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In-vitro Phytochemical and Antimicrobial Activity of *Dipsicusinnermus* in Kashmir Valley

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Abstract: ***The hydroalcholic extract of the roots of Dipsicusinermis were investigated for their in vitro phytochemical,antibacterial and antifungal activities.The preliminary phytochemical screening of plant extract shows the existence of proteins, protein sulphur, carbonate, terpenes, saponin, tanic and phenolic compounds. The disc diffusion method of the extract of Dipsicusinermis showed significant antibacterial and antifungal activities when tested against eight bacterial strains and four fungal strains.The result indicated that the extract inhibited the growth of selected bacteria and fungi. Thin layer chromatography carried out on the root extract confirms the presence of various phytochemicals.The results obtained in the present study suggest that root extract of Dipsicusinermis could be useful in treating diseases caused by the test organisms.***

Keywords: Medicinal plant, Antibacterial, Antifungal, Hydroalcohol, Dipsicusinermis, Thin layer chromatography

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Herbal medicine is the oldest from of healthcare known to mankind. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. Plants in traditional medicines contain a vast array of substances that can be used to treat chronic and infectious diseases. Plants have played a vital role in the treatment of diseases since prehistoric times and are one of the most important areas of research in the world today. From the very earliest days of civilization, mankind has turned to plants for healing, a tradition that has survived the arrival of modern medicine and found new strength at the end of 20th century(1). Traditional medicine has maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries(2). The WHO estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicine(3). It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs while in fast developing countries such as China and India, the contribution is as much as 80%. Medicinal plants constitute one of the main sources of new pharmaceuticals and health care products.

The use of crude extracts of plant parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits. The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes(4). There has been an increasing incidence of multiple resistance on human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substance from various sources like the medicinal plant(5). However, very little information is available on such activity of medicinal plant and out of the 4,00,000-plant species on earth, only a small number has been systematically investigated for their antimicrobial activity(6).

In an effort to expand the spectrum of antibacterial agents from natural resources. *Dipsicusinermis* is a species in the genus Dipsacus which contains approximately 28 to 33 species and belongs to the family of the Dipsacaceae. *Dipsicusinermis* is deciduous. The simple leaves are opposite. They are lanceolate with entire margins. Dipsacusinermis produces heads of white tubular flowers from August to September, *Dipsicusinermis* is native to the Himalaya and Myanmar.

In the current study, a screening of hydroalcoholic extracts of *Dipsicusinnermus* roots against pathogenic bacteria and fungi were done in order to detect new sources of antimicrobial agents.

2. Materials and Methods

2.1 Collection of Medicinal Plants

Fresh and healthy roots of the *Dipsicusinnermus* medicinal plant has been used in the present study and were collected in the month of July 2014 from low altitude GogaldorTangmargkashmir, India. Plant parts were collected on the basis of the information provided in the ethnobotanical survey of India. Each specimen/plant material was labelled with the date of collection, locality, and their medicinal uses were recorded.

2.2 Processing of Medicinal Plants

The roots of plants were used to prepare extracts for the study. The plants collected were washed with water to remove the soil and dust particles. Then they were dried in thoroughly shaded place, and blended to form a fine powder and stored in airtight containers.

2.3 Human Pathogenic Bacterial Species

The human pathogenic bacteria such as *Staphylococcus aureus*: ATCC (American type culture collection) 6538, *Enterococcus fecalis*: MTCC (Microbial type culture collection) 2729, *Streptococcus mutans*: MTCC 497, *Micrococcus luteus*: ATCC 6259, *E.coli:* ATCC 2065, *Pseudomonas aeruginosa*: ATCC 741, *Pseudomonas putida*: ATCC 47054, *Proteusvulgaris*: ATCC 1335, *Candida albicans*: ATCC 10231, *Aspergillus niger*: ATCC 1197, *Herpes*: ATCC 1493, *Tinea cruris*: were obtained from Institute of Microbial Technology, Chandigarh. The strains were used for evaluating antibacterial and antifungal activity.The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C, whereas the yeasts and molds were grown in Sabouraud dextrose agar at 28°C. The stock cultures were maintained at 4°C until further use.

2.4 Preparation of Plant Extract

The hydroalcoholic extracts of the medicinal plants were prepared by dissolving 100g of the dried powder of root, extracted with hydro alcoholic (Merck) using soxhletion[14]. The extraction with hydro alcoholic solvent was kept for seven to eight days to obtainextract. After that, the extract was evaporated in rotary evaporator at 55°C to obtained crude for phytochemical analysis, determination of bio-active compound and antimicrobial susceptibility. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2- to 4°C. This crude extracts of hydroalcohol was used for further investigation for potential of antimicrobial properties.

2.5 Preparation of Sterile Disc

Whatman's No.3 filter paper was punched into 5 mm disc form and they sterilized, each sterile disc was incorporated individually with 20 – 60µl of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another dose of extract was applied on discs and stored at 4°C until further use.

2.6 Phytochemical Investigation

Phytochemical analysis for Carbohydrate, Protein, Amino acid, Glycosidase, Flavonoids, Alkaloids, Tannic and phenolic compounds, Protein sulphur, Carbonate, Starch, Coumarin glycosidase, Saponin, Tannin, Terpenes, Polyphenol was conducted as per the standard protocol.[15-17]

2.7 Antibacterial Activity using Disc Diffusion Method

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petri plates. After solidification, 100µl of fresh culture of human bacterial pathogens were swabbed on the respective plates. The discs with different concentrations (mg/mL) of plant extract (25%,50%,75% and 100%) were inoculated over the agar plates using sterile forceps and incubated for 24 hours at 37^{0}C. After incubation, the zone of inhibition aroundeach disc was measured (mm) and recorded.[18]

2.8 Antifungal activity using disc diffusion method

Disc diffusion method was also used for the evaluation of antifungal activity ofhydroalcoholic root extract of*Dipsicusinnermus*. Fungal strains were cultured on Sabouraud's Dextrose Agar plates. The discs with different concentrations (mg/mL) of plant extract (25%,50%,75% and 100%) were inoculated over the SDA plates using sterile forceps and incubated for 72 hours at 28^{0}C. After incubation, the zone of inhibition around each disc was measured (mm) and recorded.

2.9 Thin Layer Chromatography

The solvent system was poured to a depth of 0.5 cm in a rectangular chromatographic glass chamber. The chamber was lined with a piece of filter paper to ensure proper saturation. The spots of extract were applied on a silica gel-G plate with the help of capillary tube. The distance between two spots was kept approximately 2.0 cm. The applied spots were dried at room temperature and the plate was gently placed inside the glass chamber. The angle of the plate with the vertical was kept approximately 15°. The chromatogram was developed till the solvent front migrated to about 10.0 cm. The plate was taken out and the solvent front was marked. The plate was dried at room temperature and inspected either under UV light or sprayed with the specific detecting reagent. The coloured spots were marked and the R_f value of each separated component was calculated.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front TLC plates}}$$

3. Results and Discussion

3.1 Preliminary phytochemical screening

It was found that hydroalcoholic root extract of *Dipsicusinnermus* contained proteins, protein sulphur, carbonate, terpenes, saponin, tanic and phenolic compounds.So, it may be due to the presence of these chemical compounds that the plant extracts inhibit the growth of the bacteria and fungi.Table-1

Table 1: Phytochemical investigation of hydroalcholic root extract of *Dipsicusinnermus*

Compounds	Present/ Absent	Compounds	Present/ Absent
Carbohydrate	Absent	Starch	Absent
Protein	Present	Coumarin glycosidase	Absent
Amino acid	Absent	Saponin	Present
Glycosidase	Absent	Tannin	Absent
Flavonids	Absent	Terpenes	Present
Alkolids	Absent	Steriod	Absent
Tannic & phenolic compounds	Present	Protein sulphur	Present
Organic, Oxalic test and Malic test	Negative	Carbonate	Present

3.2 Antimicrobial activity

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.The antibacterial screening of different concentrations of the extracts on the test isolates are shown in Table 2, 3 and 4. The results show that the increase in concentration of the extracts increased the zones of growth inhibition of the bacteria. The assessment of the antibacterial activity was based on the measurement of diameter zone of Inhibition (mm) that formed around the disc filled with the plant extract at different concentration. Maximum inhibition zone was recorded at 100 mg/ml and the minimum inhibition zone at 25 mg/ml in the bacteria.

Among the four Gram positive bacteria tested, *E. feacalis and S. mutans*gave the largest area of inhibition zone where as *S.aureus* and *M. lutious*gave the least inhibition zone against *hydroalcoholic root extract of Dipsicussinnermus* and similarly among the four Gram negative bacteria tested *E. coli* gave the largest area of inhibition zone where as *P. aeruginosa, P. putida* and *P. vulgaris* gave the least inhibition zone. This shows that hydroalcoholic root extract of *Dipsicusinnermus*had the antibacterial activity.

In the present study among the four fungal strains tested; *Herpes and T. cruris*gave the largest zone of inhibition whereas *C. albicans* and *A. niger*gave the least zone of inhibition against *hydroalcoholic root extract of Dipsicussinnermus*. The results indicated that the hydroalcoholic root extract of the plants inhibited the growth of the bacteria and fungi. Thus, inferred that the extracts contained substances that can inhibit the growth of the selected bacteria and fungi.

3.3 Thin Layer Chromatography

TLC of hydro alcoholic root extract of *Dipsicussinnermus* by using solvent system Ethyl acetate: Methanol (1:1) providedRf Value: 1.354. Figure-1

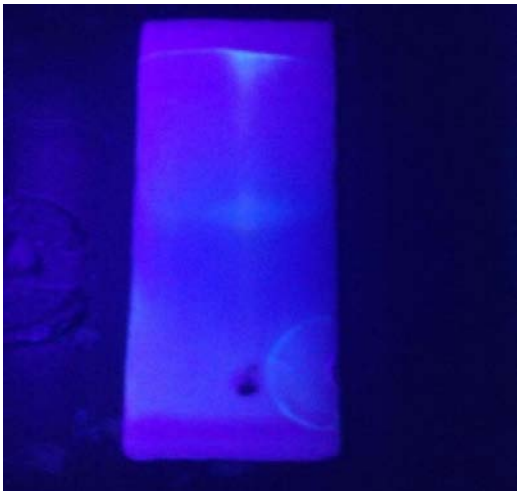

Figure 1: Qualitative analysis by Thin Layer Chromatographyof root extract *of Dipsicusinnermus* using Ethyl acetate: methanol(1:1). The Rf Value of produced content of root is 1.354.

The TLC of the extracts in ethyl acetate: methanol 1:1 solvent system confirms the presence of diverse potent bio-molecules in the plants.TLC analysis provides an idea about the polarity of various chemical constituents, in a way such that compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity.These potent biomolecules can be further used for development of different drugs in future.

4. Conclusion

In the current investigation, the hydroalcohol extract in the ratio of 50 : 50 has been selected.Hydroalcohol extract gave higher yield of constituents for this research work; the originality of work is that good results have been obtained with hydroalcohol ratio, and in comparison, of methanol or water extracts, hydroalcohol is more suitable for clinical studies. The hydroalcoholic extracts of *Dipsicusinnermus* were found to be active on most of the clinically selected bacteria and fungi. The present study justified the uses of roots in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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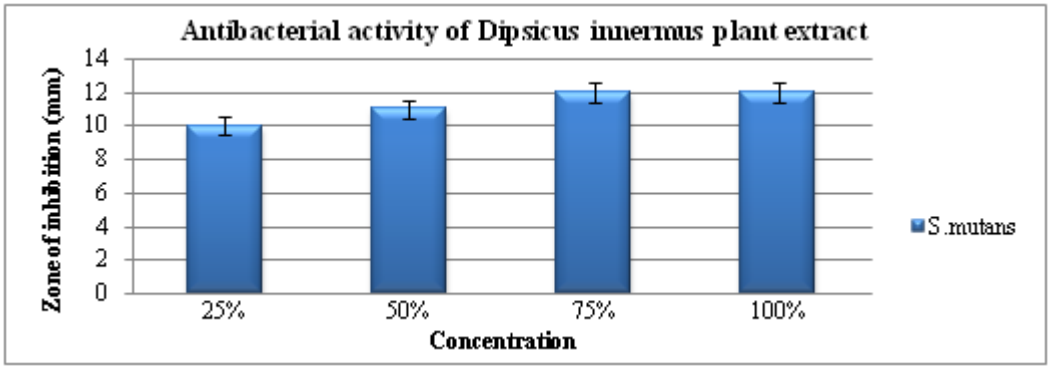
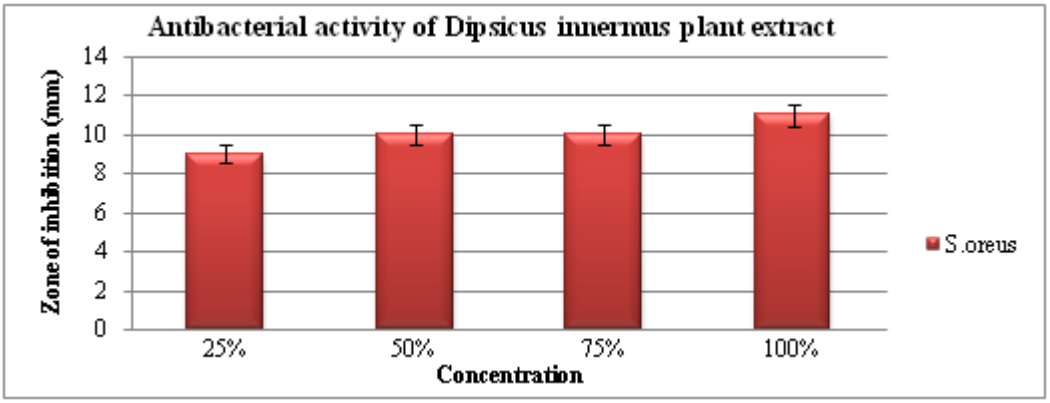
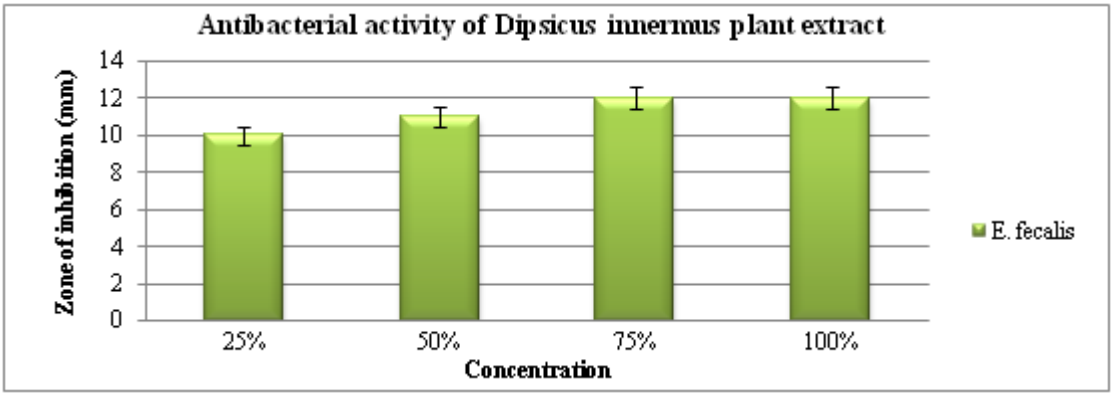
Table 2: Hydroalcholic root extract of Dipsicusinnermus. Zone of inhibition(mm) in Gram positive bacteria

Conc of extracts (mg/ml)	*S.aureus*: ATCC 6538	*E.fecalis*: MTCC 2729	*S.mutans*: MTCC 497	*M.luteus*: ATCC 6259
100	11	12	12	11
75	10	12	12	11
50	10	11	11	10
25	9	10	10	8







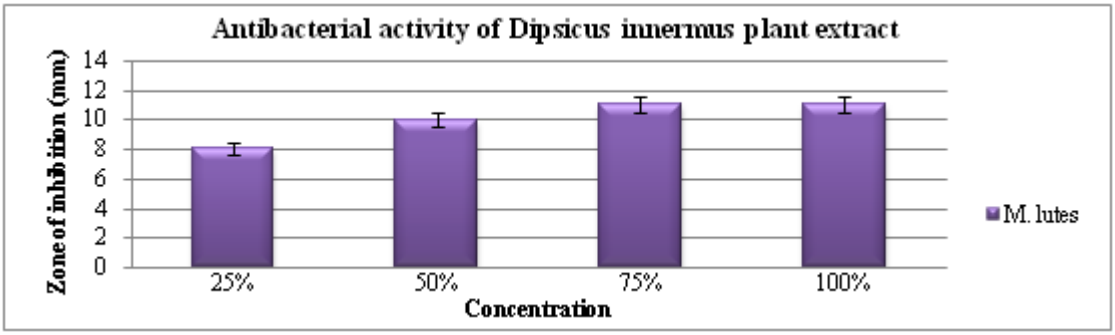
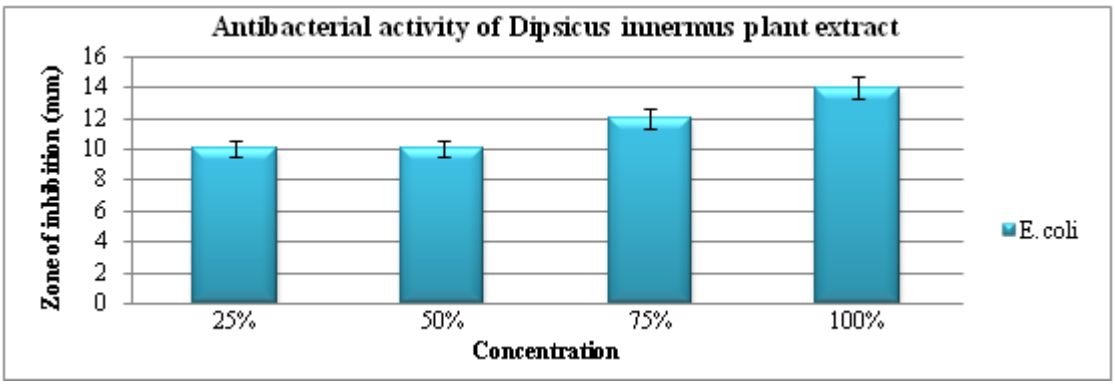
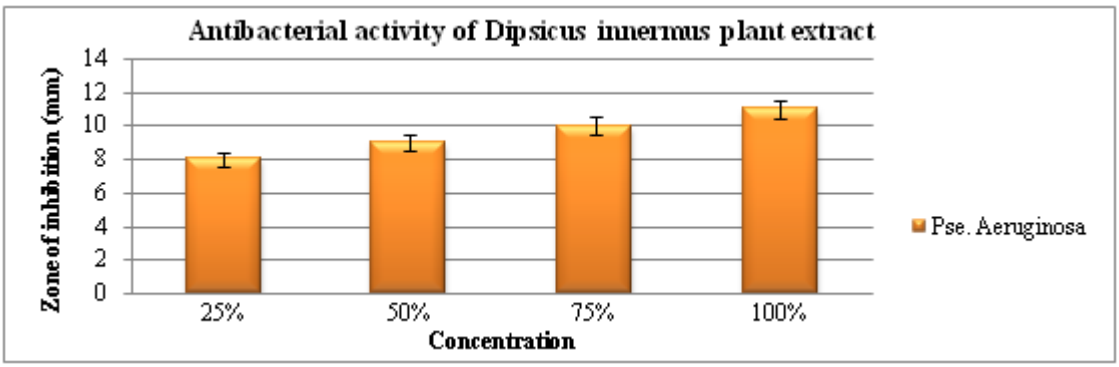
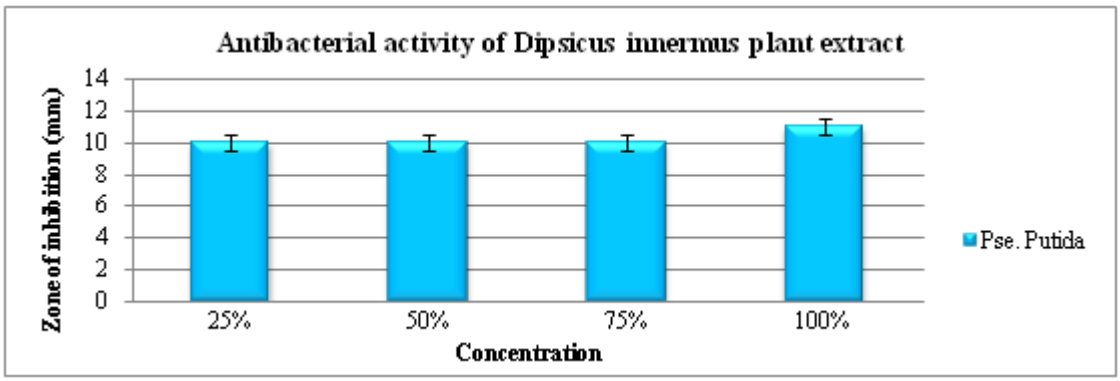


Table 3: Hydroalcholic Root extract of *Dipsicusinnermus* Zone of inhibition(mm) of Gram negative bacteria

Conc of extracts (mg/ml)	*E.coli:* ATCC 2065	*P.aeruginosa*: ATCC 741	*P.putida*: ATCC 47054	*P.vulgaris*: ATCC 1335
100	**14**	**11**	**11**	**11**
75	**12**	**10**	**10**	**11**
50	**10**	**9**	**10**	**10**
25	**10**	**8**	**10**	**9**







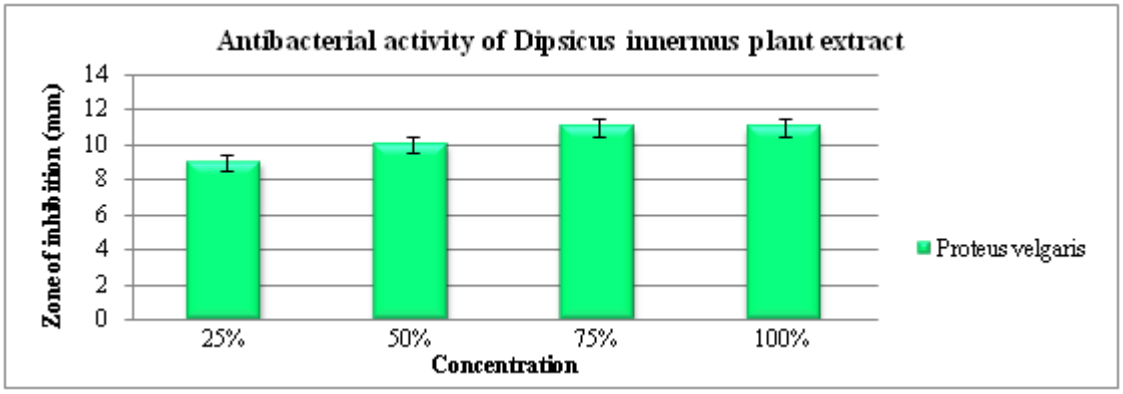
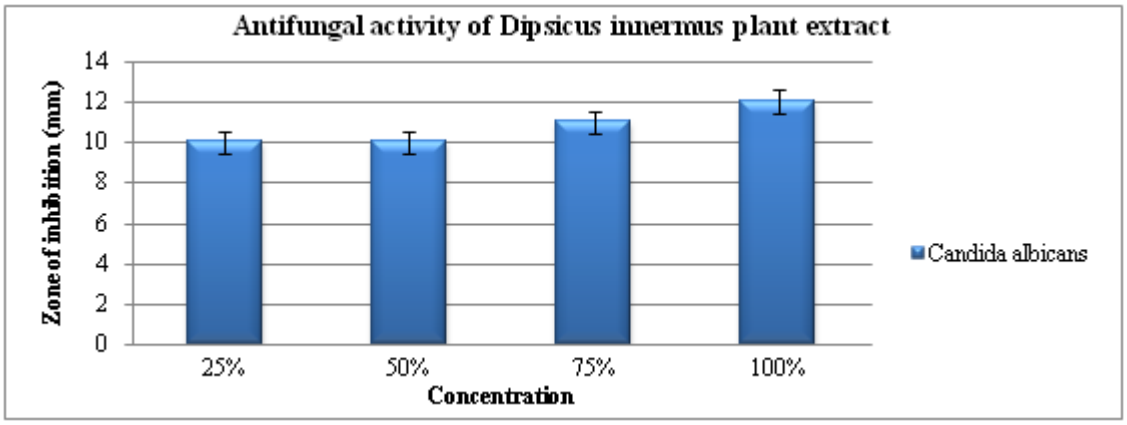
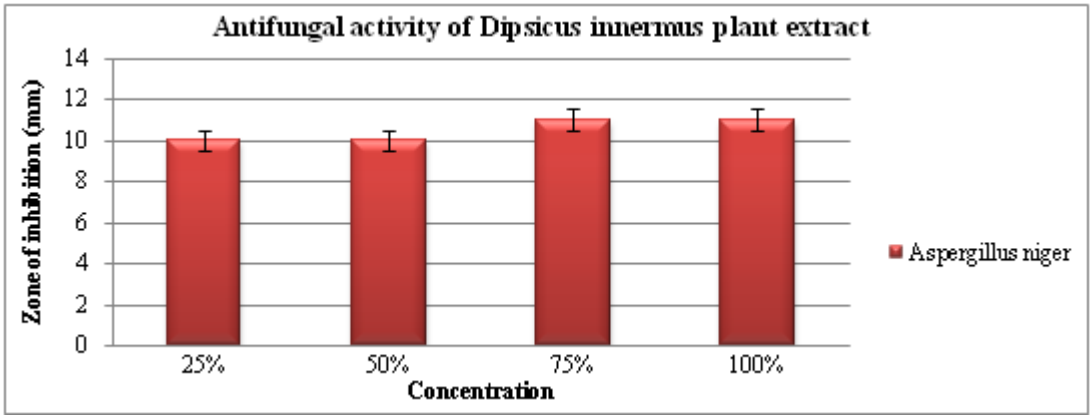
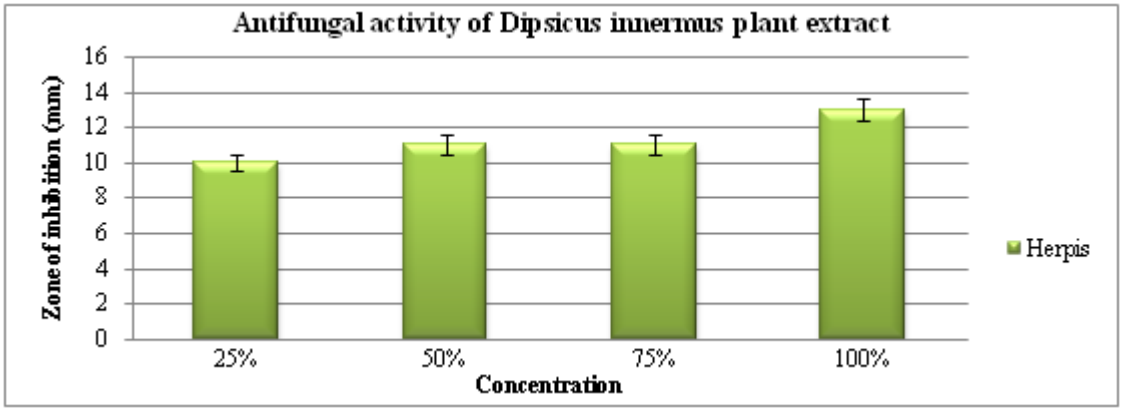


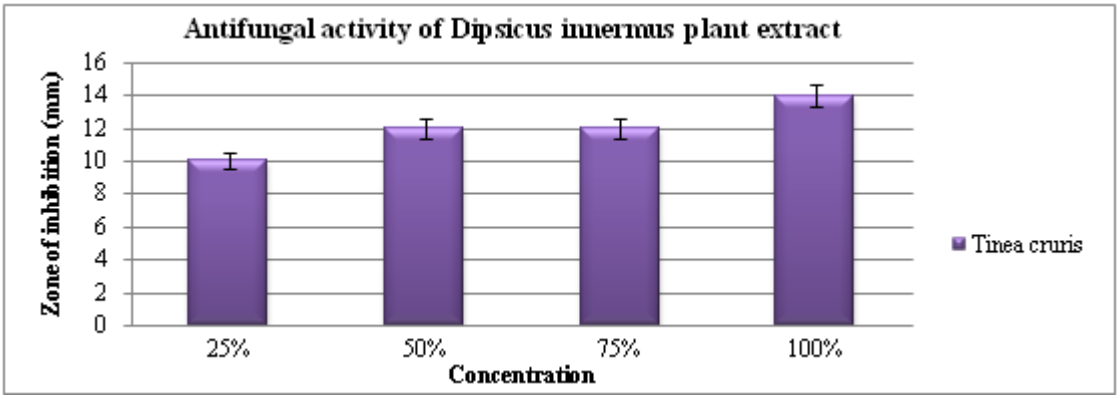
Table 4: Hydroalcholic root extract of *Dipsicusinnermus* Zone of inhibition(mm) of fungal strains

Conc of extracts (mg/ml)	*C.albicans*: ATCC 10231	*A.niger*: ATCC 1197	*Herpes*: ATCC 1493	Tinea cruris
100	**12**	**11**	**13**	**14**
75	**11**	**11**	**11**	**12**
50	**10**	**10**	**11**	**12**
25	**10**	**10**	**10**	**10**









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