipomoea crassicaulis*)

It is mainly found in wetlands such as marshes, shallow lakes and ponds, ditches, drainages, etc. Plant grows well in moist habitats usually in lowlands (Tiwari *et al.* 2005). The farmers use it as ornamental and hedge plant along the banks of irrigation and drainage canals. These ornamental uses and reproduction by seeds oftenly lead to increase the plant dissemination into new regions (Chaudhari *et al.* 1994). Recently, it has become widely spread in other terrestrial habitats.

3.3.3 *Xanthium strumarium*

Family: Asteraceae (Compositae)

Common Name: Rough cockle-Bur

Local Name: Bheda kuro, Kuro

Native place: Europe

First Reported in Nepal: Kitam. in Fau. & Fl. Nepl. Himal: 273.1955

It is mainly found in Agricultural fields and Fallowlands. Though the plants were first reported from Europe, it is probably of American origin (Munz and Keck 1973), distributed worldwide. It is a serious weed in Australia, India, South Africa, America and Nepal. *Xanthium strumarium* is distributed in open and disturbed areas particularly, along roadsides, streams and riverbanks and overgrazed pastures. It is an extremely competitive weed in agricultural field (Tiwari *et al.* 2005).

3.4 Selection of microorganism

Worldwide, infectious disease is the number one cause of death according for approximately one-half of all deaths in tropical countries.

3.4.1 Short description of microorganism involved within this study

A. Selected Pathogenic Bacteria

i) ***Klebsiella pneumoniaea*:-**This gram negative non motile capsulated bacteria causes pneumonia, urinary tract infection, septicaemia and rarely diarrhea. It is causes pyogenic infections such as abscesses, meningitis and septicaemia (Cheesbrough 1993; Chakraborty 2000)

ii) *Enterococcus faecalis*: - It is a Gram-positive, coccoid bacteria commonly isolated from clinical specimens and the human intestinal tract. It is a non motile, facultative anaerobic microbe. *E. faecalis* can cause endocarditis and bacteremia, urinary tract infections (UTI), meningitis, and other infection in human (Hidron 2008).

iii) *Escherichia coli*:-This Gram negative straight rod is part of the normal microbial flora of the intestinal tract of humans and animals often associated with opportunistic infection. The virulent straight of E. coli act as specific pathogens of the gut (enteritis) and of extra-intestinal sites (UTI, wound infection). Clinical infections caused by *E. coli* include: UTI, septic infection of wound, diarrheas, dysentery, septicaemia, pneumonia, neonatal meningitis, and abscesses in a variety of organs (Chakraborty 2000).

***iv) Bascillus subtilis*:-**It is a gram positive bacteria, cause an array of infection from ear infections to meningitis, and urinary tract infection to septicemia. Mostly they occur as secondary infection in immunodeficient hosts or otherwise compromised hosts (Baral *et al*. 2011).

v) Proteus mirabilis*:-Proteus mirabilis* actively motile Gram negative aerobic bacilli commonly causes urinary infection in the elderly and young males. It also causes abdominal and wound infections, septicaemia and occasionally meningitis and chest infection (Chakraborty 2000).

vi) ***Staphylococcus aureus*:-** This Gram positive spherical cocci causes boils, pustules, impetigo, infections of wounds, ulcers, osteomyelitis, mastitis, septicaemia, meningitis, Pneumonia and Pleural empyema. It also causes toxic food poisoning, toxic shock syndrome and toxic exfoliation (Cheesbrough 2002)

B. Selected Phytopathogenic Fungi

i) ***Alternaria brassicae*: -** Cruciferous plants (*Brassicaceae*) worldwide are severely affected by the *Alternaria brassicae* (Maude and Humpherson-Jones 1980; Humpherson-Jones 1989). *Alternaria* pathogens usually cause black spot disease, manifested by damping-off of seedlings, spotting of leaves of cab-bages, blackleg of heads of cabbages, and spot-ting/browning of cauliflower curds and broccoli florets. Black spot is the most common disease in the crucifers plantations located in tropical and sub-tropical regions. *A. brassicae* cause severe economic losses in sev-eral different ways (Humpherson-Jones and Maude 1982; Humpherson-Jones 1989).

ii) ***Botrytis cinerea*: -** *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*) family: Sclerotiniaceae is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 crop hosts worldwide. It can cause soft rotting of all aerial plant parts, and rotting of vegetables, fruits and flowers post-harvest to produce prolific grey conidiophores and (macro) conidia typical of the disease. Although there are fungicides for its control, many classes of fungicides have failed due to its genetic plasticity (Williamson *et al*. 2007).

iii) ***Fusarium oxysporum*: -** *Fusarium oxysporum* is a soilborne plant pathogen in the class Hyphomycetes, causes Fusarium wilt specifically in tomato. The first indication of this disease is a yellowing and drooping of the lower leaves. This symptom often occurs on one side of the plant or on one shoot. Successive leaves yellow, wilt and die, often before the plant reaches maturity. As the disease progresses, growth is typically stunted, and little or no fruit develops. If the main stem is cut, dark brown streaks may be seen running lengthwise through the stem (Wong 2003).

iv) *Phytophthora capsici*: - *Phytophthora capsici* is an oomycetous fungus. It can cause extensive losses, of pepper and summer squash, eggplant and watermelon because of the rapidity of disease spread during favorable weather. Diseases caused by it is phytophthora blight, Phytophthora crown root rot and Phytophthora fruit rot. *P. capsici* has shown remarkable adaptations to fungicides and new hosts. Like other members of this destructive genus, it has an explosive epidimology, rapidly producing massive numbers of asexual spores on infected host (Wenwuye 2012).

***v) Sclerotium rolfsii*: -** *Sclerotium rolfsii,* an omnivorous, soilborne fungal pathogen, causes disease on a wide range of agricultural and horticultural crops. Susceptible agricultural hosts include *Ipomea batatas*, *Cucurbita pepo* L., *Zea mays*, *Triticum vulgare* and *Arachis hypogea*. Horticultural crops affected by the fungus are included in the genera *Narcissus*, *Iris*, *Lilium*, *Zinnia*, and *Chrysanthemum* (Farr *et al.* 1989). Rapidly growing, silky-white hyphae tend to aggregate into rhizomorphic cords (Aycock 1966).

3.5 Drying Of Materials

The healthy and fresh leaves of each selected plants were washed under running tap water to remove soil particles and other dirt and then chopped into small pieces with the help of knife and then air dried in the laboratory at room temperature for few days. These plant materials were spread under shade on the news paper till they became completely dry. Exposure to sunlight was avoided to prevent the loss of active compounds.

3.6 Packing and Storage

The completely dried samples have been packed in water proof bags. The packed samples were stored in room temperature avoiding direct sunlight.

3.7 Grinding and Packing

The completely dried leaf samples of each plant was grinded separately into fine powder with the help of electric grinder. The fine powdered samples were

preserved in an air tight zipper bags to keep the samples away from moisture and microbes at the room temperature before extraction.

3.8 Phytochemical screening of Selected Species

Phytochemical screening of the extracts was done for saponins, cardiac glycosides, tannins, alkaloids, terpenoids, reducing compounds, steroids, phlobatannins and flavonoids using standard phytochemical screening methods with slight change.

3.8.1 Qualitative analysis

Preliminary qualitative phytochemical screening was carried out on aqueous and methanol extract applying the standard protocols described by Sofowara (1993), Trease and Evans (1989), and Harborne (1973) and for the result sharp change in color was noted.

A) **Terpenoids (Salkowski test):** five ml of each extract was mixed in 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 was added carefully to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

B) **Saponin:** One gram of the powered samples were boiled in 10 ml of water and methanol separately in a water bath and filtered. Five ml of the filtrate was mixed with 2.5 ml of distilled water and shaken vigorously for a stable persistent froth.

C) **Steroids:** Five ml of extract was taken and to it few drops of acetic anhydride and 5 ml of chloroform was added. Finally few drops of H_2SO_4 was added slowly along the wall of test tube. Green bluish colour indicated the presence of steroids.

D) **Cardiac glycosides (Keller- Killani Test):** Five ml of each extracts were treated with 2 ml of glacial acitic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicate a deoxysugar characteristic of

cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

E) **Flavonoids:** Five ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared after some time.

F) **Reducing sugar (such as sugar):** One ml of each extract was taken and 2 ml of water was added and then 5-8 drops of Fehling's solution was added at hot. Brick red ppt. indicated the presence of reducing compound.

G) **Tannins**: About 0.5 g of the dried powered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

H) **Phlobatannins**: When an aqueous and methanol extract of each plant sample was boiled with 1% aqueous hydrochloric acid deposition of red precipitate was taken as evidence for the presence of phlobatannins.

I) **Alkaloid**: Plant sample of 200 mg was taken in 10 ml methanol and was filtered. Mayer's Test: In 2 ml filtrate 1% HCL was added and was steamed and 1 ml of filtrate was treated with 6 drops of Mayer's reagents. Formation of yellow coloured precipitate indicated the presence of alkaloids. Wagner's Test: Filtrate was treated with Wagner reagent. Formation of brown/ reddish coloured precipitate indicated the presence of alkaloids. Dragondroff's Test: Filtrate was treated with Dragondroff's reagents Formation of red coloured precipitate indicated the presence of alkaloids.

3.8.2 Quantitative test

Alkaloid, Saponin and Flavonoid were determined in the crude form of compounds by following methods.

3.8.2.1 Alkaloid Test (Harborne method 1973)

2.5 g of the sample was weighted into a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one-quarter (25 ml) of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was completed. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighted (initial and final weight of the filter paper was calculated).

3.8.2.2 Saponin determination (Obadoni and Ochuko 2001)

The samples were ground and 10 g of each was put into a conical flask and 50 cm^3 of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 2 h with continuous stirring at about 50°C. The mixture was filtered and the residue re-extracted with another 100 ml 20% ethanol. The combined extracts were reduced to 20 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 10 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ethyl layer was discarded. The purification process repeated. 30 ml of n- butanol was added. The combined n-butanol extracts were washed twice with 5 ml of 5% of aquous sodium chloride. The remaining solution was heated in a water-bath. After evaporation the samples were dried in the oven to a constant wt; the saponin content was calculated as percentage.

3.8.2.3 Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1994)

Ten g of each plant's leaf sample was extracted with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through Sartorius

stedium, grade 292 filter paper. The filtrate was later transferred into crucible and evaporated in dryness over a water bath and weighted to a constant weight.

3.9 Preparation of extracts

A fixed weight (25 g) of dried powdered leaf samples of plant materials were soaked separately in 250 ml of methanol (95%) and distilled water for 72 h. Each mixture was stirred at 24 h interval using a sterile glass rod (Alagesaboopathi 2011). The plant samples squeezed and then filtered with the help of 3 layered cotton cloth. Water content of distilled water filtrate was evaporated on heating mantle using water bath till the solution reduced to semisolid form (Bhattarai and Shrestha 2009). The round bottom flask containing methanolic extract was fitted with rotary vaccum evaporator under negative pressure. The flask was constantly heated in rotating condition by using water bath below 55° C. Extracts were then transferred into sterile labeled bottles and they were made into semisolid form by evaporation to water bath at 50°C. The crude extracts were weighted and made the bottles air tight and stored in a refrigerator at temperature 4° C until further use (Mahida and Mohan 2007).

3.10 Antimicrobial test

3.10.1 Sterilization of equipment

Clean and clear petriplates were wrapped in newspaper and then put in hot air oven at 160°C for 2hrs for sterilization. Forceps and needle were sterilized in spirit flame. Laminar flow, spirit lamp, equipment box and other usable machines were sterilized by spirit. Used conical flasks, petriplates and beakers were sterilized in autoclave.

3.10.2 Test for Bacteria

3.10.2.1 Bacterial strain

Six different bacteria strains were selected. Among them 3 were Gram negative namely *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniaea* (ATCC 15380) *and Proteus mirabilis* (ATCC 49132) and 3 were Gram positive viz *Enterococcus faecalis* (ATCC 29212) *Bacillus subtilis* (ATCC6633) *and Staphylococcus aureus* (ATCC 25923). The bacterial strains

used for the test were brought from Department of Microbiology, Teaching hospital, Maharajgunj. They were taken on slants and later cultured in testtube having nutrient broth.

3.10.2.2 Antibacterial Test

Inhibition of bacterial growth was tested by using disc diffusion method (Peach and Tracey 1950) with some modifications according to the lab facility and the time. 24 hours old bacterial culture was taken in sterilized petriplates with the help of cotton swab and was suspended in 10 ml distilled water. This was used for the bacterial test.

3.10.2.3 Preparation of test discs

The test disc (5 mm diameter) was made by punching the filter paper Sartorious stedium 292. The discs were prepared by dipping and saturating sterilized punched filter paper in both positive and negative control and different concentrations of the plant extracts (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml). For negative control solvents i.e. methanol and distilled water were used. Ampicillin and Amoxicillin at 10 mg/ml were introduced as positive control (Oyedeji *et al*. 2010). The discs were air dried in the laminar flow.

3.10.2.4 Preparation of Nutrient Agar

Seven gram Nutrient Agar and 2 g Bacterial agar was dissolved in 250 ml of distilled water (HI media method recommended) by continuous stirring until the media started to boil. This was transferred into the conical flask and was sterilized by autoclaving at 15lbs pressure and 121˚C for 15 minutes. Then the media was transferred to laminar flow and let it cool down for some time then it was poured almost 10-10 ml in each sterilized petriplates and was left to solidify for 15-20 minutes.

3.10.2.5 Transfer of bacteria on petriplates

The bacterial strains to be tested were aseptically taken with the help of needle from primary cultured slants and were transferred to the sterilized petriplates containing nutrient broth and was inoculated overnight the incubator at 37˚C.

The Nutrient agar plates for the assay were prepared by drawing in each petriplates 6 chamber and labeling them with the date, code name of the bacteria and the concentrations code. The inoculums of bacteria were transferred into petriplates containing solidified media using sterile cotton swab. The sterile cotton swab was dipped into well mixed distilled water test culture and was spread on the media by moving the swab in Z –shape. One swab was used for singal bacterium. 7 replicates were used for each bacterium. The culture plates were allowed to dry for 5-10 minutes. Then in each petriplate in each chamber different concentrated and controls dics were put with the help of sterile forceps. Microbial growth was determined by measuring the diameter of zone of inhibition (ZOI).

3.10.3 Test for fungus

3.10.3.1 Preparation of strain

Potato Dextrose Agar (PDA) media was used for the fungal culture.100 g potato was sliced into small pieces and it was boiled into 250 ml distilled water until it became soft. This was then squeezed and filtered into a beaker using muslin cloth. The filtrate was slowly heated and 10 g Type 1 agar - agar and 10 g dextrose was added slowly and was completely dissolved. The final volume of PDA was made 500 ml by adding extra distilled water and then it was boiled. Then 5 ml of the prepared media was poured in a 20 ml of test tube and plugged the mouth using cotton plug. After that they were autoclaved at 15 lbs and 121°C for 15 minutes and let them cool in slanted position in sterilized laminar flow.

3.10.3.2 Fungal strain

Different species of fungi (*Alternaria brassicae, Botrytis cineria, Fusarium oxysporum, Phytophthora capsici* and *Scleretoium rolfsii*) obtained from Nepal Agricultural Research Council (NARC). They were brought on already made PDA slants.

3.10.3.3 Antifungal process

The antifungal activity of plant extracts was evaluated against food-associated fungi by using poisoned food technique applying the method of (Nene and Thapliyal 1979), with some modifications considering the access and availability of equipments and chemicals.

3.10.3.4 Preparation of Potato Dextrose Agar (PDA)

250 ml of PDA was prepared by taking 100 g freshly sliced potato in 250 ml of distilled water. The potato was boiled until it became soft. This was then filtered into a beaker using muslin cloth. The filtrate was slowly heated and 10 g Type 1 agar - agar and 10 g dextrose was added slowly and was completely dissolved. The final volume of PDA was made 500 ml by adding extra distilled water. The whole media was allowed to boil; this was then transferred into the conical flask and was sterilized by autoclaving at 15 lbs pressure and 121°C for 15 minute.

3.10.3.5 Subculture of fungus from slant

PDA when cooled at 50°C, 10 ml of it was then transferred into the sterilized each petriplate. The plates were then allowed to solidify for 15-20 minutes. With the help of sterilized needle each fungus mycelium transferred to already media contained and labeled petriplates. For each fungus culture needle was sterilized separately. Then the petriplates were put in incubator at (27°C) for 4 days.

3.10.3.6 Preparation of extract

Distilled water and methanol semisolid leaf extracts used for the preparation of concentrations viz. 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml. These concentrations were diluted in sterilized distilled water and methanol separately hence both distilled water and methanol were used as control.

3.10.3.7 Transfer of the fungal strains

Potato Dextrose Agar (PDA) when cooled at 50°C, a volume of 1 ml of each concentration was aseptically poured into the well labelled and sterilized petriplate followed by the addition of 9 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents (Singh and Singh 2013). The negative control plates received only sterilized distilled water or methanol in PDA without extract, while for positive control; fungiside Bavistin (Systemic fungiside) and Mancozeb (contact fungicide) were used. The plates were then allowed to solidify for 15-20 minutes. After solidification of PDA Mycelia discs of 5 mm diameter, were cut with sterilized needle from the periphery of 7 day old culture of the test organisms were aseptically inoculated upside down on the center of the PDA. Each time the needle was sterelized with flame and the whole process was done in a very asceptic and steralized condition. Seven replicates for each extract was incubated for 7 days at 27°C. The fungal growth was measured on the 7th day (Singh *et al.* 2013). Minimum and maximum readings of the colony diameter were taken using transparent millimetre ruler. The percentage of fungal growth inhibitation (growth in control – growth in treatment)/ growth in control x 100) was calculated using the formula given by Khalil and Dababneh (2007).

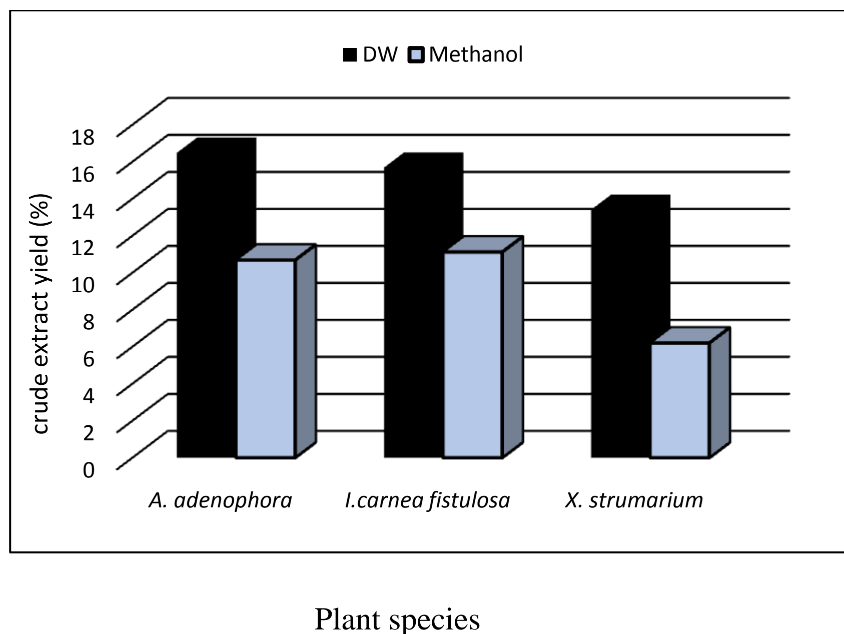
3.11 Data Analysis

The values were expressed as mean ± standard deviation (SD). Each value was a mean of seven replicates. The One – way Analysis of Variance (ANOVA) was used to determine the significant differences between the parameters and the Tukey HSD test was done to compare the differences of letters at $p < 0.05$ using statistical package SPSS version 16.

4. RESULTS

4.1 Yield of Methanol and Distilled water extracts of selected IAS

During the methanol and distilled water crude extraction of leaves, variation of extracts of IAS had resulted. The crude extracts of selected IAS plants prepared by soaking process. The weight and percentage yield of the crude extracts of both methanol and distilled water solvent is shown in fig. 1. The amount of extract varied among the plants. According to the result of this study, in methanol extract *Ipomoea carnea* ssp. *fistulosa* gave the highest yield (11.12%) followed by *Ageratina adenophora* (10.68%) and *Xanthium strumarium* (6.2%) while in distilled water extract *Ageratina adenophora* gave the highest yield (16.48%) followed by *I. carnea* spp. *fistulosa* (15.68%) and *X. strumarium* (13.4%). From both methanol and distilled water solvent the lowest yield was obtained from *X. strumarium* (Fig.1).



Plant species

Fig.1: Percentage yield of leaf extracts of selected IAS

4.2 Antimicrobial activity of selected IAS

The methanol and distilled water extracts of used part of different plants were prepared and antibacterial and antifungal activity were tested by disc diffusion method and poisoned food technique respectively. It is revealed from the tables that all the 3 plants have potential antimicrobial activity against one or more of the tested microorganisms.

4.2.1 Antimicrobial activity of *Ageratina adenophora*

4.2.1.1 Antibacterial activity

The antibacterial activity of D/W extract of *Ageratina adenophora* was ranged from (0 to 8 mm) among all 6 selected bacteria (Table 1). Among gram negative bacteria *Klebsiella pneumoniae* had the largest ZOI (7 mm to 8 mm) diameter from 200 to 250 mg/ml concentrations followed by *E.coli* (6 mm) from 150-250 mg/ml and no inhibition in *Proteus mirabilis*. Among gram positive bacteria *Enterococcus faecalis* showed the largest ZOI (6-8) mm from 50 mg/ml to 250 mg/ml followed by *Staphylococcus aureus* (6-7 mm) from 50 to 250 mg/ml and *Bacillus subtilis* (6 mm) at 250 mg/ml (Table1).

Table 1: Zone of Inhibition (ZOI) mm in distilled water leaf extract of *Ageratina adenophora*

The data were expressed as Mean ±S.D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each bacterial strain significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparision test, P< 0.05

Bacterial strain	**Zone of inhibition (ZOI) mm in methanol leaf extract of *Ageratina adenophora***									
	Concentrations (mg/ml)					control				
	50	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						DW	Ampi.	Amox.	P	F
K.p.	0±0 a	0±0 a	0±0 a	7±7 b	8±1 b	0±0 a	37±4 d	29±7 c	.000	155.65
E.f.	6±3 b	6±3 b	6±.2 b	7±.3 b	8±.3 b	0±0 a	34±.4 d	26±4 c	.000	359.58
E.coli	0±0 a	0±0 a	6±0 b	6±0 b	6±0 b	0±0 a	33±4 c	33±4 c	.000	304.51
B.s.	0±0 a	0±0 a	0±0 a	0±0 a	6±.2 a	0±0 a	28±7 b	24±7 b	.000	87.50
P. m.	0±0 a	0±0 a	0±0 a	0±0 a	0±3 a	0±0 a	27±1 c	21±1 b	.000	540.37
S.a.	6±.3 a	6±.2 a	7±.6 a	7±.8 a	7±.8 a	0±0 a	26±7 b	21±11 b	.000	32.72

Abbreviations:*K.p.=klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicates

The antibacterial activity of methanol extract of plant was ranged from (8-14 mm) diameter of all 6 selected bacteria. Among gram negative bacteria *Proteus mirabilis* showed the highest ZOI (11 to 14 mm) diameter from 50 to 250 mg/ml concentrations followed by *Klebsiella pneumoniae* (9 to 12) mm from 50 to 250 mg/ml. Among gram positive bacteria *Bacillus subtilis* showed the highest ZOI (10-13 mm) from 50 mg/ml to 250 mg/ml followed by *Staphylococcus aureus* (10-12) mm and *Enterococcus faecalis* (8-11mm). In negative control there was no ZOI observed,

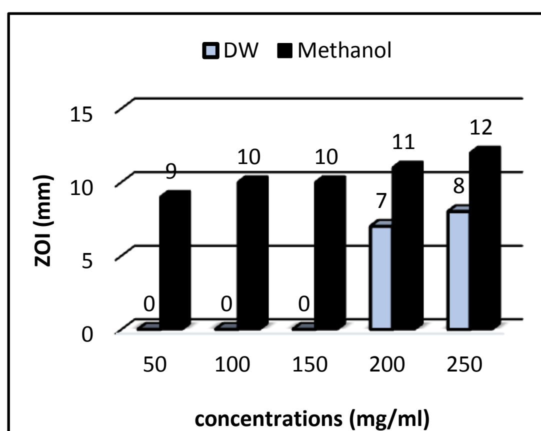
while the positive controls Amoxicillin and Ampicillin had the highest ZOI for all bacteria as compared to different concentrations (Table 2). ANOVA test showed that there was significant differences in mean value of ZOI of bacterial growth in different concentrations of plant leaf extract.

Table 2: Zone of Inhibition (ZOI) mm in methanol leaf extract of *Ageratina adenophora*

The data were expressed as Mean ±S.D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each bacterial strain significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparision test, P< 0.05

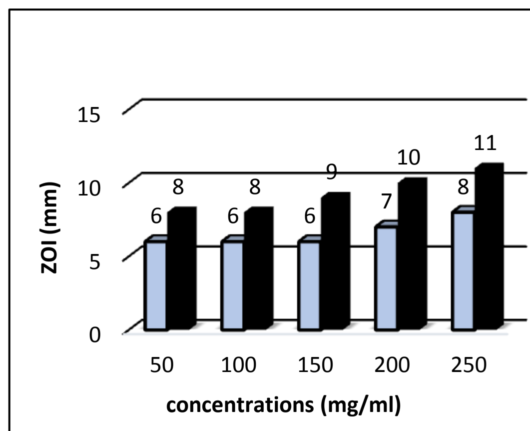
Zone of inhibition (ZOI) mm in methanol leaf extract of *Ageratina adenophora*										
	Concentrations(mg/ml)					control				
Bacterial strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						Meth.	Ampi.	Amox.	P	F
K.p.	9±1 b	10±.5 b	10±1 b	11±1 b	12±1 b	0±0 a	37±4 d	29±7 c	.000	97.15
E.f.	8±1 b	8±.5 b	9±1 b	10±1 b	11±1 b	0±0 a	34±.4 d	26±4 c	.000	250.64
E.coli	8±1 b	9±.5 b	10±1 b	11±1 b	12±1 b	0±0 a	33±4 c	33±4 c	.000	179.20
B.s.	10±.8 b	11±.6 b	12±.5 b	12±.5 b	13±.5 b	0±0 a	28±7 c	24±7 c	.000	45.57
P. m.	11±1 b	11±1 b	12±.7 b	13±.9 b	14±.9 b	0±0 a	27±1 d	21±1 c	.000	220.09
S.a.	10±.4 b	11±.6 b	12±.5 b	12±.6 b	12±.6 b	0±0 a	26±7 c	21±11 c	.000	24.72

Abbreviations:*K.p.=klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicates



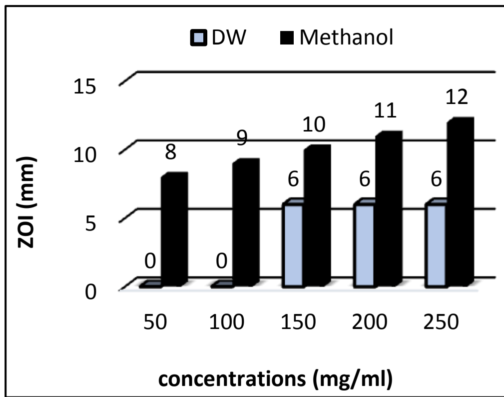
Concentrations (mg/ml) of leaf extracts of *A. adenophora*

Fig.2: Mean ZOI in *K.pneumoniae*, n=7



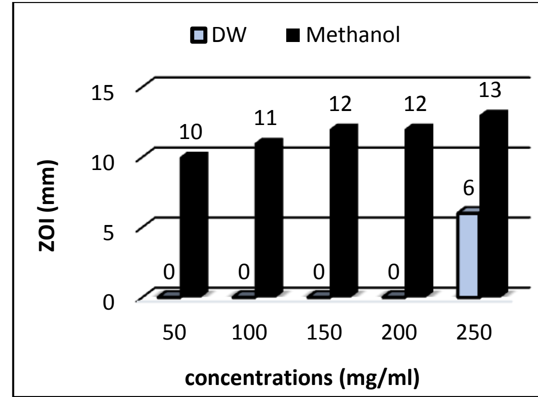
Concentrations (mg/ml) of leaf extracts of *A. adenophora*

Fig.3: Mean ZOI in *E. faecalis*, n=7



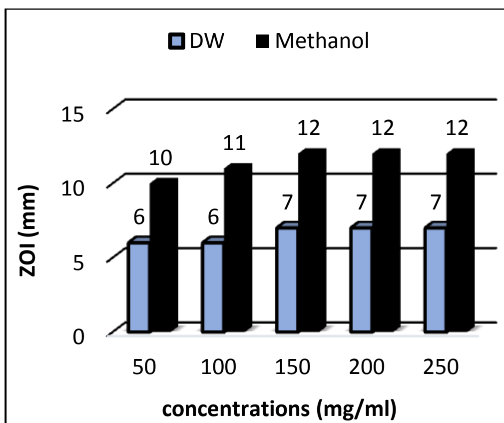
Concentrations (mg/ml) of leaf extracts of *A. adenophora*

Fig.4: Mean ZOI in *E. coli*, n=7



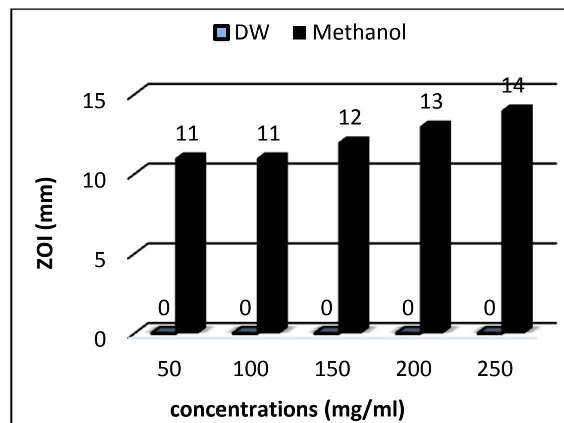
Concentrations (mg/ml) of leaf extract of *A. adenophora*

Fig.5: Mean ZOI in *B. subtilis*, n=7



Concentrations (mg/ml) of leaf extracts of *A. adenophora*

Fig. 6: Mean ZOI in *S. aureus,* n=7



Concentrations (mg/ml) of leaf extracts of *A. adenophora*

Fig. 7: Mean ZOI in *P. mirabilis*, n=7

4.2.1.2 Antifungal activity

Among five fungii D/W extract of *A. adenophora* ranged from (90-14 mm) diameter (Table 3). 90 mm was 100% because the diameter of petriplate was 90 mm. In distilled water leaf extracts *Phytopthora capsici* and *Sclerotium rolfsii* showed the highest LMG (90-30) mm diameter at (50-250) mg/ml concentrations and the least LMG shown by *Botrytis cinerea* (23-14) mm diameter at (50-250) mg/ml concentrations. There was two positive control (Bavistin and Mancozeb) used for all fungus. *Alternaria brassicae* had (37 to 21) mm at 150 to 250 mg/ml which was lesser than Bavistin (44 mm). *B. cinerea* had 15-14 mm at (200-250 mg/ml) lesser than Bavistin (16 mm), *P. capsici* had 72 mm at 200 mg/ml, lesser than Bavistin (90 mm). *S.rolfsii* had 30 mm at 250 mg/ml, lesser than Bavistin (46 mm) Table 3.

Table 3: Linear mycelium growth (mm) in distilled water crude leaf extract of *Ageratina adenophora*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strain significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05

Linear mycelium growth (mm) in D/W leaf extract of *Ageratina adenophora*										
	Concentrations (mg/ml)					control				
	50	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
Fungal strain						DW	Bavistin	Mancozeb	P	F
A.b.	56±3 d	46±4 c	37±1 b	33±2 b	21±2 a	72±6 e	44±1 c	21±1 a	.000	200.64
B.c.	23±1 e	21±.8 d	20±1 d	15±.8 bc	14±.5 ab	27±1 f	16±.7 c	13±1 a	.000	186.78
F.o.	43±.7 c	42±1 c	41±1 c	36±1 b	15±1a	76±3 d	13±1 a	15±.9 a	.000	1.21
P.c.	90±0 c	90±0 c	90±0 c	72±4 b	30±7 a	90±0 c	90±0 c	33±2 a	.000	535.07
S.r.	90±0 e	90±0 e	90±0 e	73±4 d	30±7 b	90±0 e	46±1 c	22±2 a	.000	645.91

Abbreviations: *A.b.=Alternaria brassicae, B.c.=Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii,* values are mean ± SD of seven replicates.

In methanolic extract of *Ageratina adenophora*, *S. rolfsii* showed the highest LMG (90 mm-24 mm) diameter at 50-250 mg/ml concentrations and 90 mm was 100% because the diameter of petriplate was 90 mm. The lowest mycelium growth was observed for *P. capsici and F.oxysporum,* 0 mm at higher concentrations Table 4. As compared to positive controls (Bavistin and Mancozeb), *Alternaria brassicae* had LMG (33 mm-13 mm) at 50 mg/ml-250 mg/ml which was lower than Bavistin (45 mm) and Mancozeb (21mm). *B. c*inerea had (12 mm -9 mm) at 100-250 mg/ml which was lower than Bavistin (16 mm) and Mancozeb (13 mm). *F. oxysporum* was found (13 mm -0 mm) at 150 mg/ml-250 mg/ml which was similar and lower than Bavistin (13mm) and Mancozeb (15mm) respectively. *P.capsici* had (71-0) mm at 250-250 mg/ml which was lower than Bivastin (90 mm) and Mancozeb (32 mm). *S. rolfsii* was found at 200 mg/ml -250 mg/ml LMG (37 mm -24 mm), lower than Bivastin (46 mm) Table 4. ANOVA result showed that there was significant differences, P<0.05 in mean value of LMG of fungi in different concentrations of plant leaf extract (Table 4).

Table 4: Linear mycelium growth (mm) in methanol leaf extract of *Ageratina adenophora*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strain significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05

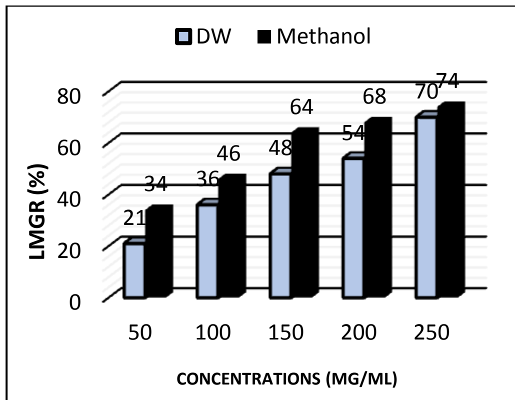
Linear mycelium growth (mm) in methanol leaf extract of *Ageratina adenophora*										
	Concentrations (mg/ml)					control				
Funga strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**		P	F
						Methanol	Bavistin	Mancozeb		
A.b.	33±1 e	27±.6 d	18±.3 b	16±.3 ab	13±.7 a	50±4 g	45±1 f	21±1 c	.000	424.68
B.c.	16±.6	12±.6	12±.4	11±.6	9±.9	22±1	16±.7	13±1	.000	157.97
F.o.	24±.6 e	16±.4 d	13±.5 bc	12±.8 b	0±0 a	31±4 f	13±1 bc	15±.9 cd	.000	181.81
P.c.	71±2 d	27±2 b	0±0 a	0±0 a	0±0 a	90±0 e	90±0 e	32±1 c	.000	5.89
S.r.	90±0 e	83±2 e	64±11 d	37±9 b	24±3 a	90±0 e	46±1 c	21±1 a	.000	206.63

Abbreviations: *A.b.=Alternaria brassicae, B.c.=Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii*, values are mean ± SD of seven replicates.

4.2.1.3 LMGR of *Ageratina adenophora* at different concentrations

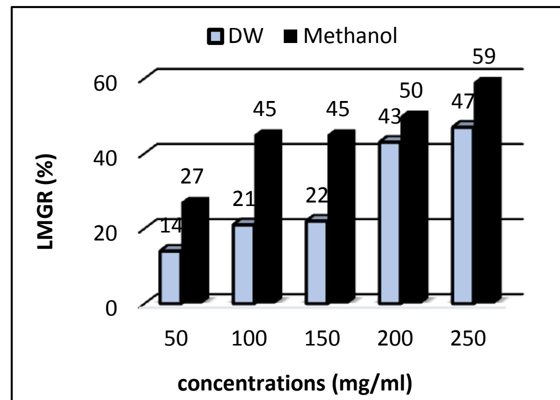
A. brassicae had 21%-70% LMGR and 34%-74% LMGR in distilled water and methanol extract respectively at concentrations 50 mg/ml-250 mg/ml (Fig. 8). *B. cinerea* had (14%-47%) LMGR and (27%-59%) LMGR in distilled water and methanol respectively (Fig. 9). *F. oxysporum* had (44%-79%) LMGR and (22%-100%) LMGR in distilled water and methanol respectively (Fig. 10). No LMGR was found in *Phytophthora capsici* in distilled water crude extract at 50 mg/ml-150 mg/ml respectively, while (19%-65%) was observed at 200 mg/ml-250 mg/ml. In methanol leaf extract *Phytophthora capsici* had LMGR (21%-70%) at 50 mg/ml and 100 mg/ml while from 150 mg/ml to 250 mg/ml 100% reduction was found (Fig.11). *S. rolfsii* had no LMGR at 50 mg/ml to 150 mg/ml in D/W leaf extract but at 200 mg/ml-250 mg/ml (19% and 65%) LMGR was observed. In methanol leaf extract at 50 mg/ml there was no LMGR found while from 100 mg/ml to 250 mg/ml LMGR was observed (7%-73%) Fig.12. *Ageratina adenophora* was found most effective against *Phytophtora capsici* at 150 mg/ml to 250 mg/ml by inhibition the growth 100% in methanol with the increasing concentrations of both methanol and distilled water

plant extract the linear mycelium growth reduction was increased. Distilled water extract had lower LMGR percentage than methanol extract at all concentrations for all fungi except *F. oxysporum* which was found with higher LMGR (44%-79%) in distilled water crude extract than LMGR (22%-67%) in methanol crude extract of plant at all concentrations (Fig. 10).



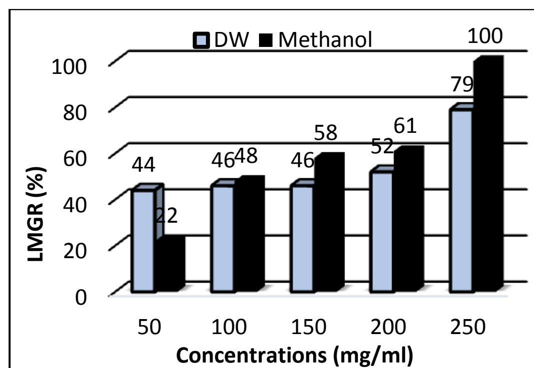
Concentrations (mg/ml) of leaf extracts in *A.adenophora*

Fig.8: Mean LMGR percentage in *A. brassicae*, n=7



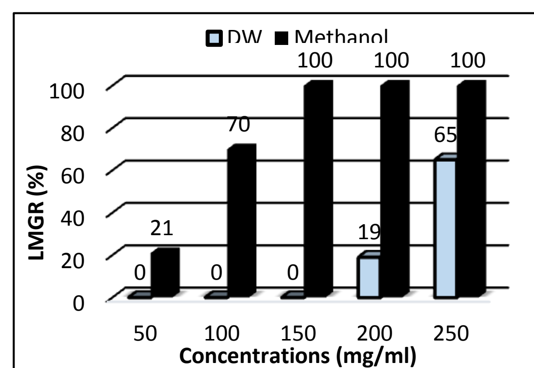
Concentrations (mg/ml) of leaf extracts in *A.adenophora*

Fig.9: Mean LMGR percentage in *B. cinerea*, n=7



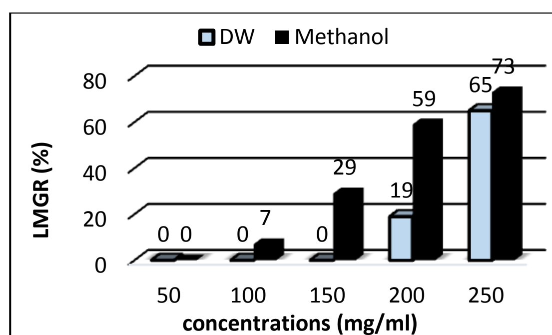
Concentrations (mg/ml) of leaf extracts in *A.adenophora*

Fig.10: Mean LMGR percentage in *F. oxysporum*, n=7



Concentrations (mg/ml) of leaf extracts in *A.adenophora*

Fig.11: Mean LMGR percentage in *P. capsici*, n=7



Concentrations (mg/ml) of leaf extracts in *A.adenophora*

Fig.12: Mean LMGR percentage in *S.rolfsii*, n=7

4.2.2 Antimicrobial activity of *Ipomoea carnea* ssp. *fistulosa*

4.2.2.1 Antibacterial activity

The ZOI of D/W crude leaf extract of *Ipomoea carnea* ssp. *fistulosa* was ranged from (0 to 12) mm diameter on all selected bacteria. Among gram negative bacteria *P. mirabilis* showed the highest zone of inhibition (6 mm -10 mm) diameter at 100 mg/ml – 250 mg/ml concentrations followed by *K. pneumoniae* (6 mm – 7 mm) diameter at 50 mg/ml to 250 mg/ml concentrations and the lowest zone of inhibition showed by *E. coli* (6 mm) diameter at 250 mg/ml concentration. Among gram positive bacteria *S. aureus* showed the highest zone of inhibition (6 mm -12 mm) diameter at 50 mg/ml to 250 mg/ml concentrations followed by *E. faecalis* (6 mm-11mm) diameter at 50 mg/ml to 250 mg/ml concentrations and *B. subtilis* (6 mm-9 mm) diameter at 100 mg/ml to 250 mg/ml concentrations. ANOVA test showed that there was significant differences in mean value of ZOI of bacterial growth in different concentrations of plant leaf extract (Table 5).

Table 5: Zone of Inhibition (ZOI) mm in distilled water leaf extract of *Ipomoea carnea* ssp. *fistulosa*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each bacterial strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05

Zone of inhibition (ZOI) mm in DW leaf extract of *Ipomoea carnea* ssp. *fistulosa*										
	Concentrations (mg/ml)					control				
Bacterial strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						DW	Ampi.	Amox.	P	F
K.p.	6±.4 b	6±.1 b	6±.3 b	7±.6 b	7±.8 b	0±0 a	37±4 d	29±7 c	.000	125.39
E.f.	6±.3 b	7±.8 bc	8±1 bc	10±.8 cd	11 ±1 d	0±0 a	34±.4 f	26±4 e	.000	282.99
E.coli	0±0 a	0±0 a	0±0 a	0±0 a	6±2 b	0±0 a	33±4 c	33±4 c	.000	293.65
B.s.	0±0 a	6±2 b	7±.1 b	9±.6 b	9±.6 b	0±0 a	28±7 c	24±7 c	.000	65.39
P. m.	0±0 a	6±.5 b	7±.2 bc	8±.7 bc	10±1 c	0±0 a	27±1 e	21±1 d	.000	348.32
S.a.	6±.7 ab	8±1 ab	9±1 b	10±1 b	12±1 b	0±0 a	26±7 c	21±11 c	.000	27.95

Abbreviations:*K.p.=klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicates

The ZOI of Methanol crude leaf extract of plant was ranged from (7 mm-14 mm) diameter of all 6 selected bacteria. Among gram negative bacteria *Escherichia coli* showed the highest zone of inhibition (11mm – 14 mm) diameter at 50 mg/ml to 250 mg/ml concentrations followed by *Proteus mirabilis* (7 mm-12 mm) diameter at 50 mg/ml to 250 mg/ml concentrations and *Klebsiella pneumoniae* showed the lowest ZOI (9 mm -11mm) diameter at 50 mg/ml to 250 mg/ml concentrations. Among gram positive bacteria, *E. faecalis* showed the highest ZOI (12 mm – 14 mm) diameter at 50 mg/ml to 250 mg/ml concentrations followed by *Bacillus subtilis* (11mm-13 mm) diameter at 50 mg/ml to 250 mg/ml concentrations and *S. aureus* (8 mm - 11mm) diameter at 50 mg/ml to 250 mg/ml concentrations. Bacterial growth was inhibited by increasing concentration from 50 mg/ml to 250 mg/ml (Table 6).

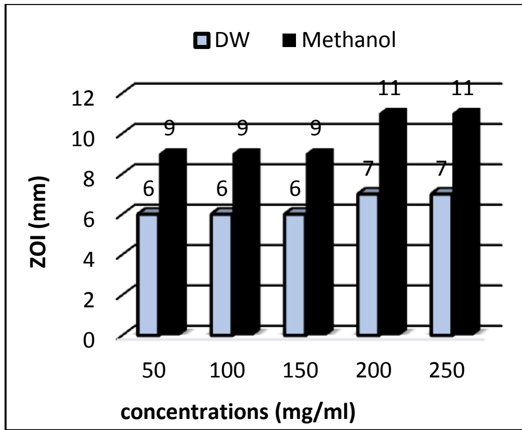
In negative control there was no ZOI observed, while the positive controls Amoxicillin and Ampicillin had higher ZOI for all bacterial strain growth as compared with different concentrations (of both methanol and DW solvents) of plant extracts (Tables 5 & 6). ANOVA test showed that there was significant differences in mean value of ZOI of bacterial growth in different concentrations of plant leaf extract.

Table 6: Zone of Inhibition (ZOI) mm in methanol leaf extract of *Ipomoea carnea* ssp. *fistulosa*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each bacterial strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05

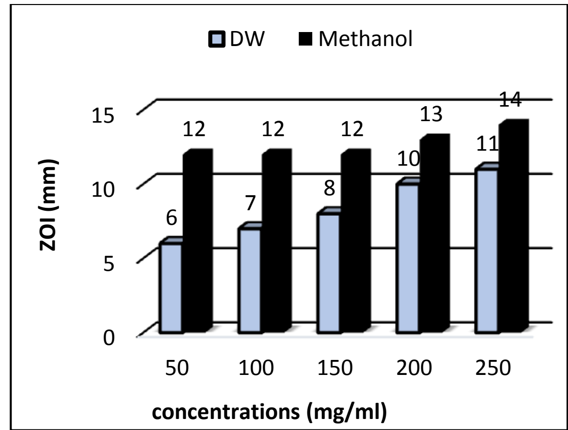
Zone of inhibition (ZOI) mm in methanol leaf extract of *Ipomoea carnea* ssp. *fistulosa*										
	Concentrations (mg/ml)					control				
Bacterial strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						Meth.	Ampi.	Amox.	P	F
K.p.	9±2 b	9±2 b	9±2 b	11±1 b	11±1 b	0±0 a	37±4 d	29±7 c	.000	88.00
E.f.	12±1 b	12±.8 b	12±1 b	13±1 b	14±.8 b	0±0 a	34±.4 d	26±4 c	.000	223.31
E.coli	11±2 b	13±1 b	13±1 b	13±1 b	14±1 b	0±0 a	33±4 c	33±4 c	.000	135.72
B.s.	11±.8 b	12±1 b	12±1 b	13±.8 b	13±1 b	0±0 a	28±7 c	24±7 c	.000	43.06
P. m.	7±.9 b	10±2 bc	11±2 bc	11±1 c	12±1 c	0±0 a	27±1 e	21±1 d	.000	161.19
S.a.	8±1 ab	9±1 b	9±1 b	10±1 b	11±1 b	0±0 a	26±7 c	21±11 c	.000	26.81

Abbreviations:*K.p.=klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicates



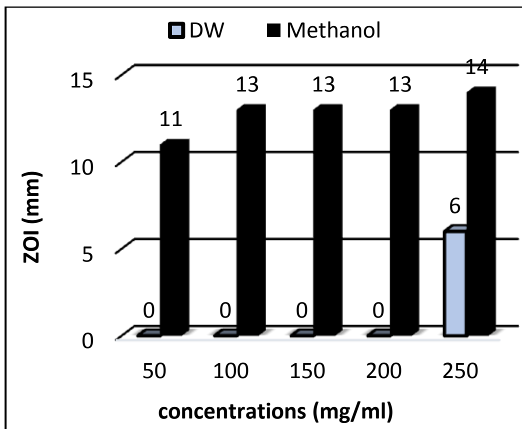
Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

Fig.13: Mean ZOI in *K.pnemoniae,* n=7



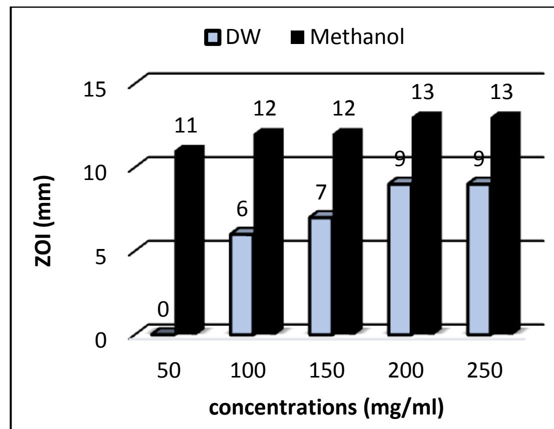
Concentrations (mg/ml) of leaf extracts of *I. carnea fistulosa*

Fig.14: Mean ZOI in *E.faecalis,* n=7



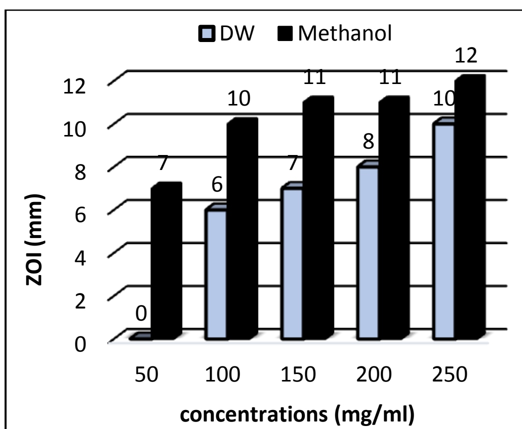
Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

Fig.15: Mean ZOI in *E.coli*, n=7



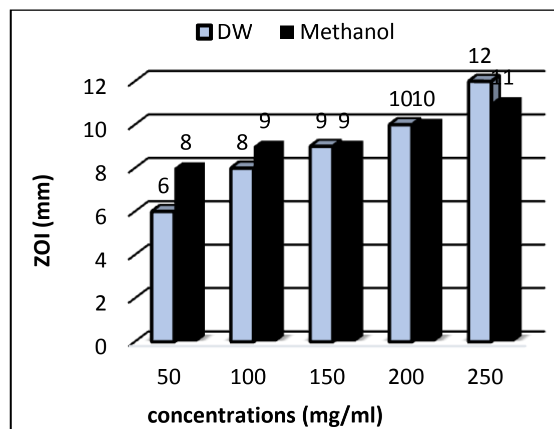
Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

Fig.16: Mean ZOI in *B.subtilis*, n=7



Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

Fig.17: Mean ZOI in *P.mirabilis*, n=7



Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

Fig.18: Mean ZOI in *S.aureus*, n=7

4.2.2.2 Antifungal activity

Linear Mycelium Growth Reduction of D/W extract of *Ipomoea carnea* ssp. *fistulosa* ranged from (90-11 mm) diameter at 50 mg/ml to 250 mg/ml concentrations among selected 5 fungi Table 7. *Phytophthora capsici* and *Sclerotium rolfsii* showed the highest LMG (0 mm -90 mm) at 50 mg/ml to 250 mg/ml and *B. cinerea* showed the lowest LMG (25-17 mm) at concentrations 50 mg/ml to 250 mg/ml.

As compared with positive controls (Bavistin and Mancozeb), *A. brassicae* showed LMG (43 mm- 27 mm) at 100 mg/ml to 250 mg/ml concentrations which was lower than Bavistin (45 mm) diameter. *Fusarium oxysporum* had lower mycelium growth 11 mm at 250 mg/ml than both bavistin (13 mm) and Mancozeb (15 mm) Table 7.

Table 7: Linear mycelium growth (mm) in distilled water crude leaf extract of *Ipomoea carnea* ssp. *fistulosa*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05.

Linear mycelium growth (mm) in D/W leaf extract of *Ipomoea carnea* ssp. *fistulosa*										
	Concentrations (mg/ml)					control				
Fungal strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						DW	Bavistin	Mancozeb	P	F
A.b.	47±1 e	43±4 de	38±.5 cd	34±1 c	27±.4 b	72±6 f	45±1 e	21±1 a	.000	192.26
B.c.	25±1 e	22±.7 d	21±.9d	19±.7 c	17±.8 a	27±1 f	16±.7 b	14±1 a	.000	171.69
F.o.	51±.6 f	40±1 e	29±1 d	21±1 c	11±1 a	77±3 g	13±1 ab	15±.9 b	.000	1.13
P.c.	90±0 b	90±0 b	90±0 b	90±0 b	90±0 b	90±0 b	90±0 b	32±1 a	.000	7.61
S.r.	90±0 c	90±0 c	90±0 c	90±0 c	90±0 c	90±0 c	46±1 b	21±1 a	.000	9.79

Abbreviations: *A.b.=Alternaria brassicae, B.c.=Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii*, values are mean ± SD of seven replicates.

LMGR of Methanol extract of *Ipomoea carnea* ssp. *fistulosa* ranged from 90 mm – 0 mm diameter at 50 mg/ml to 250 mg/ml concentrations among selected 5 fungal strain (Table 8). *Phytophthora capsici* showed the highest LMG (90 mm -83 mm) diameter at 50 mg/ml to 250 mg/ml concentrations respectively, and the lowest LMG observed in *Fusarium oxysporum* (20 mm-11mm) at 50 mg/ml-150 mg/ml and at 200-250 mg/ml concentrations the mycelium growth of fungus completely inhibited. As

compared with positive controls, *A. brassicae* showed LMG (31 mm) at 50 mg/ml which was lower than Bavistin (44 mm) diameter and at 250 mg/ml concentrations, (18 mm) lower than Mancozeb (21 mm). *B. cinerea* had lower mycelium growth (15 mm) at 200 mg/ml than Bavistin (16 mm) diameter. *Fusarium oxysporum* had lower LMG (11mm) diameter at 150 mg/ml concentration than Bavistin (13 mm). *P. capsici* showed 83 mm diameter at 250 mg/ml concentration which was lower than Bavistin (90 mm). ANOVA result showed that there was significant differences in the mean value of LMG of fungi in different concentrations of plant leaf extract (Table 8).

Table 8: Linear mycelium growth (mm) in methanol leaf extract of *Ipomoea carnea* ssp. *fistulosa*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, $P < 0.05$.

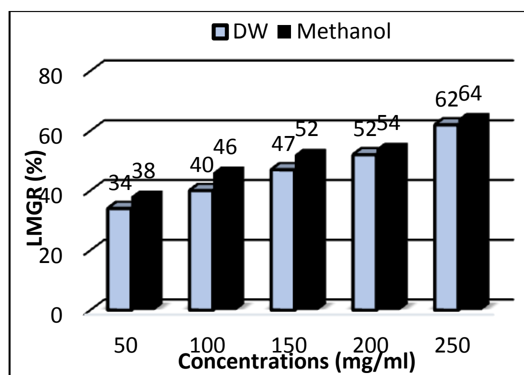
Linear mycelium growth (mm) in methanol leaf extract of *Ipomoea carnea* ssp. *fistulosa*										
	Concentrations (mg/ml)					control				
Fungal strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						Meth.	Bavistin	Mancozeb	P	F
A.b.	31±.8 e	27±1 d	24±1 c	23±.6 bc	18±.6 a	50±4 g	44±1 f	21±1 b	.000	297.76
B.c.	20±.6 e	18±.9 de	17±.6 cd	15±.7 b	13±.6 a	22±1 f	16±.7 bc	13±1 a	.000	88.22
F.o.	20±1 d	15±.7 c	11±.5 b	0±0 a	0±0 a	31±4 e	13±1 bc	15±.9 c	.000	219.31
P.c.	90±0 c	90±0 c	90±0 c	90±0 c	83±7 b	90±0 c	90±0 c	32±1 a	.000	403.92
S.r.	90±0 d	90±0 d	90±0 d	85±2 d	70±6 c	90±0 d	46±1 b	21±1 a	.000	667.41

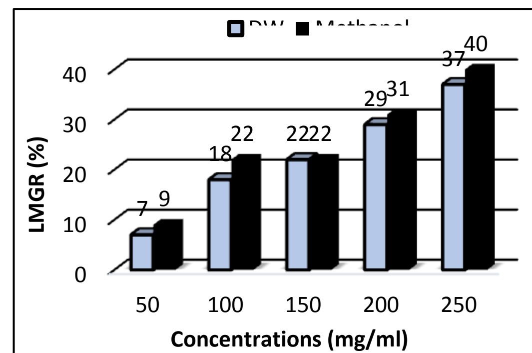
Abbreviations: *A.b.=Alternaria brassicae, B.c.=Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii*, values are mean ± SD of seven replicates.

4.2.2.3 Linear mycelium Growth Reduction Percentage of *Ipomoea carnea* ssp. *fistulosa*

A. brassicae had 38%-64% LMGR percentage in methanol extract of *I. carnea* ssp. *fistulosa* and 34%-62% LMGR percentage in D/W extract at concentrations 50 mg/ml-250 mg/ml respectively. *Botrytis cinerea* had 9%-40% and 7%-37% LMGR percentage in methanolic extract and distilled extract respective. *F. oxysporum* had 35%-100% and 33%-86% LMGR percentage in methanolic extract and distilled water extract respectively. No LMGR percentage was observed in *P. capsici* in distilled water leaf crude extract at all concentrations while in methanol 7% was observed at

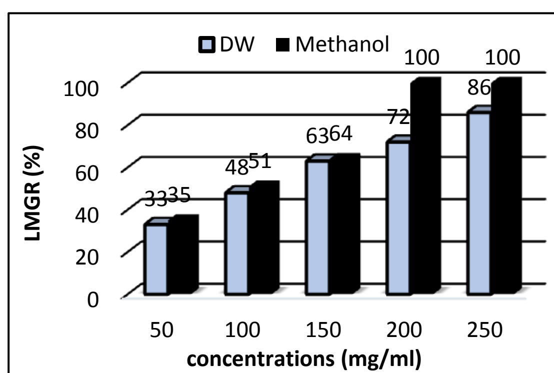
250 mg/ml. *S. rolfsii* had no LMGR percentage at 50 mg/ml-250 mg/ml in D/W while in methanol 5%-22% LMGR observed at 200 mg/ml- 250 mg/ml concentrations. *Ipomoea carnea* ssp. *fistulosa* was found the most effective against *F. oxysporum* by inhibiting 100% LMGR in methanolic extract. Distilled water extract also found effective against *F. oxysporum* by inhibiting it 85% at 250 mg/ml concentration. With the increasing concentration of both methanol and distilled water leaf crude extract the linear mycelium growth reduction was also increased. The methanolic extract was found higher than distilled water for LMGR at all concentration for all fungi (Figures 19-23).

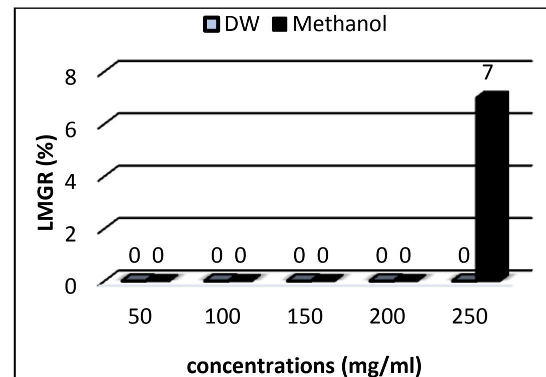




Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa* Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

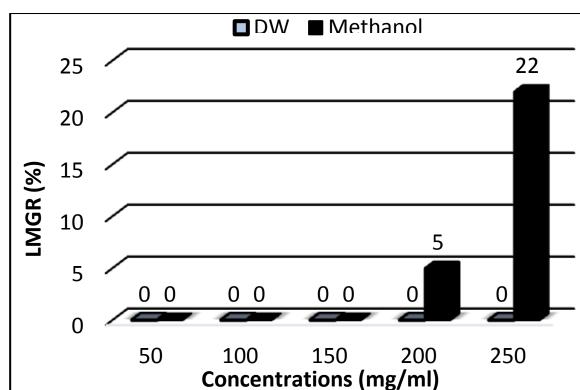
Fig.19: Mean LMGR percentage in *A.brassicae*, n=7 Fig.20: Mean LMGR percentage in *B.cinerea*, n=7





Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa* Concentrations (mg/ml) of leaf extracts of *I.carnea fistulosa*

Fig.21: Mean LMGR percentage in *F. oxysporum*, n=7 Fig.22: Mean LMGR percentage in *P.capsici*, n=7



Concentrations (mg/ml) of leaf extracts of *I.carnea* ssp. *fistulosa*

Fig.23: Mean LMGR percentage in *S. rolfsii*, n=7

4.2.3 Antimicrobial activities of *Xanthium strumarium*

4.2.3.1 Antibacterial activity

Zone of inhibition (ZOI) of D/W crude leaf extracts of *Xanthium strumarium* ranged from 0 mm to 17 mm diameter at 50 mg/ml-250 mg/ml concentrations respectively. Among gram negative bacteria *Proteus mirabilis* had ZOI (6 mm diameter) at 50 mg/ml to 250 mg/ml concentration and remaining two bacterial strain of *Klebsiella pneumonia* and *E. coli* was not inhibited by any concentration of plant.

Among gram positive bacteria *Bacillus subtilis* had the highest ZOI (12 mm-17mm) diameter at 50 mg/ml-250 mg/ml concentrations respectively followed by *Enterococcus faecalis* (9 mm-16 mm) at 50 mg/ml-250 mg/ml and *Staphylococcus aureus* had showed the lowest ZOI 6 mm diameter at 200 mg/ml-250 mg/ml concentrations. With the increasing concentrations of plant extracts the ZOI increased. ANOVA test showed that there were significant differences in mean value of ZOI of bacterial growth of different concentration of plant leaf extract (Table 9).

Table 9: Zone of Inhibition (ZOI) mm in distilled water leaf extract of *Xanthium strumarium*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each bacterial strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05.

Zone of inhibition (ZOI) mm in distilled water leaf extract of *Xanthium strumarium*										
	concentrations					control				
Bacterial strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						DW	Ampi.	Amox.	P	F
K.p.	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	37±4 c	29±7 b	.000	179.98
E.f.	9±1 b	11±2 bc	12±3 bc	14±4 bc	16±4 c	0±0 a	34±.4 e	26±4 d	.000	78.51
E.coli	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	33±4 b	33±4 b	.000	363.03
B.s.	12±1 b	13±1 b	14±2 b	16±2 b	17±2 b	0±0 a	28±7 b	24±7 b	.000	34.53
P. m.	6±0 b	6±0 b	6±0 b	6±0 b	6±.2 b	0±0 a	27±1 d	21±1 c	.000	380.49
S.a.	0±0 a	0±0 a	0±0 a	6±1 a	6±.3 a	0±0 a	26±7 b	21±11 b	.000	45.83

Abbreviations:*K.p.=klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicates.

ZOI of methanol crude leaf extract of *X. strumarium* ranged from (0 mm-30 mm) diameter at 50 mg/ml-250 mg/ml concentrations respectively. Among gram negative bacteria *K. pneumoniae* showed the largest (25 mm-30 mm) diameter at 50 mg/ml-250 mg/ml concentrations followed by *Proteus mirabilis* (6 mm-8 mm) diameter at 50 mg/ml-250 mg/ml concentrations. *E. coli* had (0 mm) which was not inhibited by any concentrations of plant leaf extracts.

Among gram positive bacteria *Bacillus subtilis* had showed the highest zone of inhibition (25 mm-28 mm) diameter at 50 mg/ml-250 mg/ml concentration followed by *Staphylococcus aureus* (25 mm-27 mm) at 50 mg/ml-250 mg/ml respectively and *Enterococcus faecalis* showed the lowest ZOI (9 mm-11mm) diameter at 50 mg/ml-250 mg/ml concentrations. With the increasing concentrations of plant leaf extracts the ZOI increased. In negative control there was no ZOI observed. As compared with both positive controls Ampicillin and Amoxicillin, *Klebsiella pneumoniae* had ZOI 30 mm diameter at 250 mg/ml concentration which was higher than ZOI of Amoxicillin (29 mm) diameter, *Bacillus subtilis* had higher ZOI at all concentrations (25 mm-28 mm) than Amoxicillin (24 mm). *S. aureus* had (25 mm-27 mm) diameter at 50 mg/ml-250 mg/ml concentrations. The lowest value had higher ZOI than Amoxicillin

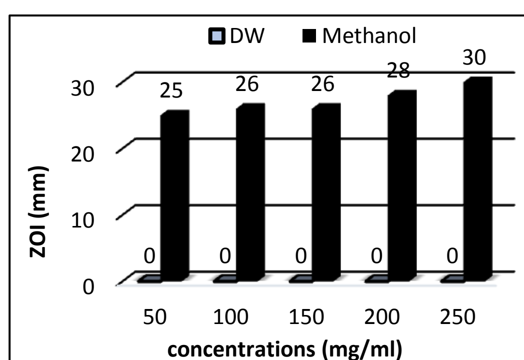
(21mm) diameter. At concentrations 250 mg/ml ZOI was higher than Ampicillin (26 mm) diameter. Methanol extract showed more effect on bacteria than D/W. Gram negative bacteria was found more resistant than gram positive bacteria (Tables 9 &10). ANOVA test showed that there was significant difference ($p<0.05$) in mean value of ZOI of bacterial growth in different concentration of plant leaf extract (Table 10).

Table 10: Zone of Inhibition (ZOI) mm in methanol leaf extract of *Xanthium strumarium*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05.

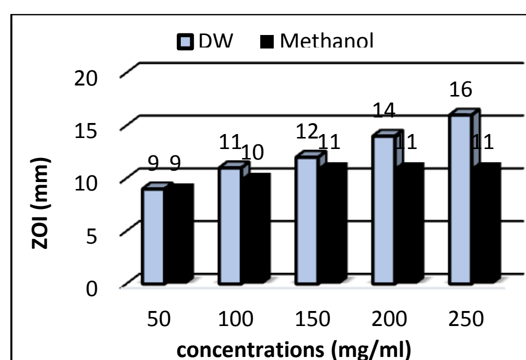
Zone of inhibition (ZOI) mm in methanol leaf extract of *Xanthium strumarium*										
	Concentrations (mg/ml)					Control				
Bacterial strail	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						Meth.	Ampi.	Amox.	P	F
K.p.	25±.1 b	26±1 b	26±1 b	28±1 b	30±1 b	0±0 a	37±4 c	29±7 b	.000	67.79
E.f.	9±.9 b	10±.9 b	11±1 b	11±.7 b	11±1 b	0±0 a	34±.4 d	26±4 c	.000	240.03
E.coli	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	33±4 b	33±4 b	.000	363.23
B.s.	25±.7 b	26±.5 b	26±.7 b	27±.6 b	28±.4	0±0 a	28±7 b	24±4 b	.000	39.76
P. m.	6±0 b	6±0 b	6±.4 b	7±.3 b	8±.2 b	0±0 a	27±1 d	21±1 c	.000	360.81
S.a.	25±.7 b	25±.6 b	26±1 b	26±1 b	27±1 b	0±0 a	26±7 b	21±11 b	.000	23.49

Abbreviations:*K.p.=klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicate



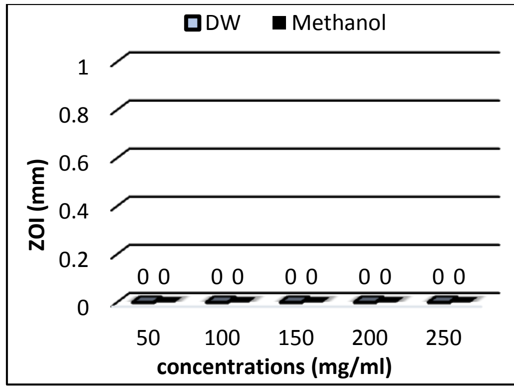
Concentrations (mg/ml) of leaf extracts of *X.strumarium*

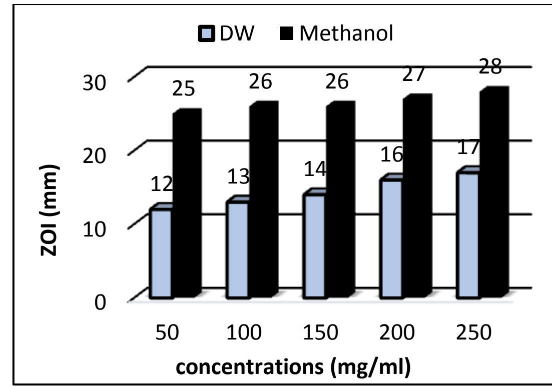
Fig.24: Mean ZOI in *K. pneumoniae,* n=7



Concentrations (mg/ml) of leaf extracts of *X.stumarium*

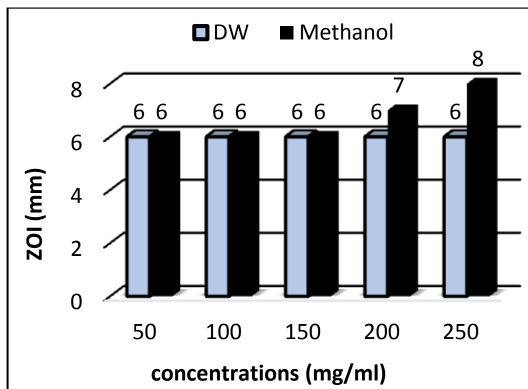
Fig.25: Mean ZOI in *E. faecalis,* n=7

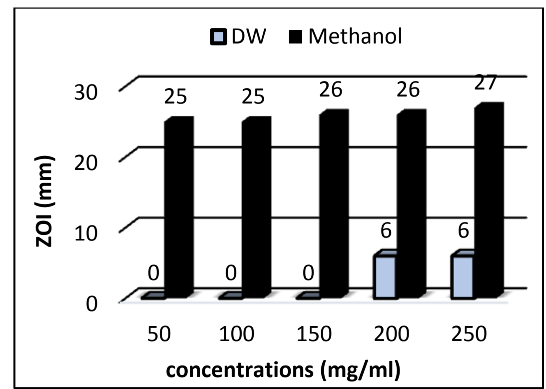




Concentrations (mg/ml) of leaf extracts of *X.strumarium*
Fig.26: Mean ZOI in *E. coli*, n=7

Concentrations (mg/ml) of leaf extracts of *X.strumarium*
Fig.27: Mean ZOI in *B. subtilis*, n=7





Concentrations (mg/ml) of leaf extracts of *X.strumarium*
Fig.28: Mean ZOI in *P. mirabilis*, n=7

Concentrations (mg/ml) of leaf extracts of *X.strumarium*
Fig.29: Mean ZOI in *S. aureus*, n=7

4.2.3.2 Antifungal activity

Linear mycelium growth reduction (LMGR) of D/W extract of *X. strumarium* ranged from 90 mm-13 mm diameter at 50 mg/ml-250 mg/ml concentrations respectively. Among fungi *P. capsici* showed the highest LMG (90 mm) diameter at all concentrations and *B. cinerea* showed the lowest LMG (24 mm-13 mm) diameter at 50 mg/ml-250 mg/ml concentrations respectively (Table11).

As compared with positive controls (Bavistin and Mancozeb), *A. brassicae* had (40 mm, 39 mm, 28 mm) diameter at 150 mg/ml-250 mg/ml concentrations respectively which was lower LMG than Bavistin which was (44 mm). *Botritis cinerea* had (15 mm, 14 mm and 13 mm) diameters a LMG at 150 mg/ml-250 mg/ml concentrations which was lower LMG than Bavistin (16mm). ANOVA result showed there was significant differences in mean value of LMG of fungi in different concentration of plant leaf extract (Table 11).

Table 11: Linear mycelium growth (mm) diameter in distilled water crude leaf extract of *Xanthium strumarium*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, $P < 0.05$.

Linear mycelium growth (mm) in D/W leaf extract of *Xanthium strumarium*										
	Concentrations(mg/ml)					control				
Fungal strain	**50**	**100**	**150**	**200**	**250**	**Negative** DW	**Positive** Bavistin	Mancozeb	P	F
A.b.	61±2 e	57±3 e	40±1 cd	39±2 c	28±3 b	72±6 f	44±1 d	21±1 a	.000	204.55
B.c.	24±2 c	17±3 b	15±3 ab	14±.8 a	13±.9 a	27±c	16±.7 ab	13±1 a	.000	55.8
F.o.	72±2 d	60±9 c	32±1 b	27±1 b	16±2 a	76±3 d	13±1 a	15±.9 a	.000	326.06
P.c.	90±0 b	90±0 b	90±0 b	90±0 b	90±0 b	90±0 b	90±0 b	32±1 a	.000	7.61
S.r.	90±0 e	90±0 e	90±0 e	86±.4 d	84±.9 c	90±0 e	46±1 b	21±1 a	.000	7.56

Abbreviations: *A.b.=Alternaria brassicae, B.c.=Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii,* values are mean ± SD of seven replicates.

Linear mycelium growth reduction (LMGR) of methanol crude leaf extract of *X. strumarium* ranged from 90 mm-0 mm diameter at 50 mg/ml-250 mg/ml concentrations respectively among all fungi. *Phytophthora capsici* showed the highest LMG ranged from 90 mm-27 mm diameter at 50 mg/ml-150 mg/ml concentrations while from 200 mg/ml-250 mg/ml the growth of fungus completely inhibited by plant extract, followed by *Sclerotium rolfsii* ranged from 87 mm-80 mm diameter at 50 mg/ml-100 mg/ml concentrations but at 150 mg/ml-250 mg/ml concentrations the growth was completely inhibited. The lowest LMG was observed in *Botrytis cinerea* (16 mm-10 mm) at 50 mg/ml-250 mg/ml (Table 12).

In the comparison of positive controls Bavistin and Mancozeb with different concentrations of plant extract, *A. brassicae* had mycelium growth 37 mm diameter at 50 mg/ml which was lower than Bavistin with mycelium growth 44 mm and at 250 mg/ml concentration (20 mm) diameter which was also lower than Mancozeb (21) mm diameter. Similarly, *Botritis cinerea* had LMG (12 mm) at 100 mg/ml concentration which was found lower than Mancozeb (13 mm). *F. oxysporum* showed 0 mm diameter at 150 mg/ml which was lower than both Bavistin (13 mm) and Mancozeb (15 mm) diameter. In the fungus *Phytophthora capsici* the lowest mycelium growth was observed 31mm at concentration 100 mg/ml which was lower than both Bavistin (90 mm) and Mancozeb (32 mm) respectively. In *S. rolfsii* the

LMG was completely inhibited from 150 mg/ml which was also lower than both Bavistin (46 mm) and Mancozeb (21mm). ANOVA result showed that there was significant difference $p<0.05$ in mean value of LMG of fungi in different concentrations of plant leaf crude extract (Table 12).

Table 12: Linear mycelium growth (mm) diameter in methanol leaf extract of *Xanthium strumarium*

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05.

Linear mycelium growth (mm) in methanol leaf extract of *Xanthium strumarium*										
	concentrations					control				
Fungal strain	**50**	**100**	**150**	**200**	**250**	**Negative**	**Positive**			
						Methanol	Bavistin	Mancozeb	P	F
A.b.	37±1 b	35±3 b	26±5 a	25±5 a	20±.8 a	50±4 d	44±1 c	21±1 a	.000	76.86
B.c.	16±1 d	12±2 bc	11±.2 abc	11±.5 ab	10±.2 a	22±1 e	16±.7 d	13±1 c	.000	89.19
F.o.	28±.8 d	22±1 c	0±0 a	0±0 a	0±0 a	31±4 d	13±1 b	15±.9 b	.000	354.7
P.c.	90±0 d	31±1 c	27±.8 b	0±0 a	0±0 a	90±0 d	90±0 d	32±1 c	.000	1.61
S.r.	87±1 e	80±6 d	0±0 a	0±0 a	0±0 a	90±0 e	46±1 c	21±1 e	.000	1.94

Abbreviations: *A.b.=Alternaria brassicae, B.c.=Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii,* values are mean ± SD of seven replicate.

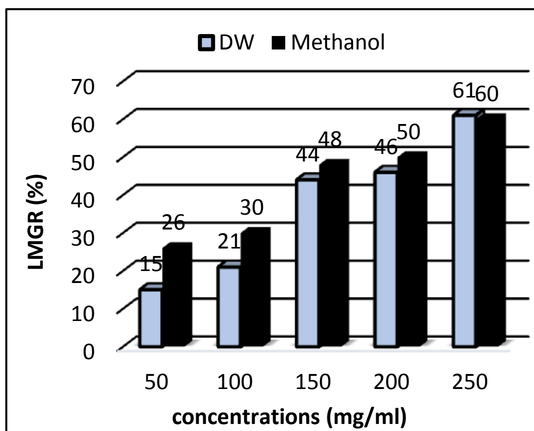
4.2.3.3. Linear mycelium growth reduction percentage of *Xanthium strumarium*

In *A. brassicae* 15%-61% linear mycelium growth reduction found at 50 mg/ml-250 mg/ml in distilled water while in methanolic extract 26%-60% was observed at 50 mg/ml-250 mg/ml fig. 30. *B. cinerea* had LMGR 11%-52% and 27%-54% at 50 mg/ml-250 mg/ml in distilled water and methanol extract respectively fig. 31. *F. oxysporum* showed linear mycelium growth reduction 5%-79% in distilled water leaf extract while in methanol extract 9%-29% LMGR was observed at 50 mg/ml-100 mg/ml concentrations and 100% reduction was observed at 150 mg/ml, 200 mg/ml and 250 mg/ml concentrations fig. 32. In distilled water crude leaf extract no LMGR was found at any concentrations for *P. capsici* while in methanol crude leaf extract at 100 mg/ml and 150 mg/ml concentrations 65% and 70% reduction was observed and 100% reduction was observed at 200 mg/ml and 250 mg/ml concentrations

respectively fig. 33. *S. rolfsii* had no LMGR at 50 mg/ml to 250 mg/ml concentrations in distilled water leaf crude extract while 4% and 6% was observed at 200 and 250 mg/ml concentration. In methanol crude extract at 50 mg/ml to 100 mg/ml concentrations LMGR (3%-11%) was observed while 100% reduction was observed at 150 mg/ml-250 mg/ml concentrations respectively fig. 34.

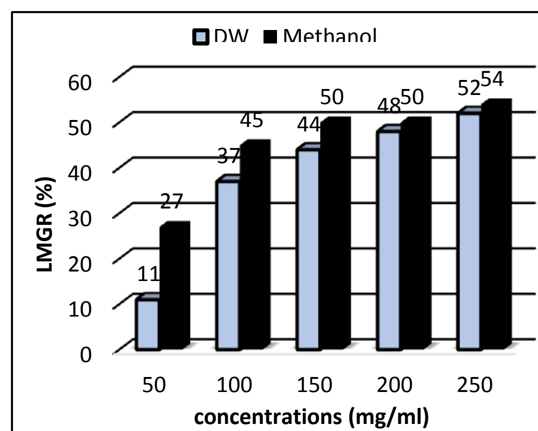
X. strumarium was found most effective against *F. oxysporum*, *P. capsici* and *S. rolfsii* by inhibiting the growth 100% in methanol extract at different concentrations. *X. strumarium* showed effect against *F. oxysporum* by inhibiting growth 79% at 250 mg/ml concentration in distilled water extract. With the increasing concentration of both methanol and distilled water leaf extract the linear mycelium growth reduction percentage increased.

In methanol leaf crude extract linear mycelium growth reduction percentage was found higher than in distilled water crude leaf extract at all the concentration for all fungi except *A.brassicae* which was found with higher LMGR 61% in distilled water crude extract than LMGR 60% in methanol crude extract of leaf at 250 mg/ml concentration (Figs.31-35).



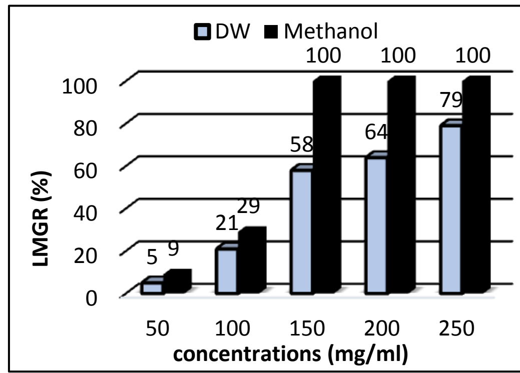
Concentrations (mg/ml) of leaf extracts of *X.strumarium*

Fig.30: Mean LMGR percentage in *A.brassicae*, n=7



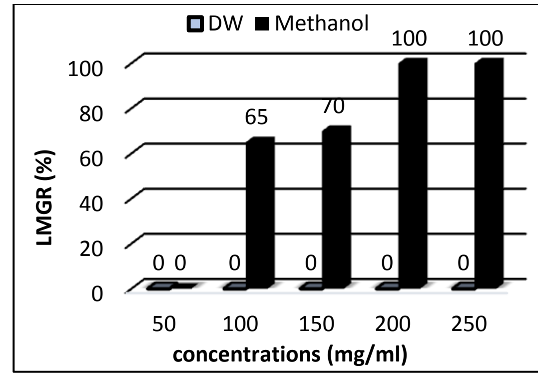
Concentrations (mg/ml) of leaf extracts of *X.stumarium*

Fig.31: Mean LMGR percentage in *B.cinerea*, n=7



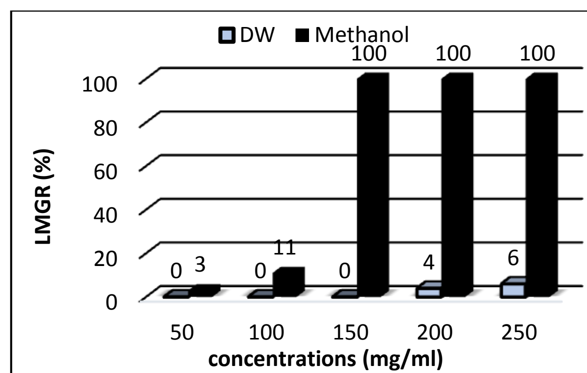
Concentrations (mg/ml) of leaf extracts of *X.strumarium*

Fig.32: Mean LMGR percentage in *F. oxysporum*, n=7



Concentrations (mg/ml) of leaf extracts of *X.strumarium*

Fig.33: Mean LMGR percentage in *P. capsici*, n=7



Concentrations (mg/ml) of leaf extracts of *X.strumarium*

Fig.34: Mean LMGR percentage in *S. rolfsii*, n=7

4.3 Antifungal properties of three IAS against tested fungi

All plant leaves extract showed positive activity against at least three fungi. Except at 50 mg/ml concentration for *S. rolfsii* methanolic extract of *A. adenophora* showed activity against all fungi, figures (8-12). Similarly, except *P. capsici* at 50 mg/ml concentration methanolic crude extract of *X. strumarium* showed activity against all fungi at all concentrations, figures (30-34). Among the selected five fungi *P. capsici* and *S. rolfsii* were most resistant fungi against distilled water extract while in methanol leaf extract *S. rolfsii, P. capsici* and *F. oxysporum* were the most susceptible fungi which inhibited their full growth by methanol extract of three plants at 150 mg/ml-250 mg/ml concentrations. *A. adenophora* completely inhibited *P. capsici* at 150 mg/ml-250 mg/ml concentrations and *F.oxysporum* at 250 mg/ml (Table 4). Methanol extract of *Ipomoea carnea* ssp. *fistulosa* completely inhibited the mycelium growth of *F. oxysporum* at 200 mg/ml and 250 mg/ml concentrations (fig.22).

Methanol extract of *Xanthium strumarium* completely inhibited the growth of *P. capsici* and *S. rolfsii* at 150 mg/ml to 250 mg/ml and for *F. oxysporum* at 200 mg/ml at 250 mg/ml (Table 12). Distilled water extract was not found completely inhibiting any selected fungus but with increasing concentration of plant extract the fungal growth decreased by both methanol and distilled water crude extract of plant. In this study among three plants methanol extract of *Xanthium strumarium* was observed more active against selected fungi followed by methanol extract of *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa*. *Fusarium oxysporum* was more susceptible fungus being inhibited by methanol extract of all three IAS tested at least two concentration (200 mg/ml-250 mg/ml). The growth of *A. brassicea* and *B. cinerea* was also decreased with increasing concentration but they were not inhibited completely by any concentration of both methanol and distilled water extract of all three tested IAS.

4.4 Antibacterial properties of three IAS against tested bacteria

In the present study, among three plants, all the tested IAS showed activity against at least two bacteria from 50 mg/ml-250 mg/ml concentration in both distilled water and methanol extract. Methanol extract of *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* inhibited all the tested bacteria (Table 6). Similarly, methanolic extract of *Xanthium strumarium* inhibited five bacteria but it was not able to inhibit the growth of *E. coli* at single concentration (fig.26). In distilled water *Ageratina adenophora* inhibited two bacteria at 50 mg/ml, 100 mg/ml, three and four bacteria at 150 and 250mg/ml respectively while at 250mg/ml five bacteria were inhibited (Table 2). Distilled water extract of *Ipomoea carnea* ssp. *fistulosa* showed activity against 3 bacteria at 50 mg/ml, five bacteria from 100 mg/ml to 200 mg/ml but at 250 mg/ml concentration all bacteria were inhibited (Table 5). Similarly, distilled water extract of *Xanthium strumarium* inhibited the growth of three bacteria at 50 mg/ml to 150 mg/ml while at 200 mg/ml to 250 mg/ml 4 bacteria were inhibited (Table 9). *Ipomoea carnea* ssp. *fistulosa* was found more active against tested bacteria (inhibited all bacteria at 250 mg/ml in distilled water extract and at all concentrations in methanol extract), followed by *Ageratina adenophora* (inhibited 5 bacteria at 250 mg/ml and at all concentrations in methanol extract) and least activity was showed by *X. strumarium* (inhibited 4 and 5 bacteria at 200 mg/m and 250 mg/ml in distilled water

respectively and 5 bacteria in 250 mg/ml in methanol). Methanol extracts were more effective against selected bacteria than distilled water extracts. And with the increasing concentrations ZOI was also increased.

4.5 Phytochemical screening

4.5.1 Qualitative test

The phytochemical screening was done to find the presence of active chemical constituents such as terpenoids, saponins, steroids, Cardiac glycosides, flavonoids, reducing sugar, tannins, phlobatannins and alkaloids.

Table 13: Preliminary phytochemical screening of leaf extract of *A. adenophora*, *I.carnea* ssp. *fistulosa* and *X. strumarium*

Solvents	**Distilled water**			**Methanol**		
Selected Plants →	*Ageratina adenophora*	*Xanthium strumarium*	*Ipomoea carnea* ssp. *fistulosa*	*Ageratina adeniphira*	*Xanthium strumarium*	*Ipomoea carnea* ssp. *fistulosa*
Terpenoids	+++	+++	+	–	+++	–
Saponins	++	++	+++	+++	+++	+++
Steroids	–	–	–	+	+++	+
Cardiac glycosides	+	+++	+	–	–	–
Flavonoids	–	–	–	+++	+++	+++
Phlobatannins	–	+	+++	–	+	++
Reducing compounds(such as sugar)	+++	+++	++	–	–	+
Tannin	+++	–	+	+++	+	+
Alkaloids	+++	++	++	+++	+++	+++

Responses to various tests were denoted by +, ++ and +++ signs indicating weak, moderate and strong reactions respectively while - for no reaction.

Distilled water extract of *Ageratina adenophora* revealed the presence of high concentration of terpenoids, reducing sugar, tannin and alkaloid while methanolic extract revealed high concentration of saponins, flavonoids, tannins and alkaloids (Table13). Distilled water extract of *Ipomoea carnea* ssp. *fistulosa* revealed the presence of high concentrations of saponins and phlobatannins while had highest reaction for saponins, flavonoids and alkaloids (Table13). Distilled water extract of *Xanthium strumarium* leaves revealed the presence of high Concentration of

terpenoids, cardiac glycosides, and reducing sugars while methanolic extract revealed the presence of high concentration of terpenoids, saponins, steroids, flavonoids and alkaloids (Table 13).

4.5.2 Quantitative test

The components quantitatively analyzed were alkaloids, saponins and flavonoids.

A.) **For Saponins**: Among three plants, *Ipomoea carnea* ssp. *fistulosa* showed the highest percentage of saponins (31.87%) followed by *X. strumarium* (16.61%) and *A. adenophora* (5.24%) Fig.36.

B.) **For Alkaloids**: *A. adenophora* showed the highest percentage of alkaloid (6.12%) followed by *X. strumarium* (1.64%) and *I.carnea* ssp. *fistulosa* (0.64%) Fig.37.

C.) **For Flavonoids:** *I. carnea* ssp. *fistulosa* showed the highest percentage of flavonoids (5.65%) followed by *X. strumarium* (4.64%) and *A. adenophora* (3.11%) Fig.38.

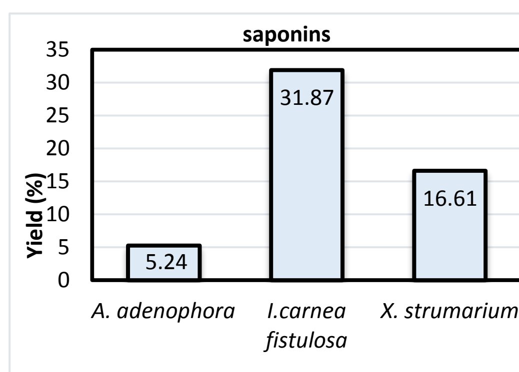


Fig. 35: Percentage yield of saponins in the leaf extracts of selected IAS

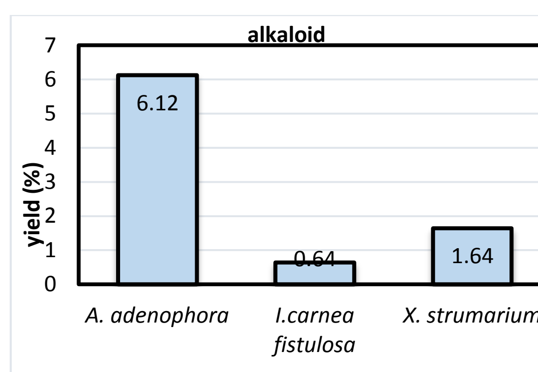


Fig. 36: Percentage yield of alkaloids in the leaf extracts of selected IAS

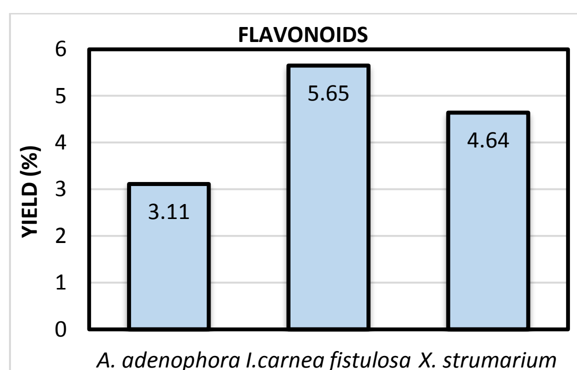


Fig.37: Percentage yield of flavonoid in the leaf extract of select

5. DISCUSSION

5.1 Extraction of crude extract from IAS

Two types of solvents (Distilled water and Methanol) were used for the extraction of compounds from the air dried leaves' powder of plant samples. The most commonly used solvents for preliminary investigations of antimicrobial activity in plants are methanol, ethanol and water (Parekh *et al*. 2006; Rojas *et al*. 2006). The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction (Das *et al*. 2010). Distilled water extract was evaporated by the help of water bath and methanol extract was recovered with the help of rotary vacuum evaporator. It was found that for the same plant, continuous extraction with different solvent viz: Methanol and distilled water gave different percentage yield. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Parekh and Chanda 2007). In the present study, the highest yield was observed from distilled water fraction of *Ageratina adenophora* (16.48%) followed by distilled water fraction of *Ipomoea carnea* ssp. *fistulosa* (15.68%) and *Xanthium strumarium* (13.4%). *Ipomoea carnea* ssp. *fistulosa* from methanol solvent gave the highest yield (11.12%) followed by *Ageratina adenophora* (10.68%) and *Xanthium strumarium* (6.2%) (Fig.1).

Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants (Eloff 1998).The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories (Parekh *et al*. 2006). The basic parameters influencing the quality of an extract are- plant part used as starting material, solvent used for extraction and extraction procedure. Effect of extracted plant phytochemicals depends on -the nature of the plant material, its origin,

degree of processing, moisture content and particle size. The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends upon-type of extraction ,time of extraction, temperature, nature of solvent, solvent concentration and polarity (Ncube *et al*. 2008).

5.2 Qualitative screening for Antimicrobial Activity

The increasing trend of resistance to the antibiotics in current use has drawn the attention of researchers to natural alternative treatments of bacterial infections. As potential sources of new, novel antimicrobial agents plants against fungi and bacteria varied with the type of microorganism and plant parts used. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan 2003). Recent work revealed the potential of several herbs as sources of drugs (Iwu 2002). The evaluation for antimicrobial agent of plant origin begins with thorough biological evaluation of plant extracts to ensure efficacy and safety followed by identification of active principles, dosage formulations, efficacy and pharmaco-kinetic profile of the new drug. Ethno pharmacolo-gists, botanists, microbiologists and natural product chemists are searching the world for phytochemicals which could be developed for treatment of infectious diseases (Tanaka *et al.* 2006).

5.3 Qualitative screening for Antibacterial Activity

In this study, screening of antibacterial activities of invasive alien species (IAS) was done to find out the presence or absence of antibacterial activity in the plant extract. The antibacterial activity of 95% methanol and distilled water crude extract of 3 invasive alien species were tested against 3 gram positive and 3 gram negative bacteria species using disc diffusion method as given by Peach and Tracey (1950) in Mueller Hinton Agar (MHA). After 24 hours incubation at 37°C the plates were examined for clear area representing zone of inhibition.

During the study it was found that the methanolic and distilled water extract of 3 plants showed different activities against an array of Gm+ve and Gm-ve bacteria. *Ipomoea carnea* ssp. *fistulosa* was observed more active by showing ZOI against

selected bacteria by inhibiting all of them in distilled water extract at 250 mg/ml (Table 5) and at all concentration in methanolic extract (Table 6). This is may be due to the weed extract-based synthesis of silver nanoparticles is very efficient against selected human pathogens (Daniel *et al*. 2012). Adsul *et al.* (2012) also found that the tested bacteria *Proteus vulgaris* was inhibited by acetone crude extract from leaves of *I. carnea* spp. *fistulosa* but it was not able to inhibit *E. coli, B. cereus*, and *S. aureus*. This differences in result may be due to the difference in time of collection of plant material, difference of solvent and habitat of plant material.

Methanolic extract of *Ageratina adenophora* produced zone of inhibition against all bacterial growth at five concentrations (Table 2) while in distilled water extract it was able to show zone of inhibition with *K. pneumoniae, E. faecalis*, *E. coli*, *B. subtilis* and *S. aureus* (Table 1). The plant was found to be ineffective against *Proteus mirabilis* at 250 mg/ml. Baral and Manandhar (2011) also found the similar result. According to their result, the methanolic extract of *Ageratina adenophora* was effective against *Bacillus subtilis, Staphylococcus aureus, K. pneumoniae,* and *E. coli* at different concentrations. But it was not able to inhibit *P.mirabilis* and extract of distilled water only inhibited *B. subtilis*. Similarly, Barajas *et al*. (2013) found that essential oil of *A. Jahnii* and *A. pichinchensis* showed the inhibition of zone against *S. aureus* and *E. faecallis* but they were not able to inhibit the growth of *E. coli, K. pneumoniae, P. aeruginosa*. Bhattarai and Shrestha (2009) reported that organic solvent ethanol extract of *A. adenophora* showed antibacterial effect towards *Proteus* spp.*, S.* spp.*, B. subtilis, S. aureus*, *P.mirabilis*, and water solvent extract showed antibacterial effect towards *E. coli, S. aureus*, *S.* spp, *P*. spp. and *B. subtilis*. These results are somewhat dissimilar with this present study which may be due to the difference in plant part used, employed bacteria, used solvent and habitat of plant.

Methanolic extract of *Xanthium strumarium* was able to show zone of inhibition with *K.pneumoniae*, *E. faecalis*, *B.subtilis*, *p.mirabilis*, *S. aureus* but it was ineffective against *E. coli* (Table 10). Similarly distilled water extract of *Xanthium strumarium* was able to show zone of inhibition with *E. faecalis*, *B.subtilis*, *P. mirabilis*, *S. aureus* and it was incapable with *E. coli* and *K. pneumoniae* (Table 9). Similar result was found by Malik *et al.* (2011). According to their result the leaf extract of *X. strumarium* in different solvents (aqueous, ethanol and n-hexane) only inhibited *S.*

aureus and it was not able to inhibit *E. coli, K. pneumoniae* and *B. subtilis*. Khuda *et al.* (2012) were found that the extract of chloroform of *X. strumarium* inhibited *S. aureus, B. subtilis* and *E. coli* and ethyl acetate extract inhibited *S. aureus*. But no inhibition of any bacterial growth was observed by aquous extract. The plants differ significantly in their activities against the micro-organisms tested (Janovska 2003).

5.3.1 Evaluation of Antibacterial Activity

The evaluation of antibacterial substances becomes the important steps during the new drugs research from selected IAS. For this, disc diffusion process was followed. In disc diffusion process, from plant the antibacterial substances present diffusing in the agar media which inhibit or kill the bacteria that are susceptible and thus the zone of inhibition appears around the disc in agar surface. Screening method can only provide an idea about killing or inhibition of particular bacteria. But if the ability of plant extract is very low, then they have very little or no importance although larger number of bacterial strain is inhibited by plant extract. During new drug research process from selected plants, evaluation of potency of plants extracts is also most important step.

Among the tested plants the highest zone of inhibition (25 mm-30 mm) diameter at 50 mg/ml to 250 mg/ml concentration was showed by methanolic extract of *Xanthium strumarium* on bacteria *klebsiella pneumonia.* On *Bacilus subtillis* and *staphyloccocus aureus*, this plant showed ZOI at 50 mg/ml to 250 mg/ml (25 mm – 28 mm) and (25 mm – 27 mm) respectively (Table 10). This may be due to the antibacterial substances present in it may have higher diffusibility. There are also reports on the antibacterial activity of xanthanolides isolated mostly from *Xanthium srtumarium* (Gupta and Gupta 1975; Saxena and Mishra 1995).

Methanolic extract of *Ipomoea carnea* ssp. *fistulosa* at 50 mg/ml - 250 mg/ml concentration has also showed the highest ZOI (12 mm- 14 mm) diameter and (11mm-14 mm) diameter on *Enterococcus faecalis* and *Escherichia coli* respectively (Figs.15&16). In distilled water extract plant observed had the highest ZOI (10 mm-12 mm) and (10 mm-11mm) diameter on *Staphylococcus aureus* and on *Enterococcus*

faecalis respectively at 200 mg/ml - 250mg/ml concentrations (Figs. 15&19). The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts (Rai and Carpinella 2006).

Similarly, methanolic extract of *Ageratina adenophora* showed the highest ZOI (11mm-14 mm) diameter on *Proteus mirabilis*, (10 mm-12 mm) diameter on *Staphylococcus aureus* at 50 mg/ml- 250 mg/ml concentration respectively. At 150 mg/ml - 250 mg/ml concentrations the ZOI was showed (10-12) diameter on *K.pneumoniae* and *E.coli*. At 200 mg/ml - 250 mg/ml concentration ZOI was showed (10 mm-11mm) diameter on *E. faecallis*. In distilled water extract *Ageratina adenophora* showed the highest ZOI (8 mm) diameter at 250 mg/ml concentration on *K.pneumoniae* and *E.faecallis*. This may be because the bioactive compounds of the leaves of this plant may differ in quantity and quality compared to other tested plants (Zakaria 2010).

The zone of inhibition against remaining bacteria was less than 8 mm diameter observed from 3 selected plants. This showed less spectrum activity and low ZOI so they have less potency in antibacterial activity.

The diameter of ZOI produced depends on several factors broadly classified as extrinsic or intrinsic parameters. The extrinsic parameters like pH of the medium, period and temperature of incubation, volume of well, concentration of plant extracts and size of inoculums can be fixed and standard during experiment hence no error results due to extrinsic factors. Intrinsic factors such as methods of plants including its components, solubility and diffusion property are predetermined (Prasai *et al.* 2008).

Some plants did not show effect against some tested bacteria. *Xanthium strumarium* did not show effect on *E. coli* at both methanolic extract and distilled water at 50- 250 mg/ml concentrations (Fig.27). *K. pneumoniae* also didn’t show inhibition with it in D/W (Table 9). Distilled water extract of *I. carnea.* ssp. *fistulosa* didn’t show effect on *E. coli* at 50 mg/ml-200 mg/ml concentrations (Fig.16) and DW extract of *A. adenophora* did not show effect on *P. mirabilis* at 50 mg/ml-250 mg/ml concentrations (Fig.6), *B. subtilis* at 50 mg/ml-200 mg/ml concentrations (Fig.5),

K. pneumoniae at 50 mg/ml-150 mg/ml concentrations (Fig.2) and *E. coli* at 50 mg/ml-100 mg/ml (Fig.4) concentration. With no antibacterial activities, extract may be active against other bacterial species which were not tested (Shale *et al.* 1999). Lack of activities can thus only be proven by using large doses (Farnswrth 1993). The inability of extract of some selected plants to demonstrate any visible activity against some bacteria may be probably due to the low concentration of extracts (Sudharameshwari and Radhika 2007).

Gram negative and gram positive bacteria were tested with 3 invasive alien species plant extract in the present study. In comparison, gram positive bacteria were found more susceptible than gram negative bacteria of the crude extracts. Similar type of result has already showed by Matu and Van Staden (2003); Parekh and Chanda (2007). These differences may be attribute 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 different resistant level between strains (Cetin and Gurler 1989). Higher the concentration of the extract showed higher inhibition zone while the lower concentration showed lower inhibition zone or no effect.

It is revealed from the Tables (1, 2, 5, 6, 9 & 10) that crude extract of all the three different IAS have potential antibacterial activity against at least 2 bacteria from 50 mg/ml to 250 mg/ml concentrations in both distilled water and methanol extract. The methanol extract had the highest activity against bacterial isolates; this may be attributed to the presence of soluble phenolic and polyphenolic compounds. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as Dettol and cresol (Kowalski and Kedzia 2007). The antimicrobial effect of methanol extract against these organisms may be due to the ability of the methanol to extract some of the active properties of these plants like phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial (Cowan 1999; Okwu and Josiah 2006).

Comparison of the efficacy of the extract with commercial antibiotics had shown that the latter is more effective in inhibiting the growth of the organisms than the formers. This may be due to the presence of stronger bioactive principles in the antibiotics and their molecular size which permit their penetration into the cells of the organisms (Oladunmoye 2006).

5.3.2 Qualitative screening for Antifungal Activity

Among three selected IAS, methanolic extract of *Xanthium strumarium* showed the highest antifungal activity by completely inhibiting the mycelium growth of *Phytophthora capsici, Sclerotium rolfsii* at 150 mg/ml-250 mg/ml concentration and *Fusarium oxysporum* at 200 mg/ml-250 mg/ml concentration (Table 12). This may be because the bioactive compounds of the leaves of this plant may differ in quantity and quality compared to other tested plants (Zakaria 2010). Distilled water extract of *X. strumarium* showed the highest inhibition of mycelium growth in *Alternaria brassicae* and *Fusarium oxysporum* (Table11) but it did not show activity on *P.capsici*. It inhibited the growth of fungus more than positive control Bivastin and Mancozeb (Table 12).

Methanolic extract of *Ageratina adenophora* showed the highest antifungal activity by completely inhibiting *P.capsici* at 150 mg/ml- 250 mg/ml and *F. oxysporium* at 250 mg/ml concentration (Table 4). It also showed activity against *F. oxysporum.* Distilled water extract of *A.adenophora* showed ability to inhibit growth of all selected fungus by increasing concentration of plant extract (Table 3). It showed mainly against *A. brassicae* and *B. cinerea*. It inhibited fungi more than fungicide in some cases (Tables 3&4).

Methanolic extract of *Ipomoea carnea* ssp. *fistulosa* completely inhibited the growth of *F. oxysporum* at 200 mg/ml-250 mg/ml concentration. In *P. capsici* and *S. rolfsii* it showed little inhibition while in *A. brassicae* and *B. cinerea* it showed high inhibition (Table 8). Distilled water extract of *I. carnea* ssp. *fistulosa* did not show activity on the mycelium growth of *Phytophthora capsici* and *Sclerotium rolfsii* but it showed activity against the growth of *Fusarium oxysporum* and *Botrytis cinerea* (Table 7). It

showed higher activity than synthetic fungicide Bavistin and Mancozeb on some fungi at higher concentrations of both methanolic and DW extract (Tables 7&8).

5.3.2.1 Linear Mycelium Growth Reduction (LMGR) Percentage

In methanolic leaves extract *Xanthium strumarium* showed 100% inhibition on *F. oxysporum* (Fig.33), *P.capsici* (Fig. 34) and *S. rolfsii* (Fig. 35) at higher concentration and least LMGR percentage was found in *A. brassicae* (60%) Fig.31 and *B. cineria* (54%) Fig.32 at 250 mg/ml concentration. In DW leaf extract *X.strumarium* showed the highest percentage of LMGR on *F. oxysporum* (79%) Fig.33 and the lowest percentage in *S. rolfsii*. (6%) Fig.35 at 250 mg/ml concentration (Table 11) while there was no LMGR% was found in *P.capsici* from 50 mg/ml-250 mg/ml (Fig.33) and in *S.rolfsii* at 50 mg/ml-150 mg/ml concentration (Fig.34). Khuda *et al*. (2012) found that *X. strumarium* inhibited *F. solani* in solvents-chloroform and n-hexane (30% inhibited) and ethyle acetate (20% inhibited). But in aqueous extract there was no inhibition observed. This dissimilar result may be due to the inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested (Kalimuthu *et al.* 2010). *Xanthium strumarium* was found more active against selected fungi followed by *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa*. The compounds xanthatin and cinnamic acid could have contributed to the antimicrobial property *of X. strumarium (*Ginesta-Peris *et al.*1994; Scherer *et al.* 2009).

In methanolic crude extract of *A.adenophora*, the highest (100%) LMGR percentage was found on *P. capsisi* at 150 mg/ml-250 mg/ml concentrations (Fig.12) and on *F. oxysporum* (Fig. 11) at 250 mg/ml concentration. The high percentage was also found in *A. brassicae* (74%) (Fig.9) and *S. rolfsii* (73%) at 250 mg/ml concentration (Fig.13). In distilled water extract the highest LMGR percentage was found in *F. oxysporum* (79%) (Fig. 11) while the lowest LMGR was observed in *B. cinerea* (47%) (Fig. 10). Bhattarai and Shrestha (2009) were reported that the total inhibition of fungal growth was observed in *Fusarium oxysporum, F. moniliformae*, and *F. eroliferum* by 100% aqueous and methanolic extract of *Eupatorium adenophorum*. Baral and Maharjan (2011) found that *F. oxysporum, F. moniliformae* and *F.*

proliferatum were inhibited by both methanolic and aqueous extract of *A. adenophora* from lower to higher concentrations while *F. eridiforme* was inhibited only at lower concentrations. *Sclerotium rolfsii* was found inhibited from lower to higher concentrations against methanolic extract. But with aqueous extract it was also inhibited in only lower concentrations. This difference in result may be due to the difference in time of collection of plant material and habitat of plant material. In distilled water extract of *A. adenophora* the LMGR (%) was observed for all selected fungi at least at higher concentrations (Figs. 9-13).

In methanolic leaf crude extract of *Ipomoea carnea* ssp. *fistulosa* the highest LMGR (100%) was found in *F. oxysporum* (Fig.22) and the lowest percentage was found in *P. capsici* (7%) at 250 mg/ml concentration (Fig.23). Similar result was found by Guleria and Kumar (2006). They have identified antifungal activity of dichloromethane leaf extract of *Ipomoea carnea* against *Alternaria alternate*. In distilled water extract the highest LMGR percentage (85%) was observed in *B.cinerea* (Fig.21) and (62%) was in *A. brassicae* at 250mg/ml (Fig.20) while there was no LMGR was observed in *P.capsici* (Fig.23) and *S. rolfsii* (Fig.24) at 250mg/ml concentration.

It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. The similar result was found by Bobbarala *et al.* (2009) on *Aspergillus nigar* examined by forty nine different medicinal plants. It was found that the fungi were inhibited by the extract and the inhibition was directly proportional to the increasing concentration of the extract (Goel and Sharma 2013). In this study the plant extracts by methanol provided more consistent antimicrobial activity compared to those extracted by distilled water. Similar result was found by Parekh and Chanda (2007) on five microorganisms methanolic and distilled water extract of twelve species of Indian medicinal plants. This tends to show that the active ingredients of the plant parts are better extracted with methanol than other solvents. The methanol extracts contain alkaloids, coumarins and tannins (Okemo 1996). Methanol has a high polarity index (Cowan 1999) than the water and thus is able to extract more phenolic and flavonoid compounds**.** The result has showed that the crude extract of selected IAS have

potential antifungal activity against tested fungi. This inhibitory effect may be as a result of the change in pH and chemical constituent(s) of aqueous and methanolic extracts of these plants. The mechanisms of action of these phytochemicals may be via lysing of cell, increasing permeability of cell wall and membrane, inhibition of protein and DNA synthesis and or inhibiting the transport of nutrient across the cell wall of membrane (Stewart and Beswick 1979). This inhibitory effect of the extract on the growth of these microorganisms could be attributed to the presence of some phytochemicals that were found present in the plant extract (Abukakar *et al.* 2008). *A. brassicae* and *B. cinerea* were found most resistant fungi against plant extract while *F. oxysporum, P.capsici* and *S. rolfsii* were found most susceptible fungi. This is might be due to the presence of more complex cell wall with rigidity and also might be due to reason that the fungi differ in optimum growth conditions such as pH, production rate of manganese and lignin peroxidases and their resistance to toxic chemicals (Rucksdeschel and Renner 1986).

Between bacteria and fungi, bacteria was found more susceptible than fungi against both distilled water and methanol extracts of selected IAS. The susceptibility and resistance vary among the organisms with the extract inhibiting the growth of the bacteria more than the fungi. This might be due to their eukaryotic nature which confers on them a more complex cell wall with rigidity and less permeability that the thin cell wall membrane of bacteria. Another factor that may be responsible for the low sensitivity of the fungi may be due to their ability to produce extracellular enzymes (Tortora *et al*. 2002).

5.4 Phytochemical screening

As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins and tri-terpenoids are producing a better opportunity for testing wide range of microorganism(Arora *et al.* 2012).

From preliminary phytochemical screening in distilled water and methanolic extract of leaves of selected IAS revealed the presence of high concentrations of terpenoids, saponins, cardiac glycosides, flavonoids, reducing compounds, tannins, phlabotannins and alkaloids. Distilled water extract of *Ageratina adenophora* revealed the presence of high concentration of terpenoids, reducing sugar, tannin and alkaloid (Table 13). Methanolic extract of *A. adenophora* revealed high concentration of saponins, flavonoids, tannins and alkaloids. Baral and Maharjan (2011) found the presence of Glycosides but negative result was observed for saponins and alkaloids. This difference in result may be due to the presence of phytochemicals varies based on the solvents used, climatic conditions of plant grown and preliminary phytochemical screening methods (Srinivas *et al.* 2011).

Distilled water extract of *Ipomoea carnea* ssp. *fistulosa* revealed the presence of high concentrations of saponins and phlobatannins (Table 13). Methanol extract of *I. carnea* spp. *fistulosa* had highest reaction for saponins, flavonoids and alkaloids. Sahayaraj and Ravi (2008) found that the distilled water extract of *I. carnea* ssp. *fistulosa* revealed the presence of phenolic compound, saponins, tannins and flavonoids but negative result was observed for steroids and alkaloids. Chloroform extract revealed the presence of steroids, alkaloids, phenolic compounds, tannins and flavonoids but there was negative result for saponins. Benzene extract revealed the presence of phenolic compounds, saponins, tannins and negative result was observed for steroids.

Distilled water extract of *Xanthium strumarium* leaves revealed the presence of high concentration of terpenoids, cardiac glycosides, and reducing sugars (Table 13); while its methanolic extract of revealed the presence of high concentration of terpenoids, saponins, steroids, flavonoids and alkaloids. Sravani and Laxshmi (2012) found that the methanol, aqueous and petroleum ether extracts of *X. strumarium* revealed the presence of flavonoids and steroids but negative results were obtained for alkaloids, terpenoids, tannins, glycosides and saponins. The result is somewhat dissimilar with this present study. This dissimilar result may be due to a large extent, the phenological stage of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction and solvent used (Felix 1982).

In the quantitative test of selected plant, *Ipomoea carnea* ssp. *fistulosa* contained the highest percentage of saponins (31.87%) while *Ageratina adenophora* contained the lowest percentage (5.24%) (Fig. 36). Test of Alkaloid revealed that *Ageratina adenophora* contained the highest percentage (6.12%) and *I. carnea* ssp. *fistulosa* contained the lowest percentage (0.64%) (Fig. 37). *I. carnea* ssp. *fistulosa* had the highest percentage (5.65%) of flavonoids while *Ageratina adenophora* had the lowest percentage (3.11%) (Fig. 38) which might be due to the reason that the effect of extracted plant phytochemicals depends on -the nature of the plant material, its origin, degree of processing, moisture content and particle size (Ncube *et al.* 2008).

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew 2002). Flavonoids, exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek *et al.* 2002). Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Saponins which are known to produce inhibitory effect on inflammation (Just *et al.* 1998). Terpenoids are sources for drugs that reduce cholesterol from blood streams, apart from their use as antimicrobial substances (Haslam 1996). The Steroids and saponins were responsible for central nervous system activities (Farooq *et al.* 2014). Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics.

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. The plant studied can be used to cure many diseases and the identification and isolation of the active compounds could lead to the new drug discovery of cheaper cost which would be useful for the patients.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Investigation on the phytochemical screening of the three selected Invasive Alien Species (IAS) *Ageratina adenophora*, *Ipomoea carnea* ssp. *fistulosa* and *Xanthium strumarium* leaves extracts revealed the presence of terpenoids, saponins, cardiac glycosides, flavonoids, reducing compounds, tannins, phlabotannins and alkaloids.

All the extracts (distilled water and methanol) showed varying degrees of antimicrobial activities on the microorganisms tested. The chance to find antimicrobial activity was more apparent in methanol than distilled water extracts of the same plants. Some of these plants were more effective at higher concentrations than traditional fungicides to combat the plant pathogenic fungi tested. Among the tested IAS *Xanthium strumarium* had the highest antimicrobial activity followed by *A. adenophora* and *I.carnea* ssp. *fistulosa*.

Gram negative bacteria *Escherichia coli* was found most resistant against the different concentrations of plant extracts and gram positive bacteria *S. aureus* was found the most susceptible among the pathogenic bacteria. *Alternaria brassicae* and *Botrytis cinerea* were found the most resistant fungi against the all concentrations of both methanolic and distilled water extracts of plants and *Fusarium oxysporum* was found the most susceptible fungus among the selected phytopathogenic fungi.

The present study has demonstrated that the antimicrobial activity of all the plant tested. This work proves that some invasive plants have potential and could be useful in combating microbial pathogens. Phyto-compounds are also expected to be more advantageous than synthetic chemicals for their steer magnitude of complexity, diversity and novelty of chemicals since they may be biodegradable in nature, non-pollutant and may possess no residual or phytotoxic properties. Therefore, plant extracts or plant secondary metabolites which are not toxic and specific in their action could be considered as an alternative to synthetic fungicides and antibiotics based on the availability of materials.

6.2 Recommendations

- The higher concentrations (150 mg/ml, 200 mg/ml and 250 mg/ml) of plant material showed promising antimicrobial activity thus these plants could be a source of new antibiotics and fungicides compounds.
- Further work is needed to isolate the secondary metabolites from the extract studied in order to test specific antimicrobial activity.
- Root, stem, flowers and seed also have to test for the investigation of new drug.
- MIC can be carried out for the extract that showed prominent activity against tested microorganisms.

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