

Full Length Research Paper

Phytochemical analysis and biological activities of selected medicinal plants

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The antibacterial and antifungal activities of water and chloroform extracts of *Acorus calamus*, *Aremisia annua*, *Chenopodium foliosom*, *Euphobia helioscopia* and *Cupressus sempervirens* were carried out against six bacterial strains *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* (Gram-negative), and fungal species *Aspergillus niger* and *Candida albicans*. Phytochemical analysis was also performed using the literature methods. Among the studied medicinal plant extracts against the tested bacterial strain, *E. helioscopia* showed very promising results against both the gram-positive and gram-negative bacterial and fungal species followed by *C. foliosom*, *A. annua* and *C. sempervirens* which have low activity against the fungal. Relatively low activity was shown by *A. calamus*. The significant antibacterial activity of active extracts was compared with the standard antimicrobics, piperacillin (100 µg/disc) and gentamicin (10 µg/disc).

Key words: Phytochemicals, *Aremisia annua*, *Bacillus subtilis*, antibacterial activity.

INTRODUCTION

Plants, the oldest friend of human are not only serving human by protecting them but also curing them from different types of ailments. Phytochemicals that are isolated from the medicinal plants have multiple functions as a drug, protecting agent, pest etc. The results provided by them are very promising and have showed no side effects or damage to other part of the body. The new drugs still remained unexplored. The synthetic drugs

potential of medicinal plants as a source for search of are not only expensive and inadequate for the treatment of diseases but also often with adulteration and side effects.

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). The increasing failure of

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chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo et al., 1996). In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Iwu et al., 1999).

Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Martins et al., 2001). Since time immemorial, man has used various parts of plants for various treatments. Therefore, the search for new drugs from plants continues to be a major source of commercial drugs.

Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease hence, further exploration of plant antimicrobials need to occur (Parekh et al., 2007). The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds (Kasamota et al., 1995). Such screening of various plant extracts has been previously studied by many researchers (Erdogrul, 2002; Parek et al., 2006). Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated (Balandrin et al., 1985). Keeping in view the mentioned facts, five medicinal plants including *Acorus calamus*, *Aremisia annual*, *Chenopodium foliosom*, *Euphobia heliscopia* and *Cupressus semperirens* were selected for the current study. The study also hopefully exposes new frontiers by improving the current applications of these plants and provides a scientific basis for the traditional claims of these ethnic medicinal plants.

MATERIALS AND METHODS

Reagents and chemicals

All the chemicals used were of analytical grade including sodium chloride, magnesium and ferric chloride; ammonium hydroxide and Mayers reagent were purchased from Merck and BDH. Nutrient agar media, sterile yeast and Mould extract agar were purchased from Defco, while hexane, ethanol, hydrochloric acid, methanol and butanol were purchased from Scharlu.

Post harvest treatment of plant materials

All the plant materials were washed in tap water and then rinsed with the deionized water properly, and dried under shade. The dried plants were pulverized by sterile electric blender to get powdered plant materials. The powdered plant materials were stored in air-

tight glass containers for further analysis.

Phytochemical analysis

Qualitative analysis

Alkaloids: The chloroform extracts were evaporated to dryness and the residues were heated with 2% HCl solution on a boiling water bath. The extracts were cooled, filtered and then treated with the Mayer's reagent. The sample was then observed for the presence of yellow precipitation or turbidity (Evans, 2000; Tyler, 1994; Harborne, 1973).

Flavonoids: 1.5 ml of a 50% aqueous methanol was added to 4 ml of plant extracts. The solution was warmed and magnesium turning was added. 5 to 6 drops of concentrated HCl was added to the solution and observed for red coloration.

Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added to it, and observed for blue or green black coloration

Saponins: 2 ml of distilled water was added to 2 ml of the test solution and shaken very well till frothing was observed.

Phenols: Ethyl alcohol was added to 2 ml of the test solution and few drops of ferric chloride solution and observed for coloration.

Quantitative analysis

Alkaloids: 5 g of the plant sample was prepared in a beaker and 200 ml of 10% CH₃COOH in C₂H₅OH was added to the plant sample. The mixture was covered and allowed to stand for 4 h. The mixture was then filtered and the extract was allowed to become concentrated by heating on a water bath until it reaches ¼ of the original volume.

Concentrated ammonium hydroxide was added until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue obtained was alkaloids, which was then dried and weighed.

Flavonoids: 10 g of the plant sample with 100 ml of 80% aqueous methanol was extracted at room temperature. The whole solution was filtered through and the filtrate was dried by evaporation using a water bath. The solution was then evaporated to dryness and weighed until a constant weight was obtained.

Tannins: 500 mg of plant sample was weighed and transferred to 50 ml flask. Then 50 ml of distilled water was added and stirred for 1 h. The sample was filtered into a 50 ml volumetric flask and made up volume to the mark with same distilled water. 5 ml of the filtered sample was pipette out into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm within 10 min.

Saponins: 20 g of each ground plant samples were put into a conical flask and 100 ml of 20% aqueous ethanol was added to the plant samples.

The said samples were heated on a water bath for 4 h at about 55°C with continuous stirring. The extracted mixture was then filtered and the residue was then re-extracted again with 200 ml of 20% aqueous ethanol. The collective residues were reduced to 40 ml over a hot water bath. The concentrated residue was then transferred to a separating funnel and 20 ml of diethyl ether was

added and shaken well. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. 60 ml of n-butanol was added and combined n-butanol extract were washed twice with 10 ml of 5% NaCl solution. The remaining solution was then heated on a water bath and after evaporation; the samples were dried in an oven to a constant weight.

Phenols: The plants sample was boiled for 15 min with 50 ml of $(\text{CH}_3\text{CH}_2)_2\text{O}$. 5 ml of the sample was pipette into 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of NH_4OH solution and 5 ml of concentrated $\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{OH}$ to the mixture was added. The sample was made up to the mark and left to react for 30 min for color development. The absorbance of the resultant colored product was measured at 505 nm using a spectrophotometer. From the calibration plot, the amounts of phenols were determined.

Antibacterial activity

Preparation of crude extract

100 g of each of the coarsely powdered plant material were taken and extracted separately with water and chloroform. The extracts were filtered and then few crystals of NaCl solution were added to the filtered extract to form precipitates.

The precipitates were then separated through filter paper; air dried and transferred to air tight amber glass container. The crude extract was dissolved in chloroform and water to make the final concentration, which was kept in refrigerator till used (Lang et al., 1990).

Preparation of standard bacterial suspension

The average number of viable, *Bacillus subtilis* (NCTC8236), *Escherichia coli* (ATCC25922), *Proteus vulgaris* (ATCC6380), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (ATCC0650) and *Staphylococcus aureus* (NCTC25953) organism per ml of the stock suspension was determined by means of the surface viable counting technique. About 10^8 to 10^9 colony forming units (CFU) per ml were used. A fresh stock suspension was prepared each time (Hanna, 2008; Lee et al., 2003).

Antimicrobial activity

The antimicrobial activity of the prepared extracts was determined by using well agar diffusion method. The standard bacterial stock suspension 10^8 to 10^9 CFU/ml was mixed with 60 ml of sterile nutrient agar thoroughly. 20 ml inoculated nutrient agar was poured into sterile Petri dishes. The agar was left to set and four well (10 mm in diameter) were made in each of these plates using sterile cork borer No. 8 and then agar discs were removed. The entire well were filled with 0.1 ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 24 h. Two replicates were also performed for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of extract was carried out as controls. After incubation, the zones of inhibition was measured (in mm) and mean value was calculated (Hanna, 2008; Lee et al., 2003).

Antifungal activity

The antifungal activities of the prepared extracts were determined

by using well agar diffusion method. 0.6 ml standard fungal stock suspension 10^8 to 10^9 CFU/ml was mixed with 60 ml of sterile yeast and mould extract agar thoroughly. 20 ml inoculated yeast and mould extract agar was poured into sterile Petri dishes. The agar was left to set and four wells (10 mm in diameter) was made in each of these plates using sterile cork borer No 8. And then agar discs were removed. The entire well were filled with 0.1 ml of each extracts using micro titer-pipette and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 25°C for 4 days for *Candida albicans* and 3 days for *Aspergillus niger*. Three replicates were also performed for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of extract was carried out as controls. After incubation, the zones of inhibition (in mm) were measured and mean value was calculated (Hanna, 2008; Lee et al., 2003).

Preparation of standard fungal suspension

The fungal cultures, *A. niger* (ATCC 9763) and *C. albicans* (ATCC7596) were maintained on saboraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in refrigerator till it was used (Hanna, 2008; Lee et al., 2003).

RESULTS AND DISCUSSION

The analytical results shown in Table 1 are the qualitative and Table 2 is the quantitative, showing various concentrations of the observed phytochemicals.

Alkaloid

Table 2 shows different concentration level of the phytochemicals obtained by different methods. High concentration 0.9% of alkaloid was found in *E. helioscopia* followed by *C. foliosum* and less concentration of 0.3% was noted in *A. calamus*

Flavonoids

Flavonoids show anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 1980). antimicrobial (Cushnie and Lamb, 2005) and anticancer activities. Flavonoids also referred to as bioflavonoids, are polyphenol antioxidants found naturally in plants. High amount of crude flavonoid 0.99% was detected in *A. annua*, 0.70% was found in *A. calamus* while the rest of the samples having concentration level of 0.22 to 0.53%.

Saponins

The saponins are naturally occurring surface-active glycosides. Many pharmacological activities have been reported about saponins such as antibiotic antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer (Oakenfull, 1986; Zhang et al., 2001). Saponins

Table 1. Qualitative analysis of phytochemicals of ten selected medicinal plants.

Sample code	Phenols	Tannin	Alkaloids	Saponins	Flavonoids
<i>A. calamus</i>	+	+	+	+	+
<i>A. annua</i>	+	+	+	+	+
<i>C. foliosum</i>	+	+	+	+	+
<i>C. sempervirens</i>	+	+	+	+	+
<i>E. helioscopia</i>	+	+	+	+	+

Table 2. Quantitative phytochemicals analysis of selected medicinal plants.

Sample code	Alkaloids (%)	Flavonoids (%)	Tannin (%)	Saponins (%)	Phenols (%)
<i>A. calamus</i>	0.3	0.70	0.20	1.2	0.028
<i>A. annua</i>	0.5	0.99	0.26	0.6	0.0063
<i>C. foliosum</i>	0.8	0.51	0.28	1.3	0.0065
<i>C. sempervirens</i>	0.7	0.22	0.31	1.9	0.067
<i>E. helioscopia</i>	0.9	0.53	0.02	2.4	0.021

Table 3. Zones of inhibitions of water extracts of selected medicinal plants in millimeters.

S/No.	Water extract of the plants	1	2	3	4	5	6	7	8
1	<i>A. calamus</i>	13	10	8	5	2	2	2	2
2	<i>A. annua</i>	9	13	19	4	3	1	2	0
3	<i>C. foliosum</i>	12	7	14	6	3	4	1	2
4	<i>C. sempervirens</i>	9	14	11	4	6	3	1	0
5	<i>E. helioscopia</i>	21	16	19	13	10	9	3	4

Gram-positive bacteria: 1, *B. subtilis*; 2, *P. vulgaris*; 3, *S. aureus*.

Gram-negative bacteria: 4, *E. coli*; 5, *P. aeruginosa*; 6, *S. typhi*.

Fungi: 7, *A. niger*; 8, *C. albicans*.

have been reputed to have important biological activities in humans including hypocholesterolaemic, haemolytic, immunostimulatory and anti-tumourigenic activities (Hostettmann and Marston, 1995), as well as chemo protective activities (Ireland and Dziedzic, 1986). The percentage of saponins 2.4% was found in *E. helioscopia* followed by *C. sempervirens* 1.9%, *C. foliosum* 1.3%, while least amount was found in *A. annua* which contains 0.6%.

Phenols

Table 2 shows a very less concentration of phenols in all the plant samples which were 0.07% in *C. sempervirens*, 0.028% in *A. calamus*, 0.02% in *E. helioscopia* and 0.01% in *A. annua*.

Water extract

As shown in Table 3, high activity 21 mm was recorded in

E. helioscopia against *B. subtilis* while less activity 9 mm was seen against *B. subtilis* in *A. annua*. Table 3 also shows high activity of *E. helioscopia* against *P. vulgaris* which is 16 mm while 14 mm was recorded against bacteria in *C. sempervirens* of water extract. *A. annua* has 13 mm activity against the *P. vulgaris*. The activities of the other water extract were less different from each other. The highest zone of inhibition are shown by water extract of *A. annua* and *E. helioscopia* that 13 mm against *E. coli* followed by *C. foliosum* 11 mm against same pathogen. Very less activity shown by all tested fraction except *E. helioscopia* for which 10 mm zone of inhibition recorded against *P. aeruginosa*. Insignificant results obtained against pathogens *S. typhi*, *A. niger* and *C. albicans* by all water extract of tested plant species between 1 to 9 mm. water extract of *C. sempervirens* and *A. annua* were found completely inactive against *C. albicans*. High activity 10 mm was shown by *E. helioscopia* against *p. aeruginosa* while 6 mm activity recorded in *C. sempervirens* against same bacteria. High activity 4 mm of *E. helioscopia* was noted against *C. albicans* while some activity of 2 mm was recorded in rest

Table 4. Zones of inhibitions of chloroform extracts of ten selected medicinal plants in millimeters.

S/No.	Chloroform extract of the plants	Bs	Pv	Sa	Ec	Pa	At	An	Ca
1	<i>A. calamus</i>	8	12	9	6	2	4	0	0
2	<i>A. annua</i>	14	9	11	4	3	5	2	2
3	<i>C. foliosom</i>	10	13	8	5	2	4	1	2
4	<i>C. sempervirens</i>	12	10	9	5	3	1	0	3
5	<i>E. helioscopia</i>	19	14	21	3	5	6	3	2

Gram-positive bacteria: 1, *B. subtilis*; 2, *P. vulgaris*; 3, *S. aureus*.

Gram-negative bacteria: 4, *E. coli*; 5, *P. aeruginosa*; 6, *S. typhi*.

Fungi: 7, *A. niger*; 8, *C. albicans*.

of the plant extracts against same fungi. It is clear from the Table that *E. helioscopia* was more active against all gram positive, gram negative bacteria and fungi as compared to all other plants.

Chloroform extract

From Table 4, it is concluded that high activity 19 mm was determined in *E. helioscopia* of chloroform extract against *B. subtilis* while the activity shown by the rest of the plant extracts were in between 1-17 mm. *E. helioscopia* was also active 5 mm against *P. aeruginosa*. Antifungal activity shown by *E. helioscopia* was determined high 3 mm against *C. sempervirens* while the activity of other plant extracts were in range from 0-2 mm. more significant result are shown by *E. helioscopia* 17 mm against *S. aureus*. Similarly the highest activity of water extract of *A. annua* recorded 14 mm against *B. subtilis* while insignificant activity of 2 mm against pathogen *A. niger* and *C. albicans*. *C. foliosom* showed 13 mm zone of inhibition against *P. vulgaris*. Water extract of *A. calamus* was completely inactive against *A. niger* and *C. albicans*. While the water extract of *C. sempervirens* found inactive against *A. niger*. Water extract of *A. calamus* showed highest 12 mm zone of inhibition against *P. vulgaris*.

CONCLUSION

Among the studied medicinal plant extracts against the tested bacterial strain, *E. helioscopia* showed very promising results against both the Gram positive and Gram negative bacterail and fungal species followed by *C. foliosom*, *A. annua* *C. sempervirens* which has low activity against the fungal.

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