ALLELOPATHIC POTENTIAL AND PHYTOCHEMICAL SCREENING OF SOME MEDICINAL PLANTS OF NEPAL

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CERTIFICATE

This is to certify that the dissertation work entitled "Allelopathic Potential and Phytochemical Screening of Some Medicinal Plants of Nepal'' submitted by Swasti Sharma has been carried out under my supervision. The entire work was based on her primary field work and has not been submitted for any other academic degrees. I therefore recommend this dissertation to be accepted for the partial fulfillment of Masters of Science in Botany from Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

The dissertation paper submitted by Swasti Sharma entitled "**Allelopathic Potential and Phytochemical Screening of Some Medicinal Plants of Nepal**" at the Central Department of Botany, Tribhuvan University has been accepted for the partial fulfillment of requirements for Masters of Science in Botany.

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ABSTRACT

The laboratory tests were conducted to evaluate the allelopathic potential and phytochemical study of the four selected medicinal plants *Ageratum conyzoides, Eclipta prostrata, Cannabis sativa* and *Woodfordia fructicosa* . The aqueous extract bioassay of the plants with leaf, stem and root were conducted on the two test seeds wheat and pea by filter paper method. Germination, seedling growth and biomass production were observed under the allelopathic study wheras under phytochemical study qualitative or presence or absence and quantitative or crude amount of the phytochemicals present were also estimated. Data analysis was done by SPSS version 16. For the allelopathic studies the germination and seedling growth of wheat and pea test species under the different concentrations 2% ,4% ,6% ,8% and 10% of the leaf, stem and root extracts of the plants under study were carried out. Increased concentration of the aqueous extracts of the plants increased the inhibitory rate or decreased the germination and seedling growth. Generally leaf extract showed lower germination rate than the other parts. There was significant difference for the plumule length and rootlegth of wheat from that of control except for the radicle length of the *C. sativa* root extract. In pea, no significant difference was seen for *A. conyzoides* root, *C. sativa* stem and root and *E. prostrata* stem extract. Biomass production was maximum in *W. fructicosa* and least in *A. conyzoides* stem extracts in pea .In wheat maximum in *C. sativa* and minimum in *E. prostrata* extracts. Wheat was found sensitive to *E. prostrata* and *W. fructicosa* extracts wheras pea plant was found more sensitive to *A. conyzoides* and *C. sativa* extracts. Alkaloid, flavonoid, saponin, tannin, phenol, glycoside and essential oil were the phytochemicals present in the plant extracts.

Key words: Allelopathic potential, Phytochemical study, medicinal, bioassay, germination, seedling, biomass production, sensitive, crude

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LIST OF ABBREVIATION AND ACRONYMS

N	North
E	East
Lat.	Latitude
Long.	Longitude
ANOVA	Analysis of variance
SPSS	Statistical Package for Social Science
p	Level of significance
d.f.	Degree of freedom
n	number of samples
et al.	and others
m asl.	metres above sea level
ml	millilitres
º C	Degree Celsius
gm	grams
h	hours

1. INTRODUCTION

1.1 Background

Medicinal plants are of great importance to the health of individuals and communities for the treatment of various diseases. Nearly 80% of the world's population relies on traditional medicine for primary healthcare most of which involves the use of plant extracts (Sandhya *et al*. 2006). Plants have been identified as having a wide array of medicinal properties for one or more over 300 different ailments and diseases (Nandakumar 2009). Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as anti-infectious agents. Although the use of synthetic compounds led to a decline in use of plants in modern medicine, because of their synthetic nature, known side effects, unpleasant taste, smell or burning sensation felt on the skin, people have started to prefer the use of natural compounds obtained from plants. Many of the currently available phytodrugs have been derived especially from wild resources of plants (Grover *et al.* 2002) of which at least 47% of which have medicinal, aromatic, cosmetic and culinary uses. The medicinal values of these plants lie in the bioactive phytochemical constituents that produce physiological effects on the human body (Koche *et al.* 2010) .The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and some other phenolic compounds (Edeoga *et al*. 2005).The effect of these chemicals are not limited to animal and human body alone but also on other plants. Many plants including medicinal plants were reported to interact chemically with other plant species. Such chemical interaction is known as allelopathy.

The term "allelopathy" was proposed for expressing the harmful, stimulatory, enhanced and beneficial effects that one plant species has on another through the formation of chemical retardants escaping into the environment (Molisch 1937). Allelochemicals are low molecular weight compounds excreted from plants during the process of secondary metabolism (Rice 1984). They are present in all parts of the plant tissues including leaves, stem, roots, rhizomes, flowers, fruits, seeds and even in pollen grains .They are released from the plants by volatilization, leaching, excudation and decomposition from plant residues (Molisch 1937). Their activity varies with temperature, photoperiod, water and soils, during natural processes with its initial

concentration, compound structure and mixed degree during functional processes with plant accessions, tissues and maturity within species (Shao-Lin *et al.* 2004). Most frequent effect on plants are inhibited or retarded seed germination, stimulatory or inhibitory effect on plumule and radicle growth, lack of root hairs, swelling or necrosis of root tips, discolouration, reduced dry weight accumulation and lower reproductive capacity (Ayeni *et al*. 1997). The concept of allelopathy was further supported and developed by Whittaker (1970) and Fischer *et al.* (1978). According to Lavabre (1991), allelopathic effects are controversial and still poorly understood.

A major tool for research in allelopathy is bioassay, which controls laboratory condition, high sensitivity gives reproducible result, take relative short time to perform. The filter paper method is a more suitable method because it can tolerate the moderate temperature during incubation (25°C) in the laboratory, the aqueous extracts remain fresh for longer period of time , easy availability and free from contamination ,easy handling and a good media for germination, high flow rate for movement of extracts and porosity (Gill *et al*. 2009). Allelopathic effect of medicinal species against temperate crop is well studied (Han *et al.* 2008 and Li *et al*. 2009). Allelopathic potential of some selected species had been studied by Maharjan *et al.* (2007), Compton *et al.* (2009) and Gyawali *et al*. (2008).

Phytochemicals are non nutritive plants chemicals that have protective or disease preventive properties. These chemicals are primarily produced by the plants for their protection but the recent research demonstrates that they can also protect humans against diseases. Alkaloids, saponins, glycosides, cardinolides and bufadignolides, flavonoids, tannins, phenolic compounds, anthraquinones, carbohydrates, fixed oils, fats and volatile oils are some phytochemicals present in plants. Phytochemical screening is the process of tracing plant constituents. There are general plant constituents that can be screened with standard tests.

1.2 Justification of study

Nepal is endowed with a great diversity of indigenous medicinal plants Medicinal plants are the integral part of diverse traditional, medicinal practices in Nepal and are codified in traditional medicinal systems such as Chinese, Ayurveda, Unani, Siddha , Homeopathy, Amchi etc (Manandhar *et al*. 2002). The local communities of Nepal

have been using the medicinal plant species for curing various diseases for a long time (Manandhar 2002). Nepal has enormous wealth of information on ethnopharmacology based remedies which are not only cheap and abundant but are culturally accepted. The diversity of medicinal plants is very high in Nepal but research on pharmacological properties is restricted only about 20% of the medicinal plants so far documented from Nepal Himalayas studied to some extent for their biochemical property (Ghimire *et al*. 2008). There is an increasing demand for medicinal plants-based drugs and pharmaceuticals in the world market. The beneficial medicinal effects of these plants typically result from the secondary compounds in the plants which are specific in certain taxa, such as family, genus and species (Parekh *et al*. 2005) and they can also behave as allelochemicals. These allelopathic compounds can also be used as natural herbicides and other pesticides. However the information on the allelopathic effects of medicinal herbs on many vegetables and cereals is limited. The purpose of this study is to carry out an evaluation on allelopathic activity of some medicinal plants for future chemical analysis which seems to be greatly significant. Since in the developing country like Nepal, most of the people rely on the medicines obtained from the plant products found in nature. The present study seems more significant as we can display the phytochemicals present in the plants for the use of the local community. These four species of medicinal plants were selected on the basis of their use in local community and lack of relevant literatures. Their allelopathic effect were assessed on the growth of two test plant species, wheat and pea. The purpose of selecting these two plants were their easy availability and quick and easy germination even at the controlled laboratory conditions.

1.3 Hypothesis and Objectives

Hypothesis

1. Inhibitory effect increases with the increase in the concentration of the extract and leaf extract show more inhibitory effect than stem and root aqueous extracts.
2. Phytochemicals type and amount vary due to different nature and aromaticity of plants.

Objectives

1. To study and compare the allelopathic effect of different plant aqueous extracts on seed germination and seedling vigour of the wheat and pea seeds by measuring root and shoot length.
2. Screening of the phytochemicals present and estimation of their crude amount present in different plant extracts.

1.4 Limitation of the Study

1. Due to high cost of the chemicals used in the phytochemical screening, the screening of more number of plants is very costly for academic research.

2. LITERATURE REVIEW

2.1 Allelopathy of medicinal plants

Allelopathy of medicinal plants is of special interest in the recent years (Han *et al*. 2008 , Li *et al*. 2009) .The phytochemicals present in the different parts of the medicinal plants are responsible for the medicinal as well as physiological activities (Sofowora 1993). These phytochemicals include phenols, tannins, flavonoids, saponins, alkaloids, carotenes, terpenoids and glycosides. These phytochemicals which remain in the synergestic or compound state show more strong allelopathic, activity on the different plants under study than the individual phytochemicals. Allelochemicals are found to reduce the photosynthetic activity by lowering the CO_2 assimilation. Allelochemicals impairs three major processes of photosynthesis; the stomatal control of CO_2 supply, light reaction and dark reaction (Zhou and Yu 2006). Allelopathic inhibition or promotion may be due to the reduction or increase in cell division and enlargement (Avers and Goodwin 1956), activity of growth retarding or stimulatory hormones, direct inhibition or promotion of nutrient uptake (Harper and Balke 1980, Quasen and Hill 1993), interference with respiration or oxidative phosphorylation or inhibition or enhancement of photosynthesis (Bhowmik and Doll 1984). Allelopathic effect generally results jointly from the synergestic effect of different compounds which stimulate the response of the plant at lower concentration whereas shows inhibitory effect as the concentration increased. Allelopathic inhibition is complex and involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone.

Jha and Dhakal (1990) found that the aqueous extract of aerial parts or roots of *Ageratum conyzoides* inhibited the germination of wheat and rice seeds. Maharjan *et al*. (2007) conducted the similar experiment; the allelopathic effect of aqueous leaf extract of *Parthenium hysterophorous* on germination and seedling growth of three cereal crops, three crucifers and two plants of wild Asteraceae. In the allelopathic study by Sugha 1980, radicle growth was upto 50% and that of plumule was 78% in case of wheat. In pea, radicle growth was enhanced by 60% wheras growth inhibition was observed in plumule upto 20%. The aqueous leaf, stem, root extract of *Ageratum*

conyzoides reduced the germination of wheat in the order of inhibition Leaf> Root >Stem. This aqueous extracts delayed germination and decreased root, shoot elongation and number of leaves in chickpea (Angiras *et al*. 1988). Kumar *et al*. (2007) on their study on the allelopathic influence of *Eupatorium adenophorum* and *Ageratum conyzoides* on different test seeds and revealed the possession of the alleopathic activity by different plant extracts. They also studied the allelopathic effect of *Ageratum conyzoides* in *Brassica campestris*. Strong inhibitory effect was shown due to the alleochemicals released by *Ageratum conyzoides*. The germination and radicle extension of *Brassica campestries* was completely inhibited and plumule extension was stimulated by 14.94% as compared to control. But at 3 g root extract concentration, it exhibited 50% growth of radicle and 70% growth of plumule. Leaves of *Ageratum conyzoides* exhibited the greater suppression of *B. campestris* than the stem and root (Xuan *et al*. 2004). Xuan *et al*. (2004) conducted an experinment on allelopathic potential of *Ageratum conyzoides* on *L. acuinoctialis* . Highest inhibition of 88% was shown by leaf extract, 78 % inhibition by root and 67% by the flower extracts. On raddish, 55% inhibition with 58.3% dry weight production at 2% concentration of leaf extract. Kong *et al*. (1999) found that the inhibition of *Ageratum conyzoides* extract were more pronounced for the shoot growth than the root growth and volatile oils showed more inhibitory effect than the fresh leaves . Boudha *et al.* (2001) studied the effect of essential oil from the leaves of *Ageratum conyzoides* , *Lantana camara* and *Chromolaena odorata* on *Sitophilus zeamais*. *A. conyzoides* was the most effective insecticide than others. Chuihua *et al*. (2011) made an allelopathic study of the volatile substances from the fresh leaves of *A. conyzoides* which showed inhibition in seedling growth of test plants. Volatile oil preocene I and II were isolated and reported that both of them especially precocene II showed strong inhibitory effect even at lower concentration on the seedling growth of radish, tomato and ryegrass.

Makkizadeh (2011) evaluated allelopathic effect of hemp (*Cannabis sativa*) on germination and growth of weeds oat (*Avena fatua),* fat hen (*Chenopodium album)* and pig weed (*Amaranthus retroflexus)* in laboratory and greenhouse and found that increasing hemp extract concentration decreased germination, dry weight and plant height of weeds. Study on the allelopathic activity of *C. sativa* on *Pisum sativum* (pea) and *Triticum aestivum* (wheat) was done by Compton *et al.* (2009). In the same work by Umer *et al*. (2010), the highest concentration 5g leaves completely retarded the

plumule and radicle growth of pea and wheat. 3g aqueous extract of leaf, stem, root and 1g leaf extract showed upto 60% growth of pea radicle. Plumule growth ranged between 38-70% above all concentrations. However 5g stem and root extracts retarded the growth of pea. In same concentration of wheat, it inhibited plumule and radicle growth upto 100 %, 3g leaf showed 100% inhibition of plumule but slight effect on radicle. Only 3g stem extract showed plumule and radicle growth ie, 58% and 39% respectively.

The aqueous leaf and stem extracts of the plant *Tinospora cordifolia* showed the allelopathic effect on the seed germination and seedling growth of two species of the weeds *Chenopodium* and *Cassia* species (Raoof and Siddiqui 2012). The inhibitory effect was concentration dependent and was found to increase with increasing concentration of the plant extracts. Plumule and radicle length, dry weight of seedlings reduced significantly with the response to the plant extracts. Maximum inhibition was seen at 4 % leaf extract of *Cassia tora* leaf extracts.

Qasem (1993) made the allelopathic study of some common weed species in cereal crops on the germination, growth and development of wheat and barley in petri dishes and green house. It showed pronounced allelopathic activities such as inhibition of germination, coleoptiles,reduced radicle length and plumule length, less dry mass of wheat and barley seedlings grown in petri dishes .The effect was more pronounced at early growth stages and increased as the incubation temperature decreased and concentration dependent. Barley was more sensitive to allelopathic effect than wheat. Roots were more affected than shoots because roots are the parts of the plant which are in direct contact with the allelochemicals present in the soil. Nazir *et al*. (2007) studied the allelopathic behaviour of three medicinal plants on traditional agricultural crops of Garhwal, India and found that germination of all the traditional food crops were reduced and the plumule and radicle growth of *Amaranthus caudatus* and *Eleusine coracana* were reduced under the aqueous extracts of all three medicinal plants.

Shrestha (2003) studied the allelopathic potential of *Lantana camara* on two test seeds rice and maize. She conducted the test on aqueous, hexane, methanol, chloroform, essential oils extracts of the plant. The overall effect of leaf was

inhibitory in both maize and rice at higher concentration while stem and root had slightly promotory effects in both. There was the presence of more inhibitory substances in leaf than in stem and root. Sukul and Chaudhary (1999) also reported that the phenolic compounds found in the leaves of *L. camara* were phytotoxic to rice, wheat and three grass seedlings.

2.2 Phytochemistry of the medicinal plants

Gyawali *et al*. (2008) on the phytochemical screening on the aqueous and alcoholic extracts of 47 medicinal plants belonging to 45 genera and 35 families revealed the presence of different secondary metabolites but in variable amounts. 81% of the species contain glycosides, 70% tannins, 66% terpenoids, 62% alkaloids, 60% flavonoids and 17% saponins. The fruit of *Woodfordia fructicosa* showed highest positive test for flavonoids along with alkaloid, saponin, tannin in moderate amount. There was a definite correlation between the traditional applications of the plants with the secondary metabolites present in them. This provides the scientific basis for the traditional medicinal system. Phenolic compounds such as phenols, saponins, flavonoids and alkaloids are one of the largest and ubiquitous groups of plant metabolites (Singh *et al*. 2007). They had several medicinal applications thus it is possible that these plants species could impact allelopathic effects on other organisms (Omulokoli *et al*.1997). They inhibited the radicle growth of alfa alfa (Ohira and Yatagai 1994).Glycosides were reported to lower the blood pressure .Terpenoids possess anti -inflammatory and analgesic activities.

Mozab *et al*. (2003) qualitatively screened 55 medicinal plants from Iran and found the presence of alkaloids in 39 plants, flavonoids in 37 plants, tannin in 20 plants and saponins in 44 plant species. Qualitative and quantitative screening of the phytochemicals present in the leaves of 18 medicinal plants was conducted by Savithramma *et al*.(2011). These leaves were rich in anthocyanins, coumarins, fatty acids, emodins, leucoanthocyanins, tannins, terpenoids, steroids and saponins and these secondary metabolites were responsible for the medicinal activity of these plants. Khan *et al* .(2011) on their similar study for the screening of phytochemicals from twenty different medicinal plants revealed the presence of phytochemicals anthraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids and cardiac glycosides .Tannin was absent in *Cannabis sativa* plant extracts.

Alkaloids, flavonoids, volatile oils, and terpenoides were the principal phytochemicals present in the qualitative screening of phytochemicals in *Chenopodium ambrosioides* by Hezagy and Farrag (2007). Monoterpenes were the phytochemicals responsible for the allelopathic activity of the plant on seed germination and seedling growth of two crop plants; *Lycopersicum esculentum* and *Beta vulgaris* and two weeds; *Meliotus indicus* and *Sonchus oleraceus*. The inhibitory effect was in the order :sterols and terpenes > oil extracts > methanol extract > water extract. Vagashiya, Dave and Chandra, 2011 made the qualitative study of phytochemicals present and determination of flavonoids and phenols in acetone and methanol extract from 53 traditionally used medicinal plants of western region of India. Alkaloid (30.82%), tannins (67.92%), cardiac glycosides (62.26%), steroids (60.38%) and saponins (39.62%) were the phytochemicals present and *Magnifera indica* and then *Woodfordia fructicosa* were reported for their highest phenolic contents among the plants studied.

Preliminary phytochemical screening and in-vitro antibacterial activity of the ethanolic extracts of the three medicinal plants *Litsea glutinosa*, *Vitex peduncularis* and *Elephantopus scaver* (Prusti *et al*. 2008) revealed the presence of secondary metabolites viz. flavonoid, saponin, steroid , alkaloid, glycoside. Highest amount was seen in *Elephantopus scaver* due to which it showed better antibacterial activity against test organisms than the other species. Arowosegbe *et al*. (2012) on their allelopathic study of *Aloe ferox* root extract on tomato showed highest inhibition at 6mg/ml concentration. Quantitative estimation revealed the presence of phenolic contents in highest amount followed by saponins, flavonoids, alkaloids, flavonoids and tannins. These phytochemicals were also reported to have allelopathic activity on some plant species (Seigler 1996).

Daniel and Dishi (2011) found the strong inhibitory activity of the neem plant *Azadirachta indica* extracts on germination and growth of several specific crops and weed species .Evaluation on the phytotoxicity showed that inhibition was more from neem bark than from leaves due to the presence of the phytochemicals in higher amount. Six phenolic compounds including gallic acid, benzoic acid, p- coumaric acid, p-hydroxybenzoic acid, vanillic acid and trans-cinnamic acid were isolated and

identified in both bark and leaves of the plant. Phenolic compounds were responsible for its phytotoxicity and inhibition on germination and growth of test seeds.

From *Ageratum conyzoides* six main alleochemicals precocene I, II ,III , 3 dimethyl -5-tertbutylindenone, β-caryophyllene, 2-brisabolene and fenchyl acetate were isolated and identified by CG-MS (Chuihua *et al*. 2011b). Their allelopathic effect on radish, mugbean and tomato were investigated through modes of volatilization, leaching and degeneration in soil. Precocene-I, preocene-II, β-caryophyllene and 3-3 dimethyl 1-5 tertbutyldenone by volatilization were highly inhibitory to seedling growth of receptor plants. A-brisbolene and fenchyl acetate didn't show inhibitory effect, however when mixed with preocene II showed inhibitory effect. Synergestic effect by volatile oils present in *A. conyzoides* were responsible for the allelopathic potential and not a single inhibitory compound must be present in large quantity in order to affect the growth of a receiving plant (Einhellig 1996).

Hu *et al.* (2002) in their experiment to find out the possibility of *A. conyzoides* as natural fungicide found that flavones released by *A. conyzoides* possibly control the fungal pathogens in citrus orchids like a natural fungicide comparable with commercial fungicide. The insecticidal activity due to the presence of essential oils mainly precocene is the most important biological activity of this species. An essential oil emulsion sprayed on a citrus orchid decreased the population of mites. Leaves of *A. conyzoides* were used as insect repellent due to presence of terpenic compounds pecocenes. Precocenes also accelerated larval metamorphosis in *Musca domestica* (Vyas and Mulchandani 1986). Precocene present in volatile oils of *A conyzoides* possess antibacterial activity (Sharma *et al.* 1979). Steroidal extracts have antibacterial, antiviral properties (Pattnaik *et al* .1996).

Haema-gluttinating properties of *Cannabis sativa* due to the presence of phytochemicals especially the essential oils in the leaves of *Cannabis sativa* correlate the indigenous use of the leaf extract to control bleeding (Bhattarai *et al*. 2010). Anxiolytic or antipsychotic actions of cannabidiol; a *C. sativa* constituent has been reported by Zuardi *et al*. (2006). A high dose of D^9-tetrahydrocannabinol present in Cannabis sativa induces anxiety and psychotic-like symptoms which are significantly reduced by cannabidiol (CBD).The volatile oils detected in the "headspace"

atmosphere surrounding *C. sativa* leaves are powerful insect repellents. Methyl ketones present in *C. sativa* (Turner *et al*. 1980) also repel many leaf-eating insects (Kashyap *et al*. 1991).

Tannin, flavonoid, coumestans, saponins and alkaloids were the phytochemicals present in the well known hepatoprotective herb *E. alba* (Dalal *et al*. 2010). In vitro antimicrobial studies were done and found that the antimicrobial activity of the herb was due to the presence of secondary metabolites coumestans/ wedelolactone.
Kumaraswamy *et al*. (2008) on their study on the floral extracts of *Woodfordia fructicosa* revealed highly potent antibacterial activity of the extract due to the presence of tannins. It also inhibits growth of fungi, bacteria, yeasts and viruses (Chung *et al.* 1998). Kumar *et al.* (2010) reviewed the hepatoprotective activity of four medicinal plants and found that petroleum ether, chloroform and ethyl alcohol flower extract of *Woodfordia fructicosa* showed the hepatoprotective activity. The antioxidant property of the flowers of *W. fructicosa* was due to the presence of phenolic compounds (Shahwar *et al*. 2012). The antioxidant activities by the methanolic extracts of the plant was due to the presence of polyphenolic compounds flavonoids(Middleton 2000 and Sharma 2009). Highest antimicrobial activity by methanolic extract of *Woodfordia fructicosa* floral extract was revealed by Bhattarai and Bhuju (2011). A wide range of compounds including flavonoids and poly-phenols have been isolated from *W. fructicosa*. Flavonoids are hydroxylated phenolic substances or polyphenols known to be synthesized by plants in response to microbial infection. It is also provides colouration to the plants. Flavonoids isolated from root extracts of *Avena* sp (Oat) have been reported to inhibit the ATPase activity of plasma membrane (Balke 1985). The allelopathic bioassay of four different medicinal plants studied in the methanolic extract, the decreasing order of the plants were *Woodfordia fructicosa* > *Pinus roxburghii* > *Senescio chrysanthemoids* > *Conyza bonariensis* (Shahwar *et al.* 2012). The alcoholic extract of the dried flower of *W. fructicosa* which had the presence of tannin show the abortifacient activity (Khushalani *et al.* 2006).

Phytochemical screening of the methanolic extract from the root of *Rumex steudelii* by Gebrie *et al*. 2005 revealed the presence of polysterols, polyphenols, saponins and tannins. Saponins can produce foam in aqueous solutions, show haemolytic activity,

cholesterol binding properties, precipation and coagulation of red blood cells (Sodipo 2000, Okwu 2004). Steroids have relation with various anabolic hormones including sex hormones. They also possess antibacterial and antiviral activity. Saponins, steroids, tannins, glycosides, alkaloids and flavonoids were the phytochemicals present in stem barks of *Jatropa curcas* (Igbinosa 2009).

3. MATERIALS AND METHODS

3.1 Species characters

a. ***Ageratum conyzoides L.*** (Family- Asteraceae) is an erect, annual, branched, slender, hairy and aromatic herb, which grows approximately 1 m in height. The stem and leaves are covered with fine white hairs. Leaves stalked, ovate, 4-10 cm long and 1-5 cm wide, with tip and base somewhat pointed with round-toothed margins. The flowers purple to white, less than 6 mm across arranged in close terminal inflorescences. The fruit black and easily dispersed while the seeds are photoblastic and often lost within 12 months. The plant is found growing commonly in the waste ruined sites. It has a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. The weed has been known since ancient times for its curative properties and has been utilized for treatment of various ailments, such as burns and wounds, for antimicrobial properties, for many infectious conditions and bacterial infections, arthrosis, headaches and dyspnea, pneumonia, analgesic, anti-inflammatory, antiasthmatic, antispasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases (Marks and Nwachuku 1986).

A wide range of chemical compounds including alkaloids, coumarins ,flavonoids, chromenes, benzofurans, sterols and terpenoids have been isolated from this species (Khamboj and Saluja 2008). Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal properties. *A. conyzoides* has been reported to have potential use in controlling pests (Shabana *et al*. 1991) .The volatile oil from *A. conyzoides* had significant biological activities on fungi , insects and plants particularly on plant diseases and insect pests. The volatile oil and its major components precocene not only had insecticidal efficacy, but also anti-feeding effect and delayed molting of insects (Vyas and Mulchandani 1986).

b. ***Eclipta prostrata*** **L.** (Family- Asteraceae) is an annual, erect or prostrate herb with the height of 2 feet. It has shallow tap root with fibrous root system. Leaves opposite, elliptic to lanceolate either without petiole or with short petiole, widely spaced toothed margin ,stem change from green to red at the nodes capable of bearing roots at the nodes. Flowers occur singly or in the clusters of 2-3 on small stalks at the

end of stem or in leaf axils (Karthikumar *et al*. 2007). *E. prostrata* grows commonly as a weed in moist places in temperate to tropical regions. The herb has been used in the treatment of infective hepatitis in India (Wagner *et al* .1986) and snake venom poisoning in Brazil . It has been reported that the leaves of this herb are used in the case of gastritis and respiratory disorders like cough and asthma . In ayurvedic medicine, the leaf extract is considered a powerful liver tonic, rejuvenative and especially good for the hair. In addition, the crude form of the herb is reported to have anti-inflammatory, anti-fungal and anti-hepatotoxic properties.

The herb *E. prostrata* mainly contains coumestans i.e, wedelolactone and dimethyl wedelolactone, polypeptides, polyacetylenes, thiophene-derivatives, steroids, triterpenes and flavonoids (Karthikumar *et al*. 2007).

c. ***Cannabis sativa L.*** (Family-Cannabaceae) is an annual herbaceous plant found growing in the wild state in the wastelands, roadsides and even cultivated in some parts of the world for its various purposes. It is an annual dioecious flowering herb, leaves palmately compound with small leaflets. Male and female flowers are found separate. Male flowers are found on loose panicles whereas female flowers are borne on racemes. Fruit is achene. Preparations from *C. sativa* were extensively used as an antiseptic agent for oral cavity, various respiratory ailments and skin infections, in cuts and wounds, boils, blisters, inflammations (Mechoulam 1986). Hot water extracts from the different parts of *C. sativa* are used for treatment of gonorrhea , dyspensia as nerve stimulant , abortifacient , antipyretic , analgesic, antifungal, antihelminthic , in piles .Smoking of the dried leaves is used as stimulant, to relieve from pain, stress etc. Approximately 500 compounds have been identified in *C. sativa.* Mainly chemicals found in *C. sativa* are terpenes and sesquiterpenes .Of particular importance are the cannabinoids, also known as phytocannabinoids, a group of C21 terpenophenolic compounds unique to *C. sativa (*Turner *et al* . 1980) . D^9-Tetrahydrocannabinol (D^9-THC) is the primary psychoactive constituent in *C. sativa* and has been the focus of a great deal of pharmacological and medicinal research (Costa 2007). Their leaf glands oozes dozens of volatile compounds, such as terpenes, ketones, and esters which produce the characteristic odor of the plant (Ross and ElSohly 1996).

d. ***Woodfordia fructicosa*** Kurz.(Family-Lythraceae) is an important traditional medicinal plant. It is a shrub 1-5 m tall. Stems and branches pendulous, long, pubescent when young, becoming glabrous. Leaves lanceolate, leathery, abaxial, apex acuminate. Inflorescences condensed, axillary shoots of 1-15 flowers. Floral tube light red, red-orange, or deep red, greenish basally, sepals oblong-ovate or deltate, epicalyx segments scarcely present. Petals 6, thin, linear-lanceolate, Stamens 12, inserted above ovary base. The flowers of *W. fructicosa* are commonly used for the treatment of several ailments which includes rheumatism, leucorrhea, menorrhagia, asthma, liver disorder, and inflammatory conditions.

The flowers possess high amount of tannins and they have astringent, acrid, refrigerant, stimulant, depurative, typtic, uterine sedative, antihelmentic, constipating, antibacterial, vulnerary and febrifuge properties(Finose and Devaki 2011). It also has antibacterial, antifertility activities. It is also used as a mordant in the preparation of dyes. The compounds identified are predominantly phenolics; phenolic acids, flavonoids and hydrolysable tannins (Khusulani 2006).

3.2 Description of the Study Area

The plant material collection was done from Tyanglaphant, Kirtipur and Kasara village, Chitwan District, Nepal. The plants under study; *Ageratum conyzoides* and *Cannabis sativa* were collected from the paddy fields and grassland of Tyanglaphant, Kirtipur respectively. Kirtipur location (27º 40.20' N 85º 17.32' E) which lies at 1300 m asl falls on sub-tropical climatic zone with characteristic monsoon rainfall and three distinct seasons: hot and dry summer, hot and moist rainy season and cold and dry winter. The minimum and maximum mean annual temperature ranges from 12.8 º C to 27.2 º C and the mean annual rainfall of 1419 mm. Silty loamy soil very suitable for paddy cultivation was found in the area. It is prone to species invasion and different aromatic invasive plants were found along the sides of the agricultural and paddy fields, along the roadsides etc.

Woodfordia fructicosa and *Eclipta prostrata* samples were collected from Kasara village, Chitwan collection site. *W. fructicosa* was collected from the grasslands at the riverbanks wheras *E. prostrata* was collected from paddy fields and nearby areas.

Kasara village location (27º21'-27º52'N 83º54'-84º48'E) lies 256 m asl. inside the Chitwan National Park. It falls under subtropical inner Terai lowlands of south-central Nepal along the bank of Rapti river. It also experiences monsoon rainfall with hot and wet summer and cold and dry winter. The minimum and maximum mean annual temperature ranges from 18 º C to 31 º C and the mean annual rainfall of 1909 mm. The vegetation pattern seemed to be influenced much by the Rapti river flowing near the site. As the sample collection site is the village inside national park area, the site falls under the buffer zone area which is protected for the specific purposes.

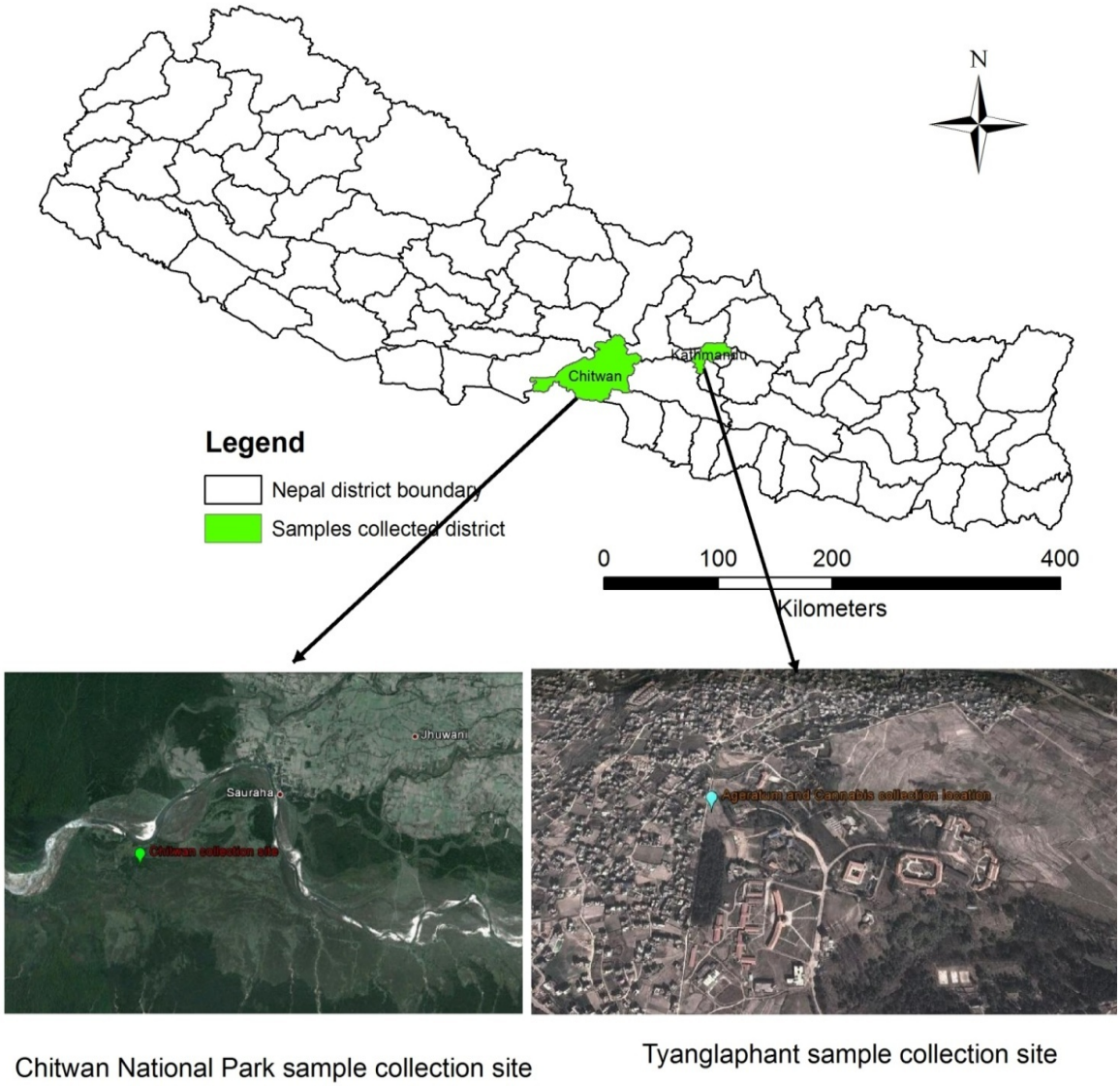


Fig 1. **Map of Nepal showing the Study area**

3.3 Collection of plant materials

Four medicinal plant species in their vegetative stages: *Ageratum conyzoides* was collected from agricultural lands and *Cannabis sativa* was collected from roadside fallow land of Kirtipur, *Eclipta prostrata* was collected from agricultural lands and *Woodfordia fructicosa* was collected from the grassland of Kasara village, Chitwan National Park during the month of June to August , 2011. Whole plants collected were brought into the Ecology laboratory and washed with running water to remove dust and all other undesired materials. Plants were separated into leaf, stem and root, finely cut into pieces and air dried for 10-15 days. Then after, the dried samples were crushed into powder in an electric grinder .Thus prepared powdered samples were kept in zipper plastic bags until they were used for the experinmental studies.

3.4 Allelopathic Potential

Ten gm powdered leaf , stem and root extracts of each plants were mixed with 100 ml distilled water and kept in dark at the room temperature for 24 hours. Aqueous extract was obtained as the filtrate of the mixture. Filtration was done by double layered muslin cloth followed by Whatmann No 1 filter paper. Final volume was adjusted to 100 ml; this gave 10% aqueous extract. The extract was considered as stock solution and a series of solution with different strengths (2, 4, 6 and 8%) were prepared by dilution. 30 ml extract for each concentration were prepared so that there would be triplicates for each concentration of the plant extracts. Fifteen uniform and surface sterilized seeds washed with 2% sodium hypochlorite for 15 minutes were kept for germination in sterilized glass petri-dishes. Petri dishes were sterilized by washing with detergent and water and then putting in hot air oven at 110 º C for 2-4 h. Petri dishes were lined double with blotting papers with thin layer of cotton at the base and moistened with 10 ml of different concentrations of aqueous extracts. 3 control sets for wheat and pea seeds each taking 10ml distilled water as the solution in each petridishes were taken. The petri dishes were kept under the laboratory condition (room temperature 25 ºC) with the diffused sunlight during the daytime for 7 days. The germination of seeds under different treatments were observed in every two days. After seven days, the number of germinated seeds were counted and the root and shoot length were measured. All root and shoot from each petridish were cut separately and oven dried at 70ºC for 48 h to get dry biomass of root and shoot; total biomass produced under each treatments were calculated for the comparative study by

weighing in the electric balance. The similar procedure was repeated for the next time also as there were two test seeds wheat*(Triticum aestivum)* and pea *(Pisum sativum)* whose allelopathic potential were studied. This experinment was repeated twice and data were pooled together before analysis.

3.5 Phytochemical Study

3.5.1 Qualitative Screening

Chemical tests were carried out in the phytochemistry lab, Ecology and resource management unit, Central Department of Botany, TU, Kirtipur. Tests on aqueous extract of powdered specimens using standard procedure were carried out to identify the constituents as described by Sofowora(1993), Trease and Evans (1989) and Harborne(1973) which was followed by Edeoga *et al.* 2005 for the screening of the phytochemicals present in some Nigerian medicinal plants.

1. Test for glycosides

The plant extract was taken in a test tube. Ammonium hydroxide was added and then shaken vigorously. Occurence of cherry red colour showed the presence of glycosides.

2. Test for phenols and tannins

In 2-3 ml of the plant extract, lead acetate solution was added. The white precipitate formation revealed the presence of tannins.

3. Test for saponins

About two gram of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing formation indicated the presence of saponin.

4. Test for flavonoids

Lead acetate solution was added to the extract. Yellow coloured precipitate revealed the presence of flavonoids.

5. Test for terpenoids (Salkowski Test)

To two ml each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (2 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

6. Test for alkaloids

Crude extract was mixed with 2 ml of 2 % HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

3.5.2 Quantitative Estimation

1. **Saponin determination by the method of Obadoni and Ochuko (2001)**

Five gram of each plant samples were mixed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hour with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 50 ml of 20% ethanol. The combined extracts were reduced to 10 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage (Obadoni and Ochuko 2001).

2. Tannin determination by Van-Burden and Robinson (1981) method

Five hundred mg of the sample was weighed into the 250 ml conical flask. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out and mixed with 3 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Van-Burden and Robinson 1981). Results were expressed as mg/g of tannic acid equivalent using the calibrated curve from the equation:

Y = 0.0593x - 0.0485; R2 =0.9826, where x was the absorbance and Y tannic acid equivalent and percentage of the crude extract was calculated out (Arowosegbe *et al.* 2012).

3. **Alkaloid determination by Harborne (1973) method**

Five gram of the sample were weighed into a 250 ml beaker. 100 ml of 20% acetic acid in ethanol was added and covered to stand for four hours. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration which is the total alkaloid present and weighed. (Harborne 1973, Obadoni and Ochuko 2001). The alkaloid content was determined using the formula:

Alkaloid (%) = final weight of sample/initial weight of extract × 100.

4. **Determinations of total phenols by spectrophotometric methods**

One gram of each samples were defatted with 50 ml of diethyl ether using a soxhlet apparatus for 2 hours. For the extraction of the phenolic component, the fat free sample was boiled with 25 ml of ether for 15 minutes. 5 ml of the extract was pipette into a 50 ml flask, then 5 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (Harborne 1973; Obadoni and Ochuko 2001). Results obtained were expressed as mg/g of tannic acid equivalent using the calibration curve from the equation: Y = 0.1216x; R2 = 0.936512, where x was the absorbance and Y the tannic acid equivalent and finally converted into percentage crude yield of the phenolic compounds (Arowosegbe *et al*. 2012).

5. Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1974)

Five gm of the plant samples were extracted repeatedly with 50 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed . Total flavonoids were calculated as mg/g of quercetin standard curve using the following calibration: Y = 0.0255x; R2 = 0.9812, where x was the absorbance and Y was the quercetin equivalent. Finally crude extract was determined in percentage. (Arowosegbe *et al*. 2012).

3.6 Data analysis

3.6.1 Growth Rate

For the study of allelopathic potential, the shoot and root length of each individual plants from all the treatments were measured. The mean value were calculated from the all the individuals of each concentration of the plant extracts. The mean shoot / root length from the control set was taken as the standard value with 100% growth.

Then, by assuming shoot and root growth in control set as 100%, the growth of the other sets were calculated by using the formula:

$$\text{Shoot growth rate in x\% treatment (\%)} = \frac{\text{Mean shoot length of the test plant under x\% treatment}}{\text{Mean shoot length of the test plant under the control set}} \times 100$$

$$\text{Root growth rate in x\% treatment (\%)} = \frac{\text{Mean root length of the test plant under x\% treatment}}{\text{Mean root length of the test plant under the control set}} \times 100$$

These values were then plotted in a graph showing the shoot and root growth rate at Y- axis and treatments done under the X-axis. This graph clearly demonstrates the growth rate of the pea and wheat at different concentrations.

3.6.2 Germination rate

Rate of germination of the different test seeds under different treatments by using the following formula:

$$\text{Germination Percentage (\%)} = \frac{\text{Total number of seeds germinated in the given treatment}}{\text{Total number of seeds inoculated in each petri dishes (15)}} \times 100$$

$$\text{Mean Germination Percentage (\%)} = \frac{\text{Germination Percentage of the test seeds under the given treatment}}{\text{Mean germination in control sets}} \times 100$$

3.6.3 Biomass estimation

Total biomass of the seedlings were calculated by taking the average of the shoot and root parts separately under each treatments. Then, by taking the biomass produced under the control sets as the standard value the biomass produced by the test seeds

under different concentration of the extract were compared by plotting a bar graph. This graph helps us to compare and relate the biomass produced in grams of shoot and root under different treatments and relate it with their allelopathic potential.

3.6.4 Phytochemical Study

For the qualitative and quantitative screening of phytochemicals, the standard procedures given by different scientists were followed as mentioned in detail earlier. The samples were then observed for the presence of precipitation. A '+' score was recorded if the reagent produced only a slight opaqueness; a '++' score was recorded if a definite turbidity, but no precipitation was observed and a '+++' score was recorded if a definite heavy precipitate was produced and '-' score if there was no precipitation.

3.6.5 Statistical analysis

The datas were analysed by using Statistical Package for Social Sciences (SPSS) version 16.00 at 0.05 level of significance using Duncan's multiple range test. Significanct difference in root and shoot length of seedlings under different treatments were tested and compared using One-way Analysis of Variance (ANOVA) and homogeneity test .In the study, the different treatments done was taken as the independent variable and the plumule and radicle length of the seedlings of different test plants at different treatments as the dependent variable. Percentage growth of shoot and root under different treatments were represented in line graphs.

4. RESULT

4.1 Allelopathic potential

4.1.1 Germination

The aqueous extract of all the plants significantly affected the germination of wheat seeds(Table-1). The inhibition effect was found to increase with increasing concentrations of different aqueous extracts. Leaf extract showed the strongest allelopathic effect on seed germination than stem and leaf extracts. Germination percentage in control was 97.8 % . Maximum germination of 100 % was observed in 2 % stem extract of *Woodfordia fructicosa* wheras minimum germination rate of 71.1 % were observed in 8 and 10 % concentration of *Cannabis sativa* leaf extract and 6 and 8 % concentration of *Eclipta prostrata* leaf extracts.

Table 1. Effect of plants aqueous extracts on germination of wheat seeds. (Mean Germination± Standard Deviation) (n=90)

Species name	Plant part	Germination%					
		Control(0%)	2 %	4 %	6%	8 %	10 %
A. conyzoides L.	Leaf	97.8±0.58	97.8±0.58	91.1±0.8	97. 8±0.58	88.9±0.58	88.9±0.58
	Stem	97.8±0.58	97.8±0.58	97.8±0.58	91.1±0.58	91.1±0.58	88.9±1.15
	Root	97. 8±0.58	95.6±0.58	93.3±0.58	95.6±1.00	88.9±1.15	88.9±1.15
C. sativa L.	Leaf	97. 8±0.58	88.9±0.58	71.1±2.08	71.1±2.08	77. 8±1.15	80±2.00
	Stem	97. 8±0.58	97.8±0.58	95.6±0.58	86.7±1.00	86.7±1.00	84.5±0.58
	Root	97. 8±0.58	95.6±0.58	93.3±0.58	84.5±0.58	91.1±0.58	82.2±1.15
E. prostata L.	Leaf	97. 8±0.58	88.9±0.58	71.1±2.08	71.1±2.08	77. 8±1.15	80±2.00
	Stem	97. 8±0.58	93.3±1.00	91.1±1.52	86. 7±1.00	84.4±2.08	80±1.72
	Root	97. 8±0.58	93.3±0.00	86. 7±0.00	84.4±0.58	82.2±1.15	80±1.00
W. fructicosa Kurz	Leaf	97. 8±0.58	95.6±1.0	95.6±0.58	91.1±0.58	88.9±1.15	80±1.15
	Stem	97. 8±0.58	100±0.0	95.6±1.15	91.1±1.15	95.6±0.58	88.9±0.58
	Root	97. 8±0.58	93.3±0.58	91.1±0.58	88.9±0.58	88.9±1.15	88.9±2.00

The aqueous extract of all the plants significantly affected the germination of pea seeds (Table-2). In pea seeds, germination percentage in control was also 97.8% Maximum germination percentage of 97.8 % were observed at 2% concentration of *Ageratum conyzoides* root, *Cannabis sativa* leaf, *Cannabis sativa* root and *Woodfordia fructicosa* leaf extracts. Minimum germination rate of 84.5% were observed in higher concentration of the plant extracts i.e, 10% concentration of *C. sativa* root extract, 8 and 10% of *E. prostrata* root extracts.

TABLE 2. Effect of plants aqueous extracts on germination of pea seeds. (Mean Germination± Standard Deviation) (n=90)

Species name	Plant parts	Germination %					
		Control (0%)	2 %	4 %	6%	8 %	10 %
A. conyzoides L.	Leaf	97.8±0.58	95.3±0.58	93.3±1.00	88.7±0.58	88.7±0.58	86.7±1.00
	Stem	97.8±0.58	95.3±0.58	93.3±1.00	93.3±1.00	93.3±1.00	88.7±1.52
	Root	97.8±0.58	97.8±0.58	91.1±0.58	91.1±1.15	91.1±1.15	86.7±0.00
C. sativa L.	Leaf	97.8±0.58	97.8±0.58	93.3±1.0	84.5±1.52	86.7±0.0	91.3±0.58
	Stem	97.8±0.58	93.3±1.0	93.3±1.0	88.7±1.15	86.7±1.0	88.7±1.0
	Root	97.8±0.58	97.8±0.58	93.3±1.0	91.1±1.52	88.7±0.58	88.7±1.0
E. prostrata L.	Leaf	97.8±0.58	93.3±0.0	93.3±1.0	91.1±0.58	86.7±1.0	88.7±1.52
	Stem	97.8±0.58	93.3±1.00	86.7±0.00	91.1±1.15	86.7±1.0	91.1±1.15
	Root	97.8±0.58	95.3±0.58	93.3±0.00	93.3±1.00	84.5±0.58	84.5±0.58
W. fructicosa Kurz	Leaf	97.8±0.58	97.8±0.58	93.3±1.00	88.7±0.58	88.7±0.58	86.7±1.00
	Stem	97.8±0.58	95.3±0.58	91.1±0.58	91.1±1.15	86.7±1.00	86.7±1.00
	Root	97.8±0.58	93.3±1.00	88.7±0.58	91.3±0.58	91.3±1.15	91.3±1.15

4.1.2 Seedling growth

There was significant difference ($p<0.001$) between the treatments in plumule and radicle length of wheat seeds (Table 3 and 4). Inhibition percentage of plumule growth in wheat ranged from 93-77% in *Eclipta prostrata*, 65-15% in *Woodfordia fructicosa* , 59-17% in *Ageratum conyzoides* and 69-43% in *Cannabis sativa*. Radicle growth inhibition percentage in wheat ranged from 93-88% in *E .prostrata*, 80-50% in *W. fructicosa* , 63-43% in *A. conyzoides* and 70-43% in *C. sativa* (Fig 2-5).

Maximum plumule inhibition (93%) in wheat was shown by 10% stem extract of *E. prostrata* (Fig- 3B) wheras minimum inhibition (15%) was shown by 4% stem extract of *W. fructicosa* (Fig 5B).

The homogeneity test showed that the plumule length of wheat at different concentration were significantly different from that of control. There was significant difference for plumule *growth* from that of control *in* leaf and stem extract of *Ageratum conyzoides* wheras in root, significance was seen only at 2% concentration of the extract as compared to the control (Table 3). In *E. prostrata,* the shootlengh at different concentrations were significantly different and increased with increasing concentration of the extract. In *Cannabis sativa*, plumule length was significantly different at all the concentrations except for 4% stem extract. *W. fructicosa,* plumule length was found significantly different from that of control except for 4 and 6% concentration of the leaf extract, 8 and 10% of stem extract and 2% root extract respectively (Table 3).

The homogeneity test for the radicle length of wheat seeds showed the significant differences at different plant extracts (Table 4). In *Ageratum conyzoides* leaf extract, significant difference was seen except for 8% concentration of the extract. In stem, significant difference was seen at 4, 8 and 10 %. There was no significant difference for radicle length in root extract from that of control. In *E. prostrata* , radicle length on leaf extract were significantly different at 2,6 and 10% , at stem extract significant difference was seen at 2,6,8 and 10% whereas significant difference was seen at all concentrations of the root extract in comparision to control. In *W. fructicosa,* significant difference was seen except for 8% and 6% concentration of the leaf and stem extracts respectively. In root significant difference was seen at 6, 8 and 10% concentrations from that of control. Maximum wheat radicle inhibition (93%) shown by 10% leaf extract of *E. prostrata* (Fig-3A).

Similarly, inhibition percentage of plumule growth in pea ranged from 84-53% in *C sativa,* 80-29% in *A conyzoides,* 47-2% in *E. prostrata* and 69% maximum inhibition and stimulatory effect was seen in *W. fructicosa.* Radicle growth inhibition percentage in pea ranged from 55-18% in *A. conyzoides* , 41-26% in *C sativa*, 31%

to 128% stimulatory effect in *E. prostrata* and 266.76% of stimulatory effect in *W. fructicosa* (Fig 6-9).

ANOVA showed significant difference ($p<0.001$) between the treatments in plumule length of pea except for the root aqueous extract of *A. conyzoides* ($p=0.502$) (Table 5). The homogeneity test showed that the plumule length of pea at different concentration of the plant extracts differed with that of control. In *A. conyzoides* leaf extract, plumule length showed significant difference at 2, 8 and 10 %. In stem extract, plumule length were significantly different at 6 and 8% and in root, significant difference was seen at 4 and 10% from that of control. Maximum plumule inhibition of pea seeds was 84% in 4% stem extract of *C. sativa* (Fig 7B). Minimum inhibition of 2% was shown by 4% root extract of *W. fructicosa* (Fig 9C).

Significant difference for the radicle length of pea plant between the treatments were seen except for *A. conyzoides* root ($p=0.230$), *C. sativa* stem ($p=0.404$) and root ($p=0.180$) and *E. prostrata* stem ($p=0.81$) extracts (Table 6). Homogeneity test showed that the radicle length of pea at different concentrations were significantly different (Table 2.4). Radicle length in leaf extract of *A. conyzoides* were significantly different from that of control except for 6% concentration of the extract. In stem extract significant difference was seen at 6 and 10% whereas in root extract no significant difference was seen. No significant difference was seen in *C. sativa* under the different treatment conditions but they were found to differ from the plumule length of control. *E. prostrata* showed significant difference at all plants extracts except for 4% concentration of leaf , 2 and 4% concentration of stem and 2, 4 and 6% concentration of root. In *W. fructicosa*, stimulatory effect was seen at all the concentrations and root length value was lower in control in comparision to the roootlength of pea under other treatments. Radicle length values were significant except for 10% of leaf extract. Maximum radicle inhibition (84%) was shown by *C. sativa* 10% stem extract (Fig 8C). Minimum inhibition (18%) was in 4% stem extract of *A. conyzoides* (Fig 6B). Stimulatory effect was shown by the *W. fructicosa* and *E. prostrata* plant extracts on their radicle growth (Fig 8 and 9). Maximum stimulatory effect was seen on 2% stem extract of *W. fructicosa* with growth percentage of 266.77%. Stimulatory effect in *W. fructicosa* was in the order root> stem>root(Fig-9).

Table 3. Effect of plant aqueous extracts on plumule length of wheat. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, *á* = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=90).

Species name	Plant parts	Plumule length (cm) at different concentration						F-Value	P-Value
		Control	2 %	4 %	6%	8 %	10 %		
A. conyzoides L.	Leaf	13.68±1.3 ^{d}	10.92± 2.12 ^{c}	8.86 ± 2.36 ^{b}	8.47 ±2.67 ^{b}	6.78 ± 1.28 ^{a}	6.16 ± 1.09^{a}	34.89	0.000
	Stem	13.68±1.3 ^{e}	11.065±3.42 ^{d}	10.01 ±2.47 ^{c}	10.87±2.0 ^{c}	8.25 ± 2.90^{a}	9.51 ± 2.14^{b}	43.355	0.000
	Root	13.68±1.3 ^{c}	11.37 ± 1.82 ^{b}	10.03±2.15b	8.89 ±2.31^{a}	9.80 ± 1.03^{a}	9.57 ± 1.22^{a}	6.714	0.000
C. sativa L.	Leaf	13.68±1.3^{d}	12.12 ±1.33^{c}	10.92 ±2.12 ^{bc}	10.40±3.1^{b}	9.92 ± 2.90^{b}	7.22 ±2.41 ^{a}	42.89	0.000
	Stem	13.68±1.3 ^{d}	11.88 ± 1.01 ^{c}	10.25 ±2.12 ^{b}	12.41±2.3 ^{c}	12.41 ± 2.13 ^{c}	9.15 ± 2.1^{a}	54.748	0.000
	Root	13.68±1.3 ^{d}	10.00 ± 0.12 ^{bc}	11.61 ± 2.2 ^{c}	11.11±1.8 ^{c}	9.34 ± 2.94^{b}	8.68 ± 2.40 ^{a}	22.079	0.000
E. prostrata L.	Leaf	13.68±13 ^{c}	3.49 ± 1.21^{b}	3.25 ± 1.02 ^{b}	1.64±0.89 ^{a}	1.63 ± 0.68^{a}	1.06 ±0.87 ^{a}	16.67	0.000
	Stem	13.68±1.3^{d}	3.09 ±0.98^{c}	2.26 ± 1.02 ^{c}	1.99 ±0.87 ^{b}	1.89 ± 0.87 $^{a\,b}$	0.91 ± 0.16 ^{a}	15.516	0.000
	Root	13.68±1.3 ^{c}	2.78 ± 0.85^{b}	2.03 ± 1.02 ^{b}	1.88 ±1.11 ^{a}	1.87 ± 0.86 ^{a}	1.02± 0.16^{a}	29.387	0.000
W. fruticosa Kurz	Leaf	13.68±1.3 ^{d}	8.03±3.85 ^{bc}	7.49±3.09 ^{b}	7.98±2.71 ^{b}	7.4±3.2^{b}	4.77±2.64^{a}	8.07	0.000
	Stem	13.68±1.3^{d}	9.12 ± 2.91^{c}	9.70 ± 3.06 ^{c}	9.97 ±2.25 ^{c}	6.80 ±2.48^{a}	7.85 ± 1.29 ^{b}	26.891	0.000
	Root	13.68±1.3 ^{d}	10.45 ± 4.62 ^{bc}	11.67 ±3.29 ^{c}	11.11±3.8^{c}	9.34 ± 4.94 ^{b}	8.68 ± 4.4^{a}	17.525	0.000

Table 4. Effect of plant aqueous extracts on radicle length of wheat. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, $\acute{a} = 0.05$). F and P values were obtained by one way analysis of variance (ANOVA). (n=90).

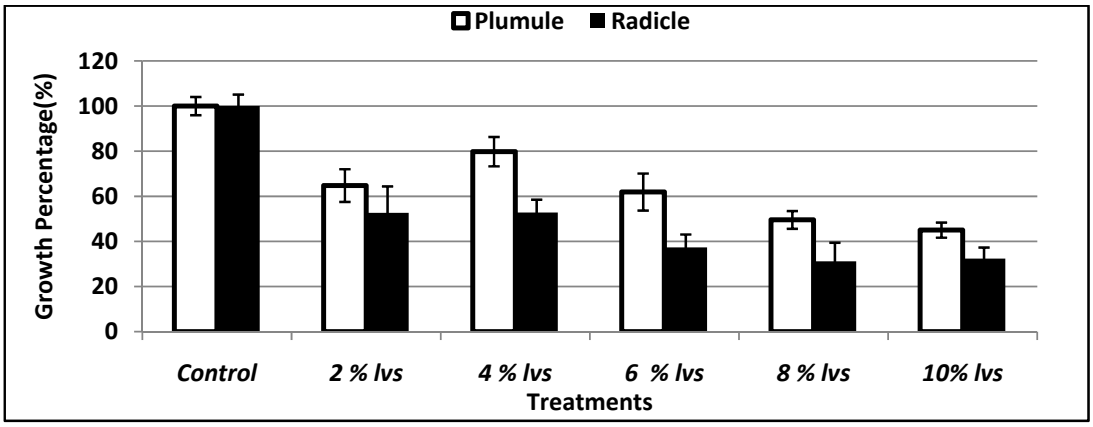
Species name	Plant parts	Radicle length (cm) at different concentration						F-Value	P-Value
		Control	2 %	4 %	6%	8 %	10 %		
A. conyzoides L.	Leaf	12.78 ± 1.1^{d}	7.78 ± 3.12^{c}	7.81 ± 1.50^{c}	5.52 ± 1.52^{b}	4.75 ± 2.20^{a}	4.78 ± 1.32^{a}	15.64	0.000
	Stem	12.78 ± 1.1^{c}	5.47 ± 1.31^{a}	7.87 ± 1.9^{b}	5.33 ± 1.24^{a}	5.73 ± 1.97^{a}	5.18 ± 1.05^{a}	7.320	0.000
	Root	12.78 ± 1.1^{d}	8.36 ± 1.21^{c}	7.05 ± 1.2^{b}	7.13 ± 2.21^{b}	6.95 ± 1.32^{ab}	6.39 ± 2.03^{a}	22.08	0.000
C. sativa L.	Leaf	12.78 ± 1.1^{c}	8.30 ± 1.23^{b}	8.41 ± 2.13^{b}	8.00 ± 2.34^{b}	7.77 ± 2.3^{b}	4.47 ± 1.21^{a}	4.21	0.001
	Stem	12.78 ± 1.1^{c}	6.83 ± 1.21^{ab}	7.00 ± 2.31^{b}	7.29 ± 1.34^{b}	7.45 ± 1.10^{b}	6.63 ± 2.14^{a}	3.595	0.004
	Root	12.78 ± 1.1^{b}	6.40 ± 2.61^{a}	6.97 ± 1.46^{a}	7.07 ± 1.87^{a}	6.72 ± 2.36^{a}	6.67 ± 1.50^{a}	2.185	0.057
E. prostrata L.	Leaf	12.78 ± 1.1^{c}	2.18 ± 1.10^{b}	1.82 ± 1.00^{a}	2.23 ± 1.21^{b}	1.79 ± 0.94^{a}	1.93 ± 1.34^{a}	20.00	0.000
	Stem	12.78 ± 1.1^{d}	3.24 ± 1.01^{c}	1.91 ± 0.98^{ab}	2.09 ± 1.21^{b}	2.22 ± 1.10^{b}	1.05 ± 0.98^{a}	37.146	0.000
	Root	12.78 ± 1.1^{d}	3.11 ± 1.03^{c}	2.76 ± 1.32^{bc}	2.35 ± 1.63^{b}	1.90 ± 0.98^{a}	1.94 ± 0.68^{a}	16.797	0.000
W. fructicosa Kurz	Leaf	12.78 ± 1.1^{d}	6.03 ± 2.66^{c}	5.62 ± 2.29^{b}	5.66 ± 2.10^{b}	6.90 ± 1.28^{c}	2.34 ± 3.17^{a}	16.43	0.000
	Stem	12.78 ± 1.1^{e}	11.57 ± 4.40^{d}	9.79 ± 2.70^{c}	6.04 ± 1.0^{b}	6.14 ± 1.70^{b}	5.54 ± 1.89^{a}	52.563	0.000
	Root	12.78 ± 1.1^{c}	6.40 ± 2.61^{a}	6.97 ± 3.46^{ab}	7.07 ± 3.87^{b}	6.72 ± 4.36^{a}	6.67 ± 4.5^{a}	19.355	0.000

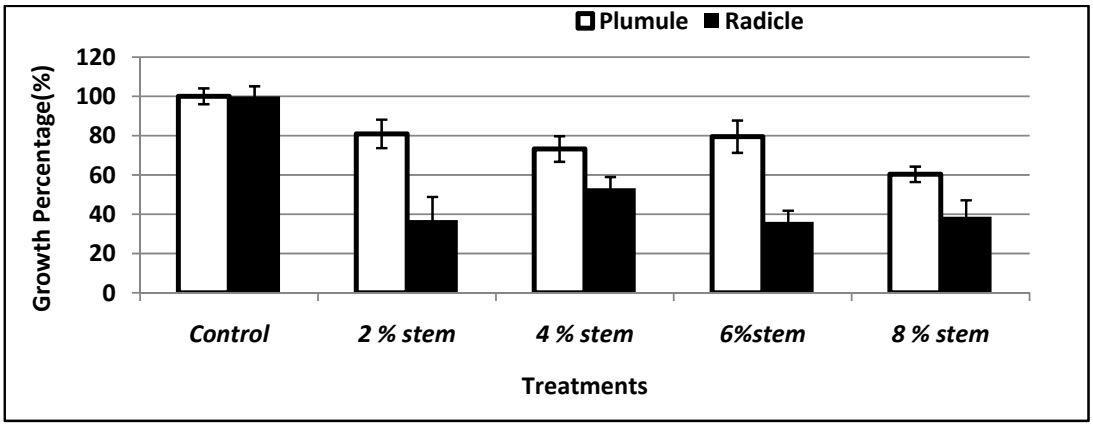
Table 5. Effect of plant aqueous extracts on plumule length of pea. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, *á* = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=90).

Species name	Plant parts	Plumule length (cm) at different concentration						F-Value	P-Value
		Control	2 %	4 %	6%	8 %	10 %		
A. conyzoides L.	Leaf	2.15±1.21 ^{c}	0.59 ±0.45^{a}	0.71 ± 0.12 ^{b}	0.74 ±0.16 ^{b}	0.69 ± 0.23^{b}	0.59 ± .23^{a}	11.99	0.03
	Stem	2.15±1.21^{c}	0.59 ±0.18^{a}	0.44 ± 0.28 ^{a}	0.67 ±0.30 ^{ab}	0.55 ± 0.30^{a}	0.80 ± 0.31^{b}	4.602	0.000
	Root	2.15±1.21^{c}	0.86 ± 0.19^{b}	0.88 ± 0.43^{b}	0.79 ±0.25^{a}	0.72 ± 0.39^{a}	0.73 ± 0.35^{a}	0.870	0.502
C. sativa L.	Leaf	2.15±1.21 ^{d}	1.28 ±0.40 ^{c}	1.0 ± 0.26^{c}	0.74± 0.42 ^{ab}	0.55 ± 0.36 ^{a}	0.62 ± 0.27 ^{ab}	9.58	0.028
	Stem	2.15±1.21 ^{c}	1.58 ± 0.40 ^{b}	1.45 ± 0.56 ^{b}	0.72 ±0.22 ^{a}	0.65 ± 0.36 ^{a}	0.64 ± 0.17 ^{a}	1.99	0.050
	Root	2.15±1.2^{d}	0.58 ± 0.20 ^{a}	1.0 ± 0.56 ^{c}	0.74 ±0.12 ^{b}	0.55 ± 0.06 ^{a}	0.62 ± 0.17 ^{b}	3.446	0.005
E. prostrata L.	Leaf	2.15±1.21 ^{b}	2.09 ± 1.02 ^{b}	1.25 ±0.53 ^{a}	1.31 ±0.63 ^{a}	2.04 ±0.76 ^{b}	1.37 ± 0.58 ^{a}	10.68	0.000
	Stem	2.15±1.21^{b}	2.10 ± 1.02^{b}	1.15 ± 0.13^{a}	1.53 ±0.03 ^{a}	2.04 ± 0.06 ^{b}	1.27 ± 0.18^{a}	17.06	0.000
	Root	2.15±1.21^{b}	1.16 ± 0.60^{a}	1.3 ± 0.57^{a}	1.56 ±0.71^{a}	1.35 ± 0.51^{a}	1.21 ± 0.44^{a}	9.248	0.000
W. fructicosa Kurz	Leaf	2.15±1.21 ^{c}	0.71 ± 0.46^{a}	1.25 ± 0.87 ^{b}	1.65 ±1.08 ^{b}	1.14 ±0.72 ^{b}	0.67 ± 0.28 ^{a}	9.87	0.004
	Stem	2.15±1.21 ^{b}	2.77 ± 1.09 ^{c}	1.27 ± 1.17 ^{a}	2.02 ±1.15 ^{b}	1.17 ± 0.98 ^{a}	1.05 ± 0.98 ^{a}	13.247	0.000
	Root	2.15±1.21^{c}	1.28 ± 0.49 ^{b}	1.53 ± 0.82^{b}	0.65 ±0.39^{a}	0.92 ± 0.19 ^{a}	1.33 ± 0.39^{b}	6.531	0.000

Table 6. Effect of plant aqueous extracts on radicle length of pea. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, *á* = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=90).

Species name	Plant parts	Radicle length (cm) at different concentration						F-Value	P-Value
		Control	2 %	4 %	6%	8 %	10 %		
A. conyzoides L.	Leaf	3.40±1.71 ^{d}	2.67 ± 1.78 ^{c}	2.80 ± 1.30 ^{c}	1.54 ± 0.13^{a}	2.01 ± 1.01^{b}	2.01 ±1.11 ^{b}	4.65	0.000
	Stem	3.40±1.71 ^{c}	1.75 ± 0.97 ^{a}	1.8 ±1.10 ^{a}	2.37 ±1.30 ^{b}	1.52 ± 0.24 ^{a}	2.27 ± 0.09^{b}	6.85	0.000
	Root	3.40±1.71 ^{a}	2.87 ± 0.55 ^{a}	2.80 ± 0.98 ^{a}	2.67 ±1.43 ^{a}	2.94 ± 0.37 ^{a}	2.89 ± 1.00 ^{a}	1.38	0.230
C. sativa L.	Leaf	3.40±1.71 ^{b}	2.01 ±1.43 ^{a}	2.52 ±1.93 ^{a}	2.46±1.49 ^{a}	2.06 ±1.31 ^{a}	2.35 ±1.26 ^{a}	2.00	0.078
	Stem	3.40±1.^{b}	2.01 ±1.22^{a}	2.52 ±1.55^{a}	2.46±1.6^{a}	2.06±1.13^{a}	2.35 ± 1.43^{a}	1.02	0.404
	Root	3.40±1.^{b}	2.01 ± 1.43 ^{a}	2.52 ±1.93^{a}	2.46±1.4^{a}	2.06±1.31^{a}	2.35 ± 1.26 ^{a}	1.53	0.180
E. prostrata L.	Leaf	3.40±1.71 ^{b}	5.36 ±1.15 ^{c}	2.76 ± 1.38 ^{a}	3.26±1.3^{b}	3.10±1.21^{ab}	2.80 ±1.30 ^{a}	14.95	0.008
	Stem	3.40±1.71 ^{b}	5.36 ± 2.15 ^{c}	2.86 ± 1.08 ^{a}	3.26 ±1.34 ^{b}	3.16±1.01^{b}	2.80 ± 1.30^{a}	2.11	0.081
	Root	3.40±1.71 ^{b}	3.33 ± 1.66 ^{b}	3.08 ± 1.48 ^{b}	2.59 ±1.24 ^{b}	3.06± 1.37 ^{b}	2.33± 0.89 ^{a}	3.05	0.011
W. fructicosa Kurz	Leaf	3.40±1.7^{a}	8.06±3.91^{d}	7.47 ±2.56^{c}	8.22±2.2^{d}	6.72±2.74 ^{b}	8.17 ± 2.42	18.94	0.000
	Stem	3.40±1.7^{a}	7.58±2.14^{d}	7.29 ±2.83^{d}	6.46±2.9^{c}	6.85±2.36^{c}	5.68 ± 1.90^{b}	21.20	0.000
	Root	3.40±1.7^{a}	6.84±0.77^{b}	8.81 ±0.62^{c}	9.07±0.4^{d}	8.18±0.75^{c}	8.96 ± 0.97^{c}	34.87	0.000





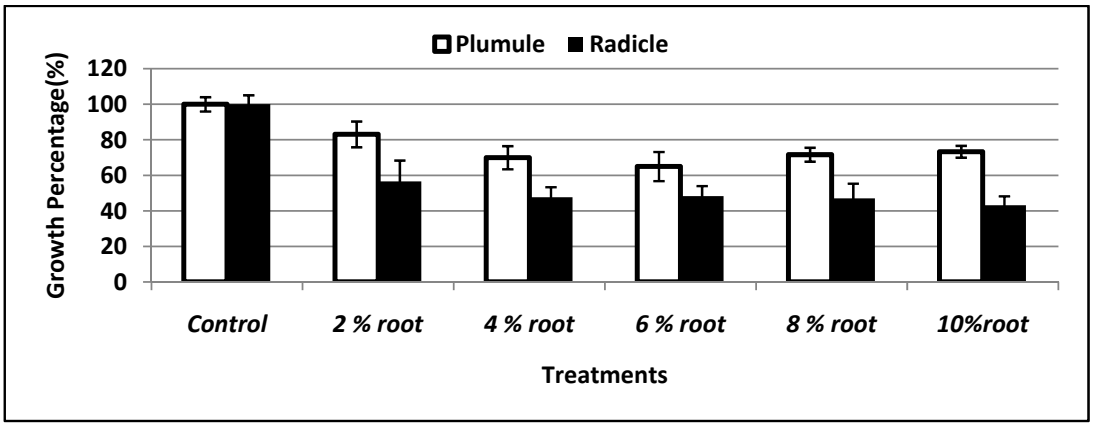


Fig 2. Effect of *Ageratum conyzoides* plant extracts on plumule and radicle growth of wheat [A-C]. [A-Leaf, B- Stem, C- Root].

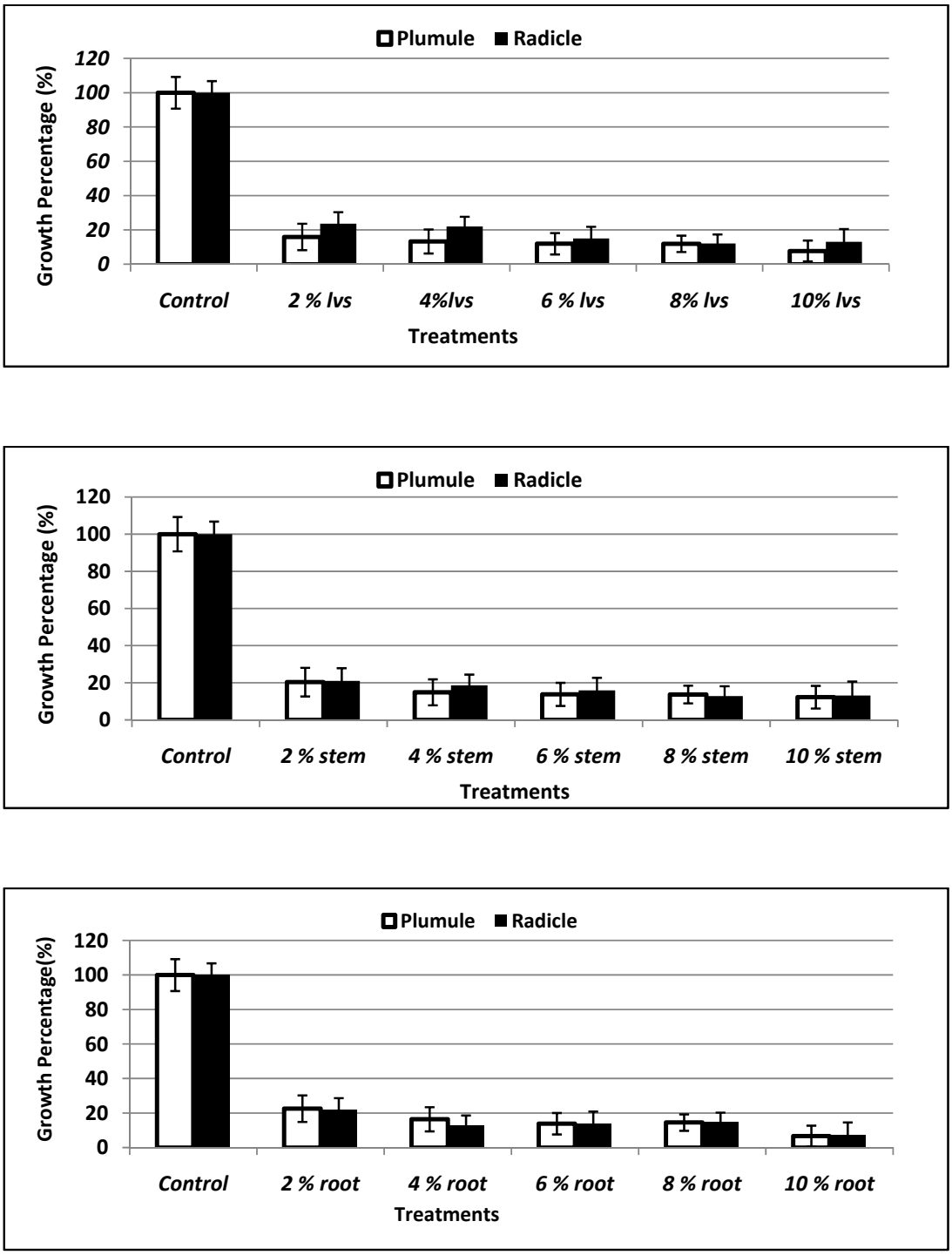


Fig 3. Effect of *Eclipta prostrata plant* extracts on plumule and radicle growth of wheat [A-C]. [A-Leaf, B- Stem, C- Root].

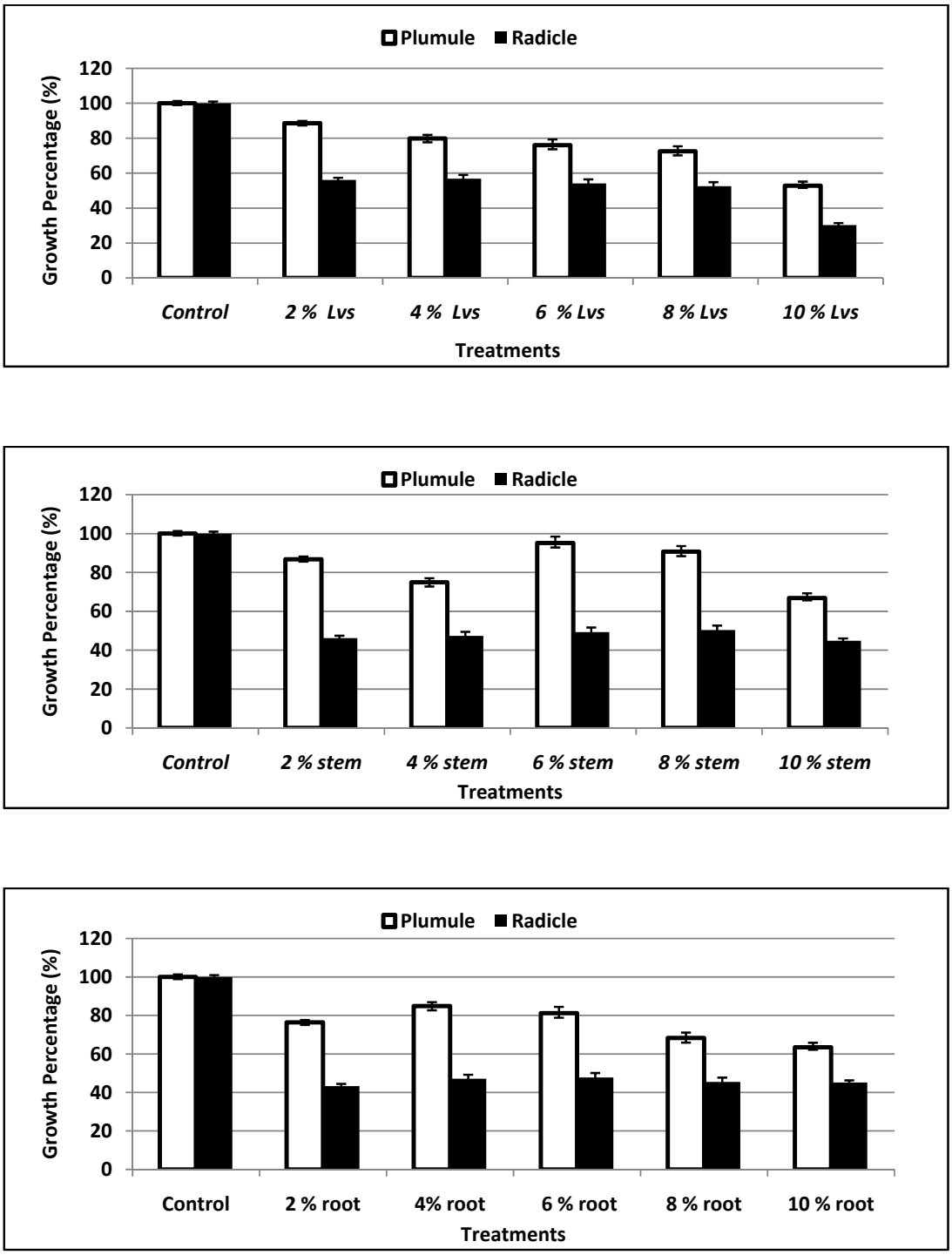
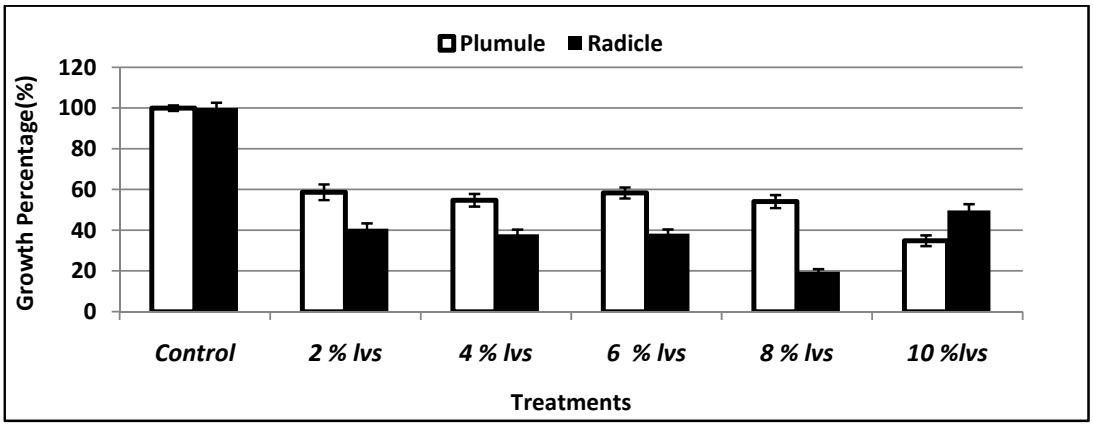
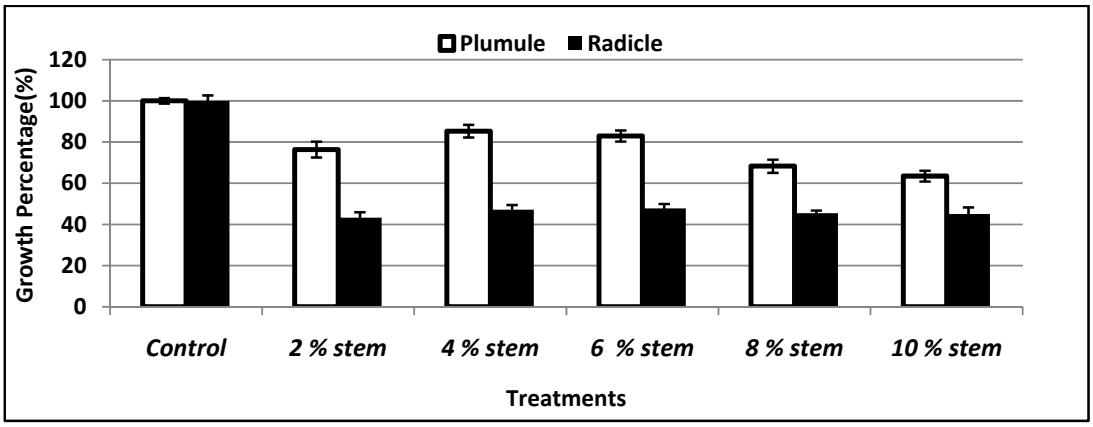


Fig 4. Effect of *Cannabis sativa plant* extracts on plumule and radicle growth of wheat [A-C]. [A-Leaf, B- Stem, C- Root].





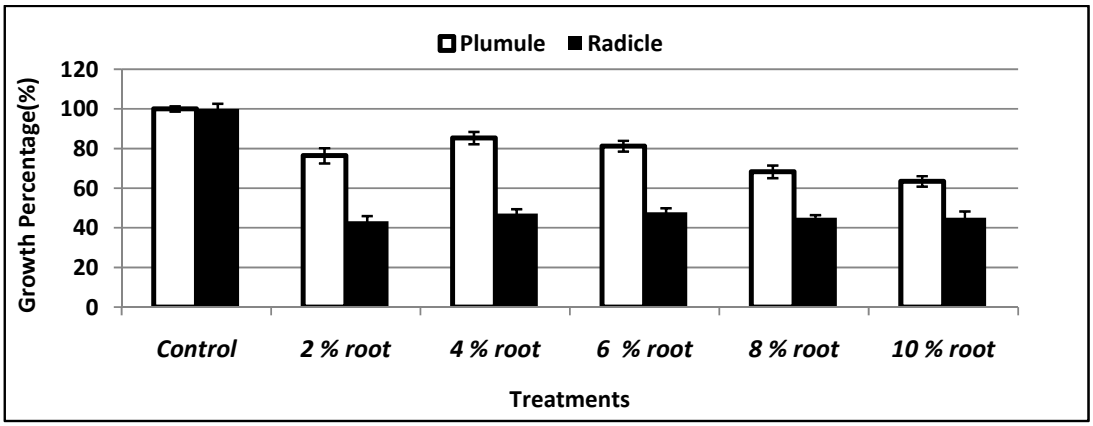


Fig 5. Effect of *Woodfordia fructicosa plant* extracts on plumule and radicle growth of wheat [A-C]. [A-Leaf, B- Stem, C- Root]

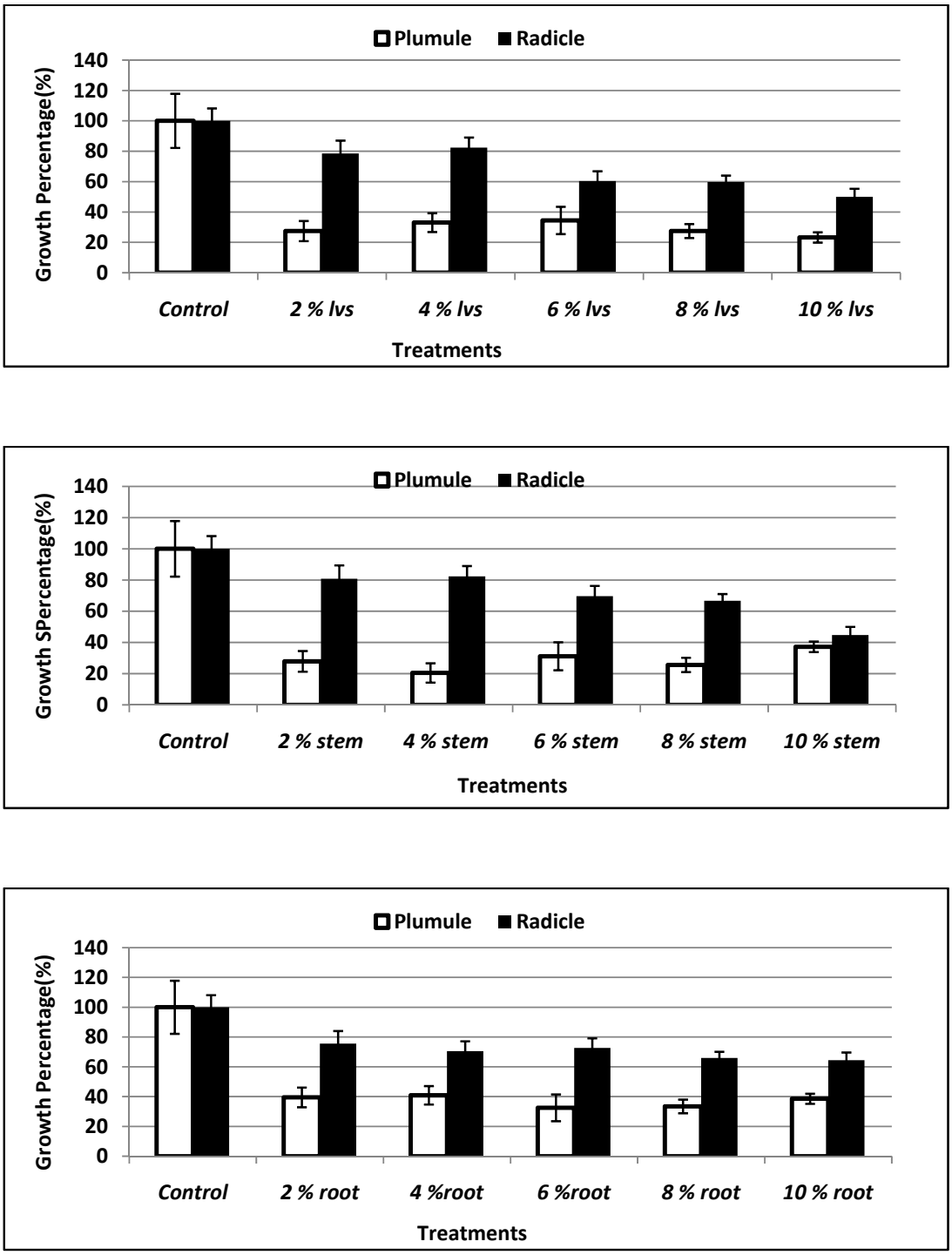


Fig 6. Effect of *Ageratum conyzoides plant* extracts on plumule and radicle growth of pea. [A-C]. [A-Leaf, B- Stem, C- Root].

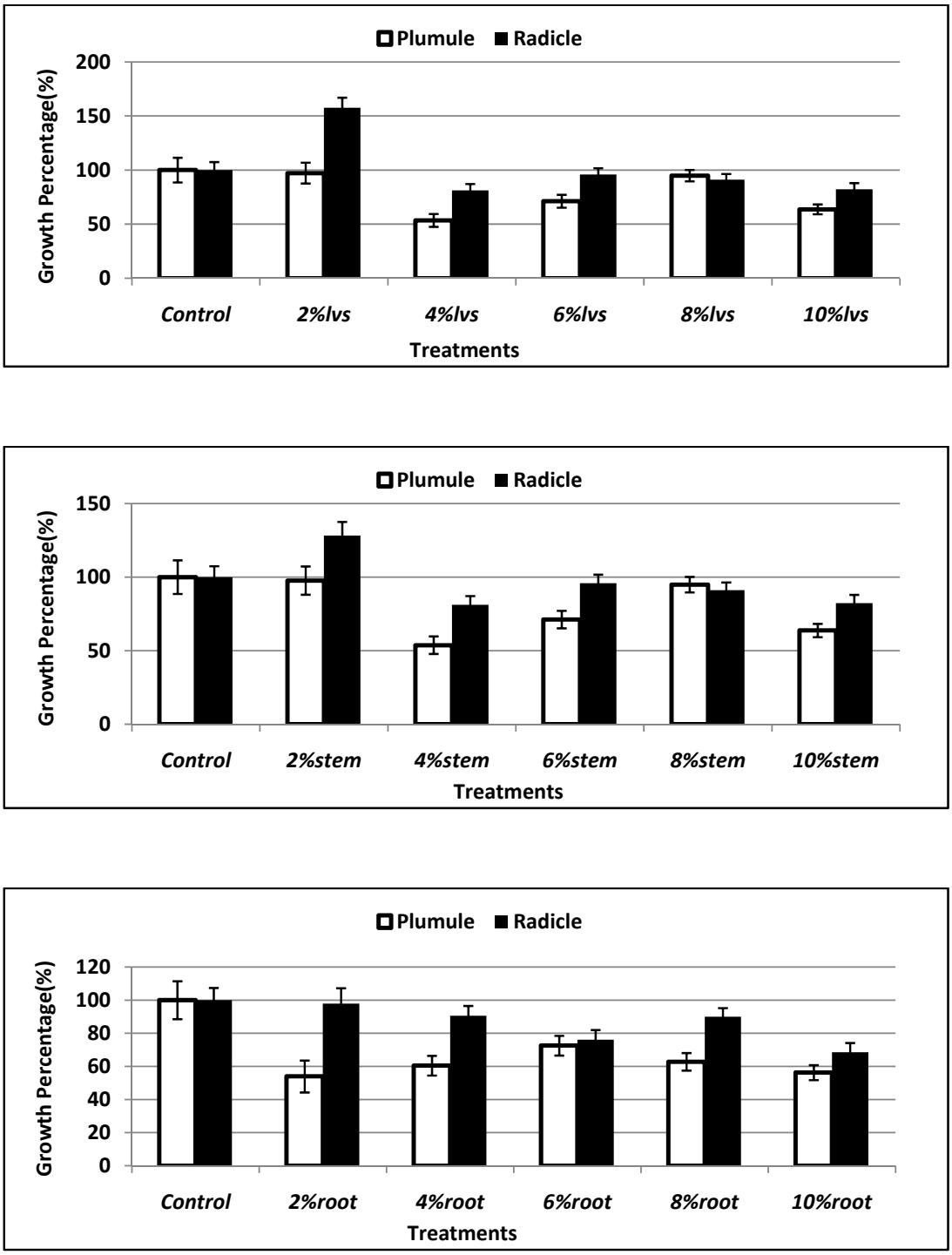
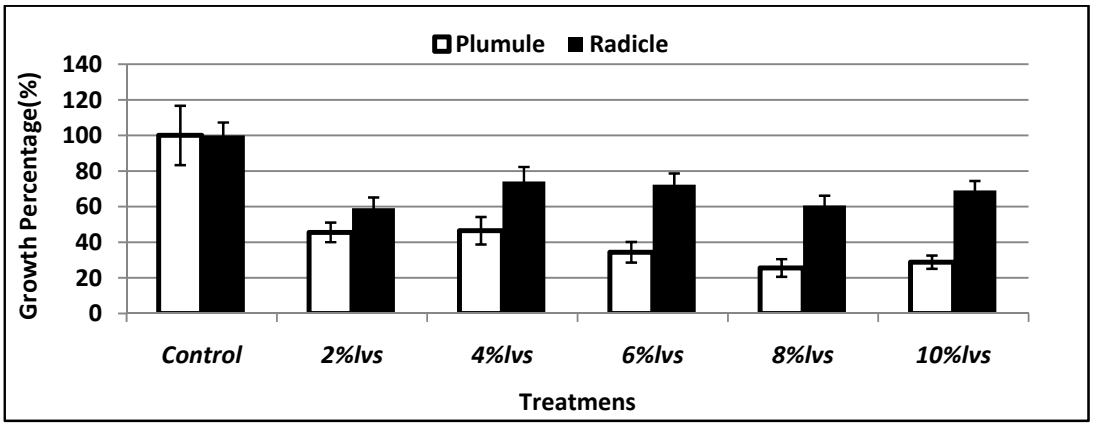
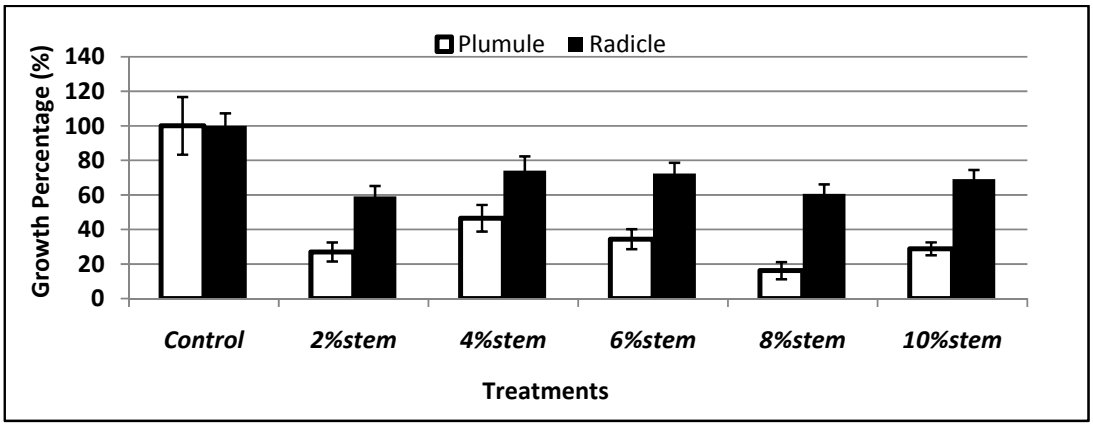


Fig 7. Effect of *Eclipta prostrata plant* extracts on plumule and radicle growth of pea.[A-C]. [A-Leaf, B- Stem, C- Root].





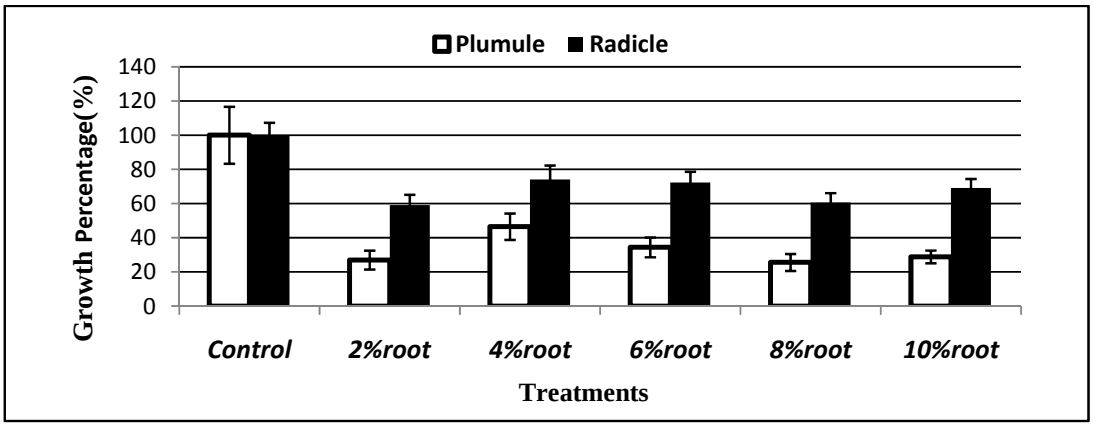


Fig 8. Effect of *Cannabis sativa plant* extracts on plumule and radicle growth of pea.[A-C]. [A-Leaf, B- Stem, C- Root].

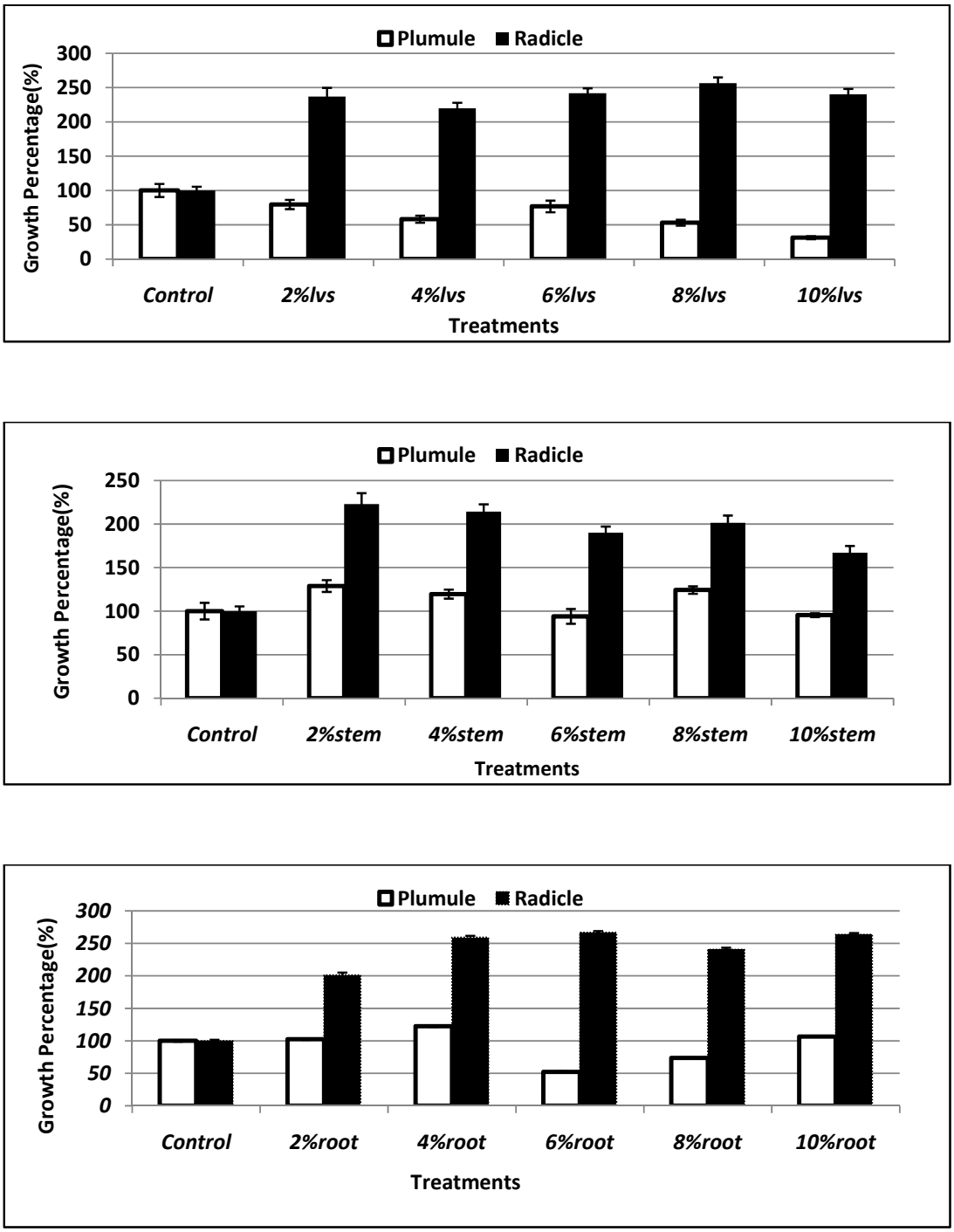


Fig 9 . Effect of *Woodfordia fructicosa plant* extracts on plumule and radicle growth of pea.[A-C]. [A-Leaf, B- Stem, C- Root].

4.1.3 Biomass production

Total biomass production on the different plant aqueous extracts in pea seeds is given in Fig 10**.** Maximum shoot and root biomass production were in case of *Woodfordia fructicosa* stem (0.153 gm) and leaf extract (0.256 gm) whereas minimum in *Ageratum conyzoides* stem (0.079 gm and 0.089 gm). In the control set there was 0.15 gm shoot and 0.146 gm root production. So, overall biomass production was maximum in *W. fructicosa* and least in *A. conyzoides.*

Fig 11 represents the total biomass production on the different plant aqueous extracts in wheat seeds. Maximum shoot and root biomass production were in case of *Ageratum conyzoides* stem extract (0.125 gm) and *Woodfordia fructicosa* leaf extract (0.17 gm) wheras minimum in *Eclipta prostrata* leaf and stem extracts (0.03 and 0.04 gm). In control set 0.127 gm shoot and 0.14 gm root production was observed. But overall biomass production was maximum in *Cannabis sativa* plant extract after control and least in *Eclipta prostrata* plant extracts.

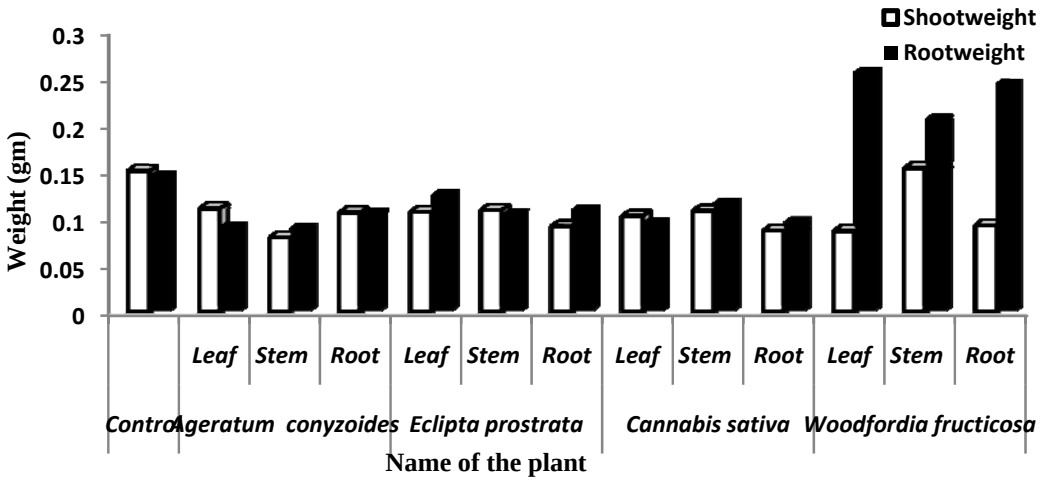


Fig 10. Total shoot root biomass production on the different plant extracts on pea seeds

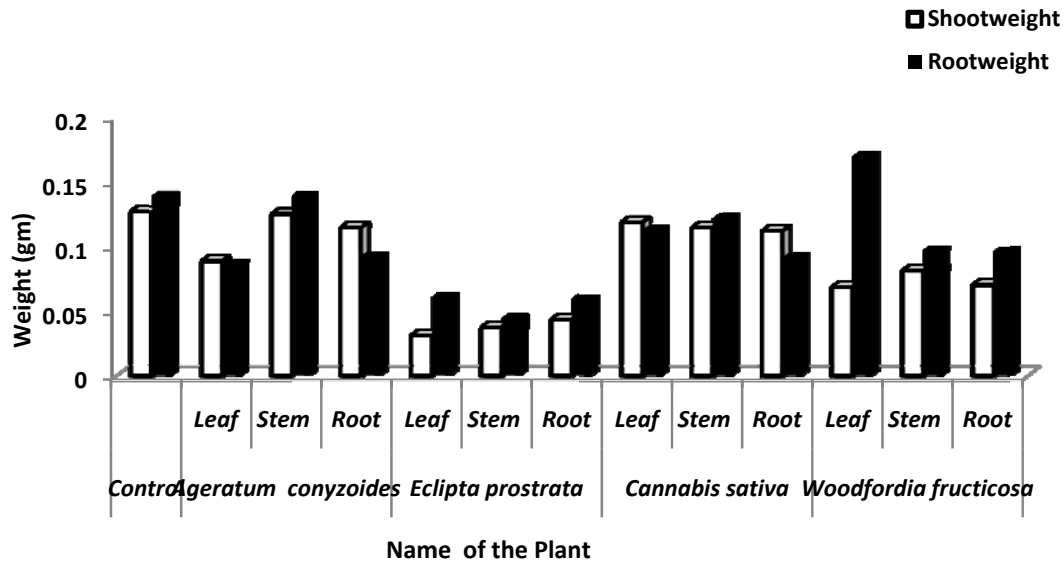


Fig 11. Total shoot root biomass production on the different plant extracts on wheat seeds

4.2. Phytochemical study

4.2.1 Qualitative screening

Phytochemical screening of the plants under study is given in Table 7. Alkaloids, saponins, tannins, terpenoids, phenols, essential oils, glycosides and flavonoids were the phytochemicals present in the plants. Flavonoids, terpenoids were present in all parts of the plant except in roots of *Cannabis sativa*. Tannin was present in all parts of the plant except in leaf and stem of *Ageratum conyzoides* and *Cannabis sativa*. Alkaloids were present in all parts of the plant except in the root and stem of *A. conyzoides* and *C. sativa.* Saponins were present only in *A. conyzoides* and *C. sativa*. Glycosides were present only in *W. fructicosa* and *E. prostrata.*

Table 7. Phytochemical constituents of selected medicinal plants studied.

Name of the species	Plant parts	Phytochemical constituents							
		Alkaloids	Flavonoids	Tannins	Terpenoids	Glycosides	Saponins	Phenols	Essintial oils
Ageratum conyzoides	Leaf	++	++	+++	+	-	+++	+	++
	Stem	++	++	-	++	-	++	+++	++
	Root	++	+	-	+	-	++	++	++
Cannabis sativa	Leaf	++	++	++	++	-	+++	++	+++
	Stem	+	+	++	++	-	++	+++	+
	Root	+	+	-	-	-	++	++	++
Eclipta prostrata	Leaf	+	+	++	++	++	-	++	++
	Stem	+	++	+++	++	+	+++	+	++
	Root	+	++	+	+	+	-	+++	++
Woodfordia fructicosa	Leaf	+++	+++	+	+	+	+	+++	+++
	Stem	+++	+++	++	++	+	-	++	+
	Root	+	+++	++	++	+	-	+++	++

If PPT is slight ‘ +’ , Medium ‘++’ , Heavy ‘+++’ , Not detected‘ –’.

4.2.2 Quantitative estimation

Quantitative crude estimation of phytochemicals present in the plants studied is given in Table 8. Phenolic compounds were present in highest amount (11.11%) in *Ageratum conyzoides* stem extract. Tannin even though present only in leaves, it's amount was maximum (5.6%) in *A. conyzoides* leaf extract. Essential oil (12.65%) and saponins (25.8%) were present in highest amount in *Cannabis sativa* leaf extract .Alkaloid (12.6%) and flavonoids (20.82%) were present in highest amount in the leaf extract of *Woodfordia fructicosa*. Phytochemicals were present in moderate amount in *Eclipta prostrata* and *Cannabis sativa* plant extracts.

Table 8. Quantitative crude estimation of phytochemicals present in the plants studied.

S. No	Name of the species	Plant part	Crude estimated amount of phytochemicals					
			Alkaloid(%)	Flavonoid(%)	Saponin(%)	Tannin(%)	Phenol(%)	Essintial oil (%)
1	*Ageratum conyzoides*	Leaf	7.44	8.74	18.86	5.6	2.98	2.8
		Stem	4.64	5.4	14.58	-	11.31	2.6
		Root	6.12	2.6	12.14	-	3.33	5.3
2	*Eclipta prostrata*	Leaf	3.6	2.6	-	3.7	4.11	5.3
		Stem	4.12	7.12	-	4.04	2.17	2.15
		Root	5.92	8.18	-	0.95	10.87	18
3	*Cannabis sativa*	Leaf	8.2	6.44	25.8	2.6	4.63	12.65
		Stem	4.36	3.78	15.38	2.3	9.57	0.3
		Root	3.12	3.2	12.54	-	3.32	7.4
4	*Woodfordia fructicosa*	Leaf	12.6	20.82	-	0.89	8.87	2.15
		Stem	10.36	13.42	-	2.6	4.57	0.45
		Root	3.04	16.16	-	2.9	10.17	3.9

'-'not detected.

5. DISCUSSION

5.1 Allelopathic potential

5.1.1 Germination

Allelopathy in agricultural practices has become more important with the main objectives of using this phenomenon in biological control of weeds (Rice 1984). As a possible approach, this fact shall be further evaluated and utilized for screening allelopathic plant species (Leather 1982). The growth inhibitory effects on four Nepalese medicinal plants were confirmed by two test plant species in the present research. Plants exhibited allelopathic activity due to release of allelochemicals of different chemical classes mainly polyphenolic compounds (flavonoids and tannins), cyanogenic glycosides and alkaloids (Einhelling 1996). The inhibitory effect of the plant extracts on seed germination and seedling growth may be due to the presence of putative allelochemicals. Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, phenols and essential oils in aquoues extracts of all four medicinal plants (Table 7). In the present study, allelopathic effect of selected medicinal plants can be attributed to the presence of phenolic compounds such as flavonoids, tannins and phenols. The effect may be due to synergistic effect rather than single constituent. The inhibitory effect increased with increasing concentrations of aqueous extracts (Table 3 and 7). It was also reported that effectiveness of receiver plants to allelochemicals was concentration dependent of inhibitory substances with a response threshold (Lovett *et al*. 1989). Inhibitory effects of these medicinal plants were different on test plant species. The variation might be attributed to the differences in kind, total amount as well as properties of allelochemicals produced by different species used in this study. Chon *et al*. (2005) reported that the extracts from lettuce plant had potent allelopathic activity and the activity differed depending on cultivar, extract or fraction.

Higher rate of germination were observed at lower concentration of the plant extracts whereas lower rate of germination were observed at higher concentration of the extracts (Table 1 and 2). This may be due to the presence of phytochemicals in more amount in the concentrated extracts than in the extracts at lower concentration. In wheat seeds, minimum germination rate (71.1%) were observed at higher concentration of *Cannabis sativa* and *Eclipta prostrata* leaf extracts (Table 1).

Maximum germination rate (100 %) in stem extract of *Woodfordia fructicosa* must account for the overall stimulatory effect resulting from the synergistic effect of the phytochemicals present in the stem extract of *Woodfordia fructicosa* . Phytochemical study revealed the presence of phytochemicals especially the phenolic compounds in higher amount in leaves of the plant than in stem than in root. This phenolic compounds interfere with the activities of respiratory enzymes in seed germination thereby causing inhibitory effect on its germination (Muscolo *et al*. 2001). Alteration in the activities of the growth harmone Giberellic acid (responsible for stimulation of seed germination) in the seed may be due to the presence of phenolic compounds (Olofsdotter 2001). Tefera (2002) also found that the inhibitory allelopathic impact of leaf extract was more powerful than of other vegetative parts. But in case of pea seeds the result was slightly different. Maximum germination rate (97.8 %) were observed at lower concentration of leaf and root extracts of the plants (Table 2). Minimum germination rate (84.5 %) were observed at higher concentration of *Cannabis sativa* and *Eclipta prostrata* root extracts .Wheat and pea seeds germination were found more sensitive to *Cannabis sativa* and *Eclipta prostrata* plant extracts .This may be due to higher allelopathic potential of these two plants.

5.1.2 Seedling Growth

The overall effect of the aqueous plant extracts under different treatment conditions were inhibitory due to the presence of phytochemicals in different parts of the plants. The effect of the presence of phytochemicals were not limited to germination inhibition alone , it also brings about impairment in the metabolic activities of the targeted plants leading to decrease in their root and shoot length and biomass production (Abu-Romman *et al*. 2010). As the concentration of the extract increased the seedling growth decreased (Fig 2-9). These types of growth inhibition by the allelopathic plants extract was also reported by Sisodia and Siddiqui (2008) , Inderjit and Keating (1999). Among the four studied plants *A. conyzoides* and *E. prostrata* belongs to family Asteraceae, *C. sativa* belong to family Cannabaceae whereas *W. fructicosa* belong to family Lyrthaceae. Asteraceae family was found to show strong allelopathic effect. *E. prostrata* plant showed maximum allelopathic effect on wheat plant. *C. sativa* and then *A. conyzoides* showed maximum allelopathic effect on the pea plant. In the similar allelopathic study by Umer *et al*. (2010), they reported *Ageratum conyzoides* as the plant with highest allelopathic potential in their

comparative study for ten medicinal plants. The higher allelopathic effect of Asteraceae family was due to higher aromaticity of the plant due to the presence of phenolic compounds at its different parts with highest concentration of the compounds in the leaf aqueous extracts. The allelopathic effect of the plants were in the order Leaf>Stem>Root. This can be explained by the fact that more amount of phytochemicals are present in leaf than in stem and than in root (Kanchan and Jayachandra 1980, Maharjan *et al*. 2007). Foliar leachates have been regarded to be most phytotoxic in nature (Xuan *et al*. 2004) probably owing to their proportionately greater biomass and with greater metabolic activity or production of more metabolites.

The growth response of the different plants studied revealed that *E. prostrata* was the plant with highest allelopathic potential with 93% inhibition rate of plumule and radicle growth of wheat plant. Wheat seeds were found more sensitive to *E. prostrata* and *W. fructicosa* plant extracts. Pea seeds were found more sensitive to *A. conyzoides* and *C. sativa* plant extracts. Radicle growth was found to be more suppressed by the different plant extracts in the wheat plant. Root length was the best indicator of allelopathic effects of plant extracts because radicle growth has been reported to be more sensitive to phytotoxic compounds than plumule growth in alfalfa. Furthermore, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Nishida *et al*. 2005). This might be due to direct contact of root with the extract and subsequently with inhibitory chemicals as described earlier with various crops and weeds by Bhowmik and Doll (1984), Quasem (1995). This might also because wheat being a monocot plant has fibrous root system where numerous roots arise from the same axis and are of equal size higher accumulation of allelochemicals in the root tissues causing the retardation of root more than the shoot. Maharjan *et al*. (2007) also reported the strong inhibitory effect by the aqueous plant extract of *Parthenium hysterophorus* on root elongation of seedlings in cereals and shoot elongation in crucifers and wild Asteraceae. But in case of pea plumule or shoot growth was found more suppressed than the radicle or root growth.

There was significant difference between the plumule and radicle growth of wheat plant (Table 3 and 4). Radicle length of wheat in *C. sativa* and *A. conyzoides* were not significant in root extracts. In *E. prostrata* there was significant difference within the

different concentrations of the plant extracts but difference was low from that of control which may be due to maximum inhibitory effect resulted from the combined effect of the allelochemicals (Arowosegbe *et al* . 2012).

In pea, significant difference in plumule length was seen at all the concentrations except for *A. conyzoides* and *E. prostrata* root extract from that of control (Table 5). For radicle length, significance difference were not observed in *A. conyzoides* root, *C. sativa* stem and root and *E. prostrata* stem extracts (Table 6). Higher significance on the plumule length and radicle length of the test seeds at leaf and stem extracts may be due to the presence of higher amount of growth inhibitors in leaf and stem extracts than in root extracts. The radicle length being more or less similar at different concentration of the plant extracts but different from that of control may signify that even in the presence of growth inhibitors in smaller amount, they are equally efficient to inhibit the growth of the plant equally as the higher concentration of the extracts (Arowosegbe *et al*. 2012).

The presence of phenolic compounds in highest amount in *E. prostrata* and *A. conyzoides* were responsible for the least biomass production in wheat and pea respectively. Maximum biomass production of wheat seeds was in *C.sativa* plant extract. This may be lesser sensitivity of the wheat seeds towards the *C. sativa* plant extracts. In pea seeds *W. fructicosa* biomass production was maximum.

5.2 Phytochemical Study

Phytochemical screening was carried out on four traditionally used medicinal plants of Nepal. Investigation revealed the presence of plant secondary metabolities in all the species but their concentration varied (Table 8). All these constituents which are known to exhibit medicinal as well as physiological activities (Sofowora 1993). Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids and essential oils. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.* 2007). They possess biological properties such as antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*. 2007).Several studies have described the antioxidant properties of medicinal plants which are rich in

phenolic compounds . Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al*. 1994) . Alkaloids are the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications. Several workers have reported on the analgesic properties of alkaloids (Harborne 1973) as well as the anti-inflammatory and anti-bacterial properties of tannins. These classes of compounds are known to show curative activity against several bacteria and it is not surprising that these plant extracts are used traditionally by herbalists to cure bacteria related ill-health. Tannins with its protein-precipitating and vaso-constriction effect could be advantageous in preventing ulcer development. Li and Wang (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention. The diuretic and antibacterial activity of plant extracts containing flavonoids have been documented (Sofowora 1993). The alkaloids contained in plants are used in medicine as anaesthetic agents. The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs .The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the parts of the plants studied.

The phenolic compounds present in *Ageratum conyzoides* may be responsible for the strong allelopathic activity of the plant especially on pea seeds and also the least biomass production by the stem extract. Phenolic compounds such as flavonoids, tannins and phenols were reported as the most common and widely distributed water soluble alleochemicals, they are released to soil by volatilization process and mix easily in the soil nutrients which may be the reason for their higher allelopathic activity (Rice 1984). The presence of phenols, saponin and essential oil in highest amount in leaf of *C. sativa* and overall combined effect of the phytochemicals present in the plant extracts of *Eclipta prostrata* and *Cannabis sativa* must be responsible for their strong allelopathic activity. In *Woodfordia fructicosa* highest amount of alkaloids and flavonoids were present even though inhibitory effect was seen at wheat seeds stimulatory effect was also seen at the pea test seeds.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The present study on the allelopathic potential and phytochemical study of the different medicinal plants studied revealed that the phytochemicals present in different parts of the plant were alkaloids, saponins, tannins, flavonoids , essential oils and phenolic compounds which were reported for their medicinal uses. They were also responsible for the alteration of different physiological activities like seed germination and seedling growth which finally affect biomass production .The sensitivity of seeds to the allelochemicals present under different treatments of the plant extracts and their extent of inhibition varied within the species and part of the test species taken . So, from the present study it can be concluded that the presence of higher amount of phytochemicals mainly the phenolic compounds ; phenols, tannins and flavonoids were responsible for the allelopathic activity of the plants . Higher inhibitory effect on the germination of test seeds by the leaf extracts must be due to the presence of phytochemicals especially the phenolic compounds in higher concentration in leaf than in other parts. Seedling growth inhibition was maximum by *E. prostrata* extracts in wheat seeds and by *C.sativa* and A. *conyzoides* plant extracts in pea seeds. Overall inhibitory effect in wheat plant was highest for *E. prostrata* plant extracts and in pea plant was highest for *C.sativa* plant extract . Presence of phenolic compounds and tannins in highest amount in *A. conyzoides* and *E. prostrata* were responsible for the least pea biomass production in *A. conyzoides* stem extract and least wheat biomass production in *E. prostrata* extracts. Flavonoids present in highest amount in *W. fructicosa* did not show inhibitory effect rather stimulatory effect and maximum biomass production, germination and seedling growth was shown by the combined effect of all the phytochemicals present in pea test seeds . Maximum wheat biomass was produced in *C.sativa* plant extracts.

So the phytochemicals phenolic compounds, alkaloids and saponins present in the plants under study which show different medicinal properties were responsible for allelopathic activities of the plants. So, the presence of allelopathic phytochemicals in the four medicinal plants under study *A. conyzoides, C.sativa, E. prostrata and W. fructicosa* were responsible for the inhibitory effect of the plants extracts on seed germination and seedling growth of these plants.

The compounds responsible for allelopathy could probably be same as those that showed medicinal effects. (Mao *et al*. 2006). So, the results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. There was definite co-relation between traditional application of plants and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system. This result may be useful to future workers to select a group of plants having similar constituents to isolate biologically active principle or prepare remedies for particular case.

6.2 Recommendation

Higher allelopathic potential of the plants under study was due to the presence of phenolic compounds in higher amount. So, the traditional medicinal practice as well as further work to isolate, purify, and characterize the active constituents of these plants is recommended strongly as they can be the potential source of useful drugs.

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APPENDICES

Preparation of reagents

1. **Meyer's Reagent**: Mercuric chloride (0.679 gm) was weighed in a 50 ml volumetric flask and dissolved in distilled water.To this solution, potassium iodide (2.5 gm) was added. The scarlet red precipitate was dissolved by shaking and then diluted with distilled water upto the mark of volumetric flask.
2. **Wagner's Reagent**: 1.27 gm of iodine and 2 gm of potassium iodide were dissolved in 5 ml water and final volume of 1000 ml was prepared.
3. **Fehling's solution**: 34.66 gm Copper sulphate was dissolved in water and final volume of 500 ml was made. (I). Similarly 173 gm of potassium sodium tartarate and 50 gm sodium hydroxide was dissolved in water and final volume of 500 ml was made (II). These two solutions I and II were mixed in equal proportions to form fehling's solution.
4. **0.1 M Ferric chloride**: 0.1 gm ferric chloride was dissolved in 50 ml of distilled water .
5. **0.1 N Hydrochloric acid**: 0.1823 gm of hydrochloric acid was dissolved in 50 ml of distilled water.
6. **0.008 M Potassium ferrocyanide**: 0.1689 gm of potassium ferrocyanide was dissolved in 50 ml distilled water.

PHOTOPLATE I

Whole plant of *Triticum aestivum* (Wheat) test seeds under the present study.

Whole plant of *Pisum sativum* (Pea) test seeds under the present study.

Whole plant of *Ageratum conyzoides* under the present study.

Whole plant of *Eclipta prostrata* under the present study.

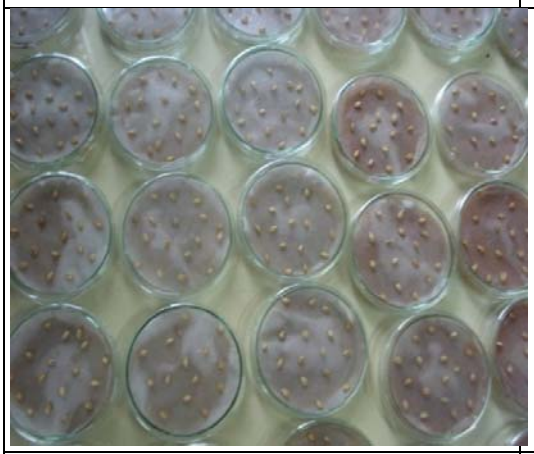
Whole plant of *Cannabis sativa* under the present study.

Whole plant of *Woodfordia fructicosa* under the present study.

PHOTOPLATE II

Powdered extracts of the plants under study in zipper plastic bags

Plant aqueous extracts of different concentrations prepared for the allelopathic study



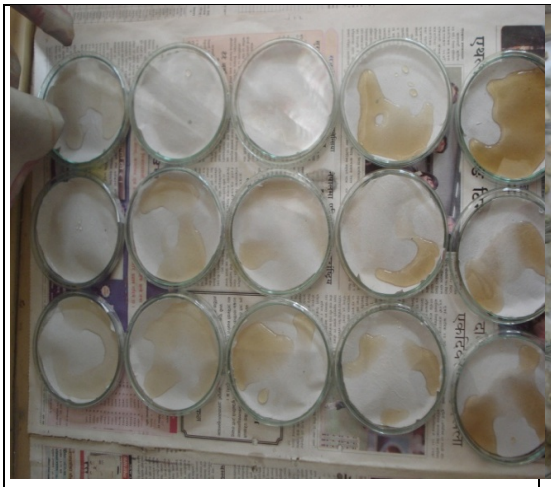
Allelopathy test set up in petridishes for wheat seeds under different treatments

Allelopathic effect of *Woodfordia fructicosa* aqueous stem extract on wheat seeds

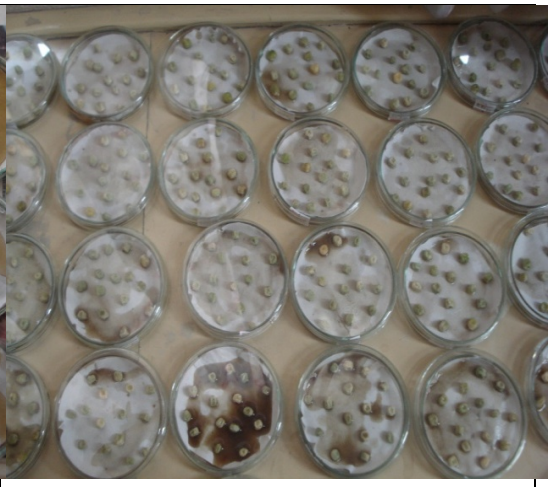
Allelopathic effect of *Ageratum conyzoides* aqueous leaf extract on wheat seeds

Allelopathic effect of *Cannabis sativa* aqueous leaf extract on wheat seeds

PHOTOPLATE III



Plant extracts 10 ml each ready for allelopathy test in petri dishes



Allelopathy test set up in petridishes for pea seeds under different treatments

Allelopathic effect of *Eclipta prostrata* leaf aqueous extract on pea seeds

Allelopathic effect of *E. prostrata* stem aqueous extract on pea seeds

Allelopathic effect of *E. prostrata* root aqueous extract on pea seeds

Allelopathic effect of *Woodfordia fructicosa* stem aqueous extract on pea seeds

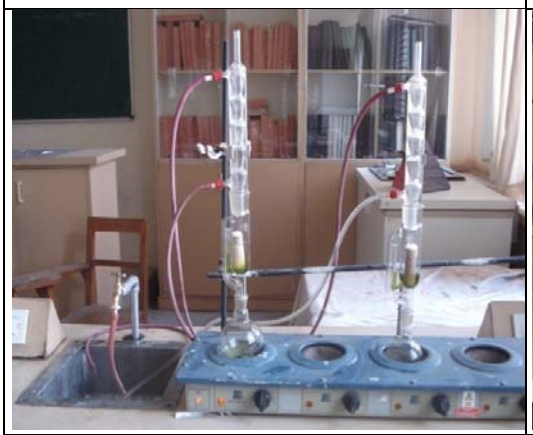
PHOTOPLATE IV

Different plant powdered extracts weighed for the phytochemical study

Aqueous extracts of *E. prostrata*

Plant extracts for alkaloid test

Crude estimation of alkaloid from different plant extracts



Soxhlet apparatus for extraction of phenol and essintial oil

Heating to dryness at electric heater for crude estimation of flavonoids