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ANTIBACTERIAL POTENTIAL IN *PARTHENIUM HYSTEROPHORUS, STEVIA REBAUDIANA* AND *GINKGO BILOBA*

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Abstract

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. In the present study the, *In vitro* antibacterial activity of 3 medicinal plants viz., *Parthenium hysterophorus*, *Stevia rebaudiana* and *Ginkgo biloba* were evaluated by using different solvent extracts. The extracts were tested against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Enterococcus* spp., and *Staphylococcus aureus* by using agar well diffusion method. Azithromycin and cepaxim were used as standard antibiotics. Some of the solvent extracts of the plant showed the highest activity against some bacteria than standard antibiotics used. The findings provide support for the use of these plants in traditional medicine.

Introduction

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine (Sukanya *et al*., 2009). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Farnsworth, 1994, Srivastava *et al*., 1996). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Prusti *et al*., 2008). Antimicrobial compounds of plant origin may be found in plant stems, roots, leaves, bark, flowers, or fruits (Beuchat *et al*., 1994; CAST, 1998). In the present study the leaves crude extract of three plants *Parthenium hysterophorus L*., *Stevia rebaudiana* and *Ginko biloba* were used.

Parthenium hysterophorus L., (Asteraceae) is a common weed distributed worldwide. It´s decoction has been used in traditional medicine to treat fever, diarrhoea, neurologic disorders, urinary infections, dysentery and malaria and as emmenagogue (Ramos *et al*., 2001; Kalsi *et al*., 1995; Surib-Fakim *et al*., 1996).

Stevia rebaudiana, a natural alternative to artificial sweetener is found to contain over 100 phytochemicals including well characterized stevioside and rebaudioside A (Komisseranko *et al*., 1994; Ghosh *et al*., 2008).

Ginko biloba is one of the oldest living tree species, dating back over 300 million years. In China, the ginkgo trees have been used for 5000 years to treat lung ailments such as asthma and bronchitis and also as a remedy for cardiovascular diseases (Perry *et*

al., 1999). Extracts from the leaves of *Ginkgo biloba* have been used therapeutically in China from ancient times and in Western countries such as France and Germany from the 1960s for the treatment of atherosclerotic diseases of peripheral vascular and cerebrovascular insufficiency (Kleijnen & Knipchild, 1992; Xie *et al*., 2003).

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). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004).

The objective of the present study was to evaluate the potential of these plant extracts used in traditional healthcare system, for antibacterial activity against important human pathogenic bacteria. Thus it is a logical approach in drug discovery to screen traditional natural products.

Materials and Methods

Plant material: Fresh leaves of *Parthenium hysterophorus* L., *Stevia rebaudiana* and *Ginkgo biloba* were collected from different nurseries of Islamabad during 2010. The plants were identified taxonomically by the Department of Plant sciences Quaid-I-Azam University Islamabad, Pakistan. Fresh leaves were washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade-drying. They were than powdered and used for extraction.

Test microorganisms: Human pathogenic bacteria such as *E. coli, P. aeruginosa, K. pneumoniae, B. subtilus, Enterococcus* spp., and *S. aureus,* were collected from Microbiology Lab, Quaid-i-Azam University Islamabad. All the test bacterial species were maintained on nutrient agar media.

Preparation of solvent extractions: 25 g of the shade dried, powder of plant materials were filled separately in the thimble and extracted with 150 ml each of methanol, ethanol and dichloro-methane using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4^{o}C in airtight bottles until further use. 15mg of each solvent residue was dissolved in 1ml of DMSO as a solvent and were used as the test extracts for antibacterial assay.

Anti-bacterial assay: Antibacterial activity of solvent extracts; methanol, ethanol and dichloro-methane were determined by Well-diffusion method on nutrient agar medium. Well were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all solvent extracts were poured in the wells of the inoculated plates. The treatments also included 50 µl of solvents served as control and antbiotics Azitromycin and Cipaxim as a standard control. The plates were incubated for 24 h at 37ºC and zone of inhibition if any around the wells were measured in mm.

Data analysis: Each treatment consists of three replicates and repeated twice. Data are presented in Tables. Mean and standard deviation was determined by using the statistical software SPSS. Zone of inhibition which was determined is based on the readings. Graphs were prepared using the Excel Spread sheet.

Results

To search for traditionally used medicinal plants with potent antibacterial properties against gram negative and gram positive bacteria, three important plants viz., *Parthenium hysterophorus*, *Stevia rebaudiana* and *Ginkgo biloba* were screened. The extract was very effective against some bacteria and showed the highest activity than standard antibiotics that were used in the study as shown in Table 1.

The inhibitory zone for methanolic crude extract of *Parthenium hysterophorus* was scored as 13.00±0.12, 14.00±0.06, 16.00±0.03, 17.00±0.12 and 26.00±0.12 mm against *E. coli, P. aeroginosa, B. subtilus, Enterococcus* spp., and *S. aureus* respectively. The activity was nil for *K. pneumoniae.* The zone of inhibition in ethanolic extract was 13.00±0.09, 12.00±0.12, 12.00±0.05 and 22.00±0.14 for the tested organisms *E. coli, P. aeroginosa, B. subtilus* and *S. aureus* respectively. The extract was not active against *K. pneumoniae* and *Enterococcus* spp. The dichloro-methane extract was effective against *E. coli, P. aeroginosa, Enterococcus* spp., and *S. aureus* and the zone of inhibition was scored as 32.00±0.14, 12.00±0.09, 16.00±0.09 and 26.00±0.09 respectively and no effect on *K. pneumoniae* and *B. subtilus.* (Table 1)

Among the *Stevia rebaudiana* methanolic leaves extracts, the *In vitro* inhibition of tested bacteria, *E. coli, K. pneumoniae, Enterococcus* spp., and *S. aureus* was scored as 26.00±0.11, 16.00±0.03, 17.00±0.07 and 12.00±0.06 mm respectively. *P. aeroginosa* and *B. subtilus* showed no activity, whereas in the case of ethanolic extract the zone of inhibition was scored (16.00±0.11) for *P. aeroginosa*, (14.00±0.10) *B. subtilus* and (14.00±0.10) for *Enterococcus* spp. It showed no activity in the ethanolic extract against *E. coli, K. pneumonia* and *S. aureus*. The extract of Dichloromethane showed activity only against *Enterococcus* spp., which was 14.00±0.04mm but no activity against other tested organisms as shown (Table 1).

The inhibitory and lethal effect of *Ginkgo biloba* in the methanolic extract against *P. aeroginosa* and *K. pneumoniae* was scored as 11.00±0.02 and 19.00±0.01 mm respectively but no zone was found for *E. coli, B. subtilus, Enterococcus* spp., and *S. aureus.* The Ginkgo ethanolic extract zone of inhibition was found 20.00±0.05, 17.00±0.08 and 18.00±0.03mm for *P. aeruginosa, K. pneumoniae* and *Enterococcus* spp., respectively. The extract in Dichloromethane also showed the activity against *P. aeruginosa, K. pneumoniae* and *Enterococcus* spp., which is 17.00±0.20, 19.00±0.12 and 20.00±0.05 mm respectively. Both the ethanolic and dichloromethane extracts of *G. biloba* was not effective against *E. coli*, *B. subtilus* and *S. aureus* as show in (Table 1).

Discussion

The leaves extracts of *Parthenium hysterophorus, Stevia rebaudiana and Ginkgo biloba* using methanol and ethanol as extracting solvents presented a better inhibitory effect on the test organisms. This could be attributed to the concentration of the active substance causing the inhibitory effect which could have been higher in the leaves. The use of methanol and ethanol as extracting solvents proved to be more efficient in extracting the active compounds. This could be ascribed to the alcoholic aqueous environment which promotes easy extraction as reported by (Nostro *et al*., (2000).

Table 1. Antibacterial potential in *Parthenium hysterophorus L Stevia rebaudiana* and *Ginkgo biloba* against different bacteria.

Plant used	Extracts (15mg/ml	Zone of inhibition in mm					
		E. coli	*P. aeruginosa*	*K. pneumoniae*	*B. subtilus*	*Enterococcus* spp.	*S. aureus*
Parthenium hysterophorus	Methanol	13.00 ± 0.12	14.00 ± 0.06	---	16.00 ± 0.03	17.00 ± 0.12	26.00 ± 0.12
	Ethanol	13.00 ± 0.09	12.00 ± 0.12	---	12.00 ± 0.05	---	22.00 ± 0.14
	Dichloro-methane	32.00 ± 0.14	12.00 ± 0.09	---	---	16.00 ± 0.09	26.00 ± 0.09
	C 1	22.00 ± 0.07	32.00 ± 0.05	15.00 ± 0.11	29.00 ± 0.09	---	26.00 ± 0.07
	C 2	16.00 ± 0.04	36.00 ± 0.08	18.00 ± 0.09	18.00 ± 0.02	---	20.00 ± 0.17
	C 3	---	---	---	---	---	---
Stevia rebaudiana	Methanol	26.00 ± 0.11	---	16.00 ± 0.03	---	17.00 ± 0.07	12.00 ± 0.06
	Ethanol	---	16.00 ± 0.11	---	14.00 ± 0.10	14.00 ± 0.10	---
	Dichloro-methane	---	---	---	---	14.00 ± 0.04	---
	C 1	13.00 ± 0.13	36.00 ± 0.07	21.00 ± 0.20	28.00 ± 0.06	---	24.00 ± 0.01
	C 2	26.00 ± 0.03	36.00 ± 0.10	13.00 ± 0.06	20.00 ± 0.12	---	14.00 ± 0.20
	C 3	---	---	---	---	---	---
Ginko biloba	Methanol	---	11.00 ± 0.02	19.00 ± 0.01	---	---	---
	Ethanol	---	20.00 ± 0.05	17.00 ± 0.08	---	18.00 ± 0.03	---
	Dichloro-methane	---	17.00 ± 0.20	19.00 ± 0.12	---	20.00 ± 0.05	---
	C 1	--	30.00 ± 0.14	22.00 ± 0.03	25.00 ± 0.13	---	17.00 ± 0.02
	C 2	12.00 ± 0.08	33.00 ± 0.11	16.00 ± 0.06	20.00 ± 0.04	---	16.00 ± 0.05
	C 3	---	---	---	---	---	---

All of the plant extracts tested for antibacterial potential showed varying degree of antibacterial activities against the test bacterial species (Table 1). The antibacterial activities of the Ethanolic, Dichloro-methane and Methanolic extracts compared favorably with these of two standard antibiotics (Azithromycin and Cepaxim) and have appeared to be broad spectrum as its activities were independent on gram reaction. The inhibition zone of methanolic extract of *P. hysterophorus* leaves was found nil for *K. pneumoniae* and highest for *S. aureus*. The methanolic extract was found to be more effective than the ethanol extract against all the organisms. The results are in agreement with that of Igbinosa *et al*., (2009). The dichloro-methane extract of *P. hysterophorus* leave showed the highest activity against *E. coli* (32.00±0.14). The current study is in close proximity with that of Sukanya *et al*., (2009). They evaluated the antibacterial activity of methanolic extract of *P. hysterophorus* against *E. coli* which was 9 mm but no activity against *S. aureus* but the authors evaluated the activity against *E. coli* and *S. aureaus* viz., 13.00±0.12 and 26.00±0.12, respectively.

The comparative antibacterial activity of methanolic, ethanolic and dichloro-methane extracts of *S. rebaudiana* and standard antibiotics (Azithromycin and Cepaxim) against *E. coli, P. aeruginosa, B. subtilus, Enterococcus* spp., and *S. aureus* is shown in the Fig. 2 and Table 1. The highest antibacterial activity of methanolic extract was found against *E. coli* (26.00±0.12 mm), but they have no activity against *P. aeruginosa, B. subtilus*. The zone of inhibition in mm for *E. coli, K. pneumoniae, Enterococcus* spp., and *S. aureus* was 26.00±0.11, 16.00±0.03, 17.00±0.07 and 12.00±0.06 mm respectively. Similarly the highest activity in ethanolic and dichloro-methane extracts was found against *P. aeruginosa,* (16.00±0.11) and *Enterococcus* spp., (14.00±0.04) respectively and no zone of inhibition for *K. pneumoniae.* Over all the *S. rebaudiana* leaves extract in dichloro-methane was not effectve against the tested organisms. The results are in consistent with that of (Kunle & Egharevba, 2009; Kunle *et al*., 2010) but the plant they used were different.

The methanolic extract of Ginkgo leaves showed the highest activity against *K. pneumoniae,* 19.00±0.01 and *P. aeruginosa* 11.00±0.02 mm, but no activity against other tested organisms. The activity of the ethanolic extract was found 20.00±0.05, 17.00±0.08 and 18.00±0.03 mm for *P. aeruginosa, K. pneumoniae* and *Enterococcus* spp., respectively. Similarly the activity of dichloro-methane extracts was 17.00±0.20, 19.00±0.12 and 20.00±0.05mm *P. aeruginosa, K. pneumoniae* and *Enterococcus* spp., respectively and no activity against the other tested organisms as shown in the Table. The results indicated in the present work are in close proximity with that of (Nair & Chanda, (2006), Choi *et al*., (2009) and Igbinosa *et al*. (2009).

Conclusion

The tested plant showed the highest activity against certain bacteria than the standard antibiotics used, indicating that these plants are good source of antibiotics for their treatment of certain bacterial diseases. However, further experimental and research efforts on these plants and their extracts are needed to be able to specify the pharmacological implication. Other details needed will include tests using other solvents, infrared spectrometry, MS and NMR of the constituents of the extracts.

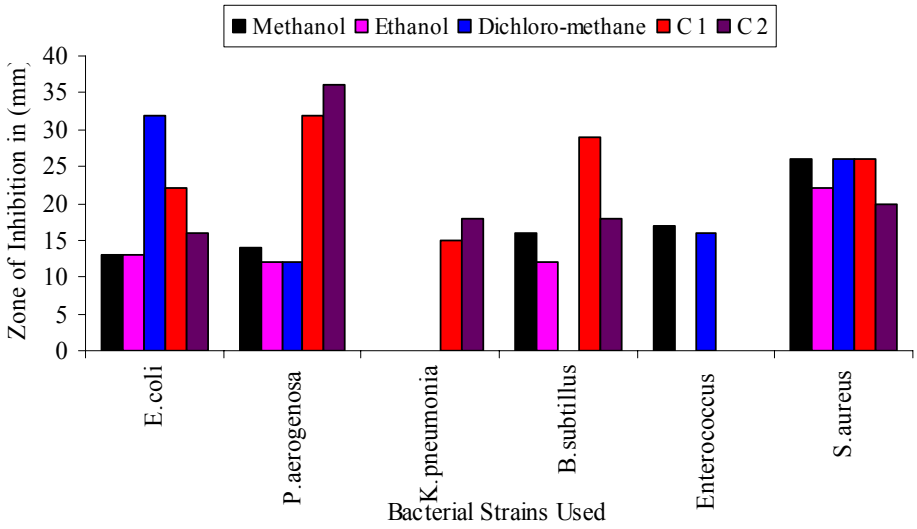

Fig. 1. Graphical presentation of antibacterial activities of different solvent leaves extracts of *Parthenium hysterophorous*.
C1= Azithromycin, C2= Cepaxam

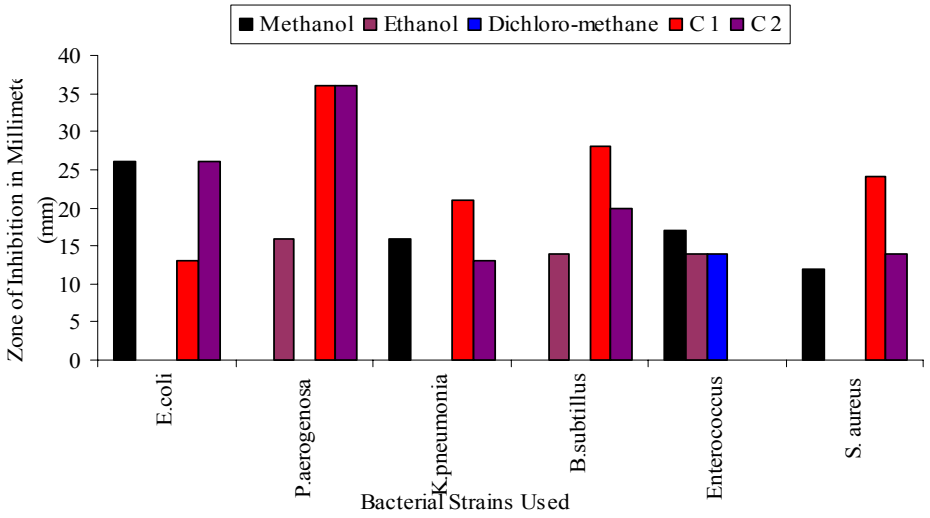

Fig. 2. Graphical presentation of antibacterial activities of different solvent leaves extracts of *Stevia rebaudiana.*
C1= Azithromycin, C2= Cepaxam

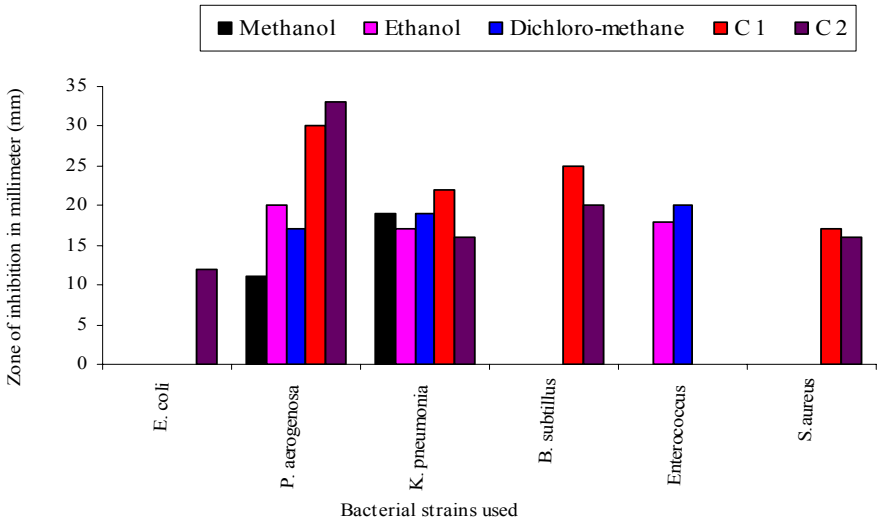

Fig. 3. Graphical presentation of antibacterial activities of different solvent leaves extracts of *Ginkgo biloba*.
C1= Azithromycin, C2= Cepaxim

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