ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 5, 2013

ISSN - 0974-2441

Research Article

IN VITRO ANALYSIS OF PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *PARTHENIUM HYSTEROPHORUS* L. AGAINST PATHOGENIC MICROORGANISMS

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Received: 16 September 2013, Revised and Accepted: 26 October 2013

ABSTRACT

An extract from the *Parthenium hysterophorus* L. leaves were screened for their phytochemical constituents and its antimicrobial activity against clinical isolates of bacterial and fungal cultures. Bacterial culture includes both Gram positive and Gram negative bacterium. The solvent used for the study was methanol. Phytochemical screening revealed the presence of Alkaloids, Carbohydrates, Cardiac Glycosides, Flavonoids, Glycosides, Phenols, Proteins, Saponins, Terpenoids, Tannins and Steroids. Agar well diffusion method was used to test the antimicrobial activity of both the standard antibiotic and plant extract. The antimicrobial activity exhibited by the methanol leaf extract has shown significant potential in inhibiting various pathogens.

Keywords: Antimicrobial, Antibiotics, Phytochemical screening, *Parthenium hysterophorus* L., Pathogens, Well diffusion method.

INTRODUCTION

The life of each organism present in the world is based on the green vegetation which it lives around. Every organism in this universe has a specified role to play. Many play a conservative role, among those plants are being the prime base as they are sustaining our environment. Even several centuries before the invention of modernized equipments and drugs, plants provided cures for many severe medical illnesses. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries.[1]

In the recent years we were hugely depended on the commercial and synthetic drugs which have resulted in the adverse side effects, resistance among several pathogenic organisms and much more. This scenario pushed us to go back to our mother of all producers, the plants to look for effective medicine of lesser or no side effect. *Parthenium hysterophorus* L. from the family of Asteraceae, popularly known as Congress weed, Carrot weed, Star weed, Fever few, White top, Chatak Chandani, Bitter weed. The ability of its seeds to germinate in any season of the year, makes it a constantly flourishing component of the vegetation.[2]

Parthenium is also reported as a promising remedy against hepatic amoebiasis.[3] South American Indians use the decoction of roots to cure ambiotic dysentry,[4] whereas parthenin, a toxin of *Parthenium,* is found pharmacologically active against neuralgia and certain types of rheumatism. It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments.[5,6] In this study, we had evaluated the phytochemical screening and antimicrobial activity of methanol extract of *Parthenium hysterophorus* L. leaves against various clinical isolates of pathogenic bacteria and fungi.

MATERIALS AND METHODS

Plant materials

The *Parthenium hysterophorus* L. plant were collected from the foothills of Western Ghats, Kandiyur, Tamil Nadu. The samples were authenticated by the Botanical Survey of India, Coimbatore, India. The authentication number for the plant is BSI/SRC/5/23/2012-13/Tech/116.

Extract preparation

Parthenium hysterophorus L. leaves were collected from well grown plant. Collected leaves were washed thoroughly with running tap water then with distilled water and dried at room shade for a week. Then it was homogenized to a fine powder and stored in an airtight glass container, protected from sunlight, stored at 4°C until the further process. The methanol extraction of the plant leaves were carried out using a Soxhlet extractor in the ratio 1:10(w/v). The extract liquid was subjected to rotary evaporation in order to remove the solvent. The semisolid extract obtained was stored in an airtight container at 4º C in freezer for further use. The dried extract were exposed to UV light (200-400 nm) for 24 hrs and checked frequently for sterility by streaking on nutrient agar plates. [7] For antimicrobial activity, a volume of 20mg of the extract was dissolved in 1ml of 5 per cent dimethyl sulphoxide (DMSO). It was sterilized by filtration using 0.22 µm millipore filter.[8]

Phytochemical analysis

The preliminary phytochemical analysis was carried out on the methanol extract using standard procedures [9-12] to identify the phytochemical constituents. They are

Detection of Alkaloids: 0.5g of each sample were dissolved individually with 5ml of 2N HCL and filtered.

Dragendroff's reagent: Filtrate was treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

Test for Flavonoids: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for Phenols: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Carbohydrates: **Benedict's test:** To 1 ml of the filtrate, 5 ml of Benedict's reagent were added. The mixture was heated; appearance of red precipitate indicated the presence of reducing sugars.

Test for Glycosides: 0.5g of each extract was stirred with 10ml of boiling distilled water. This was filtered and 2ml of the filtrate hydrolized with a few drops of concentrated HCL and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2ml of Benedict`s qualitative reagent and boiled. Appearance of reddish brown precipitate showed the presence of glycosides.

Test for Cardiac glycosides: Legal's test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Test for Terpenoids: Five ml of extract was mixed in 2ml of chloroform, and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Saponins: The extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Steroids: The extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Test for Proteins: Millon's test: Small portion of the extract when mixed with 2ml of Millon's reagent, White precipitate appeared which turned red upon gentle heating that confirmed the presence of Protein.

Test for Tannins: The extract was mixed with 2 ml of Ferric chloride solution. A blue-green coloration indicated the presence of Tannin.

Collection and maintenance of pathogens

Eight pathogenic bacteria used for the study were *Staphylococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Escherichia coli, Streptococcus* sp., *Enterobacter* sp., and *Citrobacter* sp. and the two fungal cultures were *Candida albicans* and *Candida glabrata* isolated from patients had been collected from "Kovai Medical Center and Hospital", Coimbatore and maintained on nutrient agar slants, blood agar slants and SDA slants in cold room at 4°C.

Culture Media

Muller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20ml per plate in petri dishes. Set plates were incubated overnight to ensure sterility before use [13].

Antibacterial assay

Using the well diffusion method, Twenty-four-hour broth culture of the respective bacteria organisms were adjusted to a turbidity of 0.5 McFarland standards. In brief, 0.2 ml broth culture of the respective microbial strain was dispensed into 20ml sterile nutrient broth and incubated for 24 hr at 37° C and standardized at 1.5x 10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm and performed on UV/VIS- Spectrophotometer [14]. Each of this bacterial culture was swabbed over sterile MHA plates separately by using sterile cotton swabs. A well with diameter 6mm was made using sterile cork borer. The bottoms of the wells were sealed by pouring 20 - 50 µl of molten MHA into the scooped out wells. Subsequently, from the prepared extract in DMSO, 50µl was added to the each well, by this final concentration was made up to 1mg/ml. The plates where kept at 4°C for 1hr for diffusion of extract, thereafter the plates were incubated at 37°C for 24hrs [15]. Erythromycin (1mg/ml) was served as positive reference standard to determine the sensitivity of the tested microbial strains. The Antibacterial activity was determined by measuring the diameter of Zones of inhibition (mm) produced after incubation and results were expressed in millimetres (mm).

Antifungal study

In vitro screening of antifungal activity was carried out against 2 pathogenic fungal strains using agar well diffusion method. The culture of organisms was maintained on Sabouraud dextrose agar. Activated cultures of fungal strains in Sabouraud's broth were adjusted to 1x 10^8 cfu/m as per Mcfarland standard.[16] Each of the diluted culture were swabbed on sterile SDA plates separately by using sterile cotton swabs. The plate, were dried for 30 minutes at room temperature. A well with diameter 6mm was made using sterile cork borer. The bottoms of the wells were sealed by pouring 20 - 50 µl of molten SDA into the scooped out wells. From the prepared extract in DMSO, 50µl was added to each wells, by this final concentration was made up to 1mg/ml. Fluconazole (1mg/ml) was used as positive reference, and the plates were kept at 4°C for 1hr for the prediffusion of the extract. Then the plates were incubated for 48 - 72 hours at 37°C. The zone of inhibition on fungal growth was measured in diameter (mm).

RESULTS

The methanol extract of *Parthenium hysterophorus* L. leaves were subjected to various qualitative tests for phytochemical constituents which revealed the presence of diverse constituents are shown in Table 1. The inhibition zone produced by methanol extract of *Parthenium hysterophorus* L. leaves against each of the bacterial pathogens and standard antibiotic were observed and noted in Table 2. The Antifungal activity of methanol extract of *Parthenium hysterophorus* L. leaves against each of the fungal pathogen and standard fungicide were observed and noted in Table 3. Control tests with the solvent DMSO (5%) employed to dissolve the plant extract were performed for all assays and showed no inhibition of microbial growth.

Table1: Phytochemical screening of methanol extract of *Parthenium hysterophorus* L.

S No.	Phytochemicals	*Parthenium hysterophorus* L.
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Carbohydrate	+
5	Glycosides	+
6	Cardiac glycosides	+
7	Terpenoids	+
8	Saponins	+
9	Steroids	+
10	Protein	+
11	Tannins	+

Keys Presence of the compound; - Absence of the compound.

Table2: Antibacterial activity of methanol extract of *Parthenium hysterophorus* L. against different pathogenic bacteria

S No	Bacterial cultures	*Parthenium hysterophorus* L (in mm)	Erythromycin (in mm)
1	*Staphylococcus* sp.	14	20
2	*Klebsiella* sp	12	10
3	*Pseudomonas.* sp.	20	-
4	*Proteus* sp.	10	11
5	*Escherichia coli*	13	-
6	*Enterobacter* sp.	12	-
7	*Streptococcus* sp.	10	8
8	*Citrobacter* sp.	9	8

Table3: Antifungal activity of methanol extract of *Parthenium hysterophorus* L. against different pathogenic fungi

S No	Fungal cultures	*Parthenium hysterophorus* L. (in mm)	Fluconazole (in mm)
1	*Candida albicans*	17	20
2	*Candida glabrata*	19	23

DISCUSSION

In the present study, we have investigated the qualitative phytochemical analysis of methanol extract of *Parthenium hysterophorus* L. leaves. Earlier Gupta *et al.* (1977) [17] reported the presence of Amino acids, Carbohydrates and Saponins in the methanol extract of *P. hysterophorus.* In the present study along with

the above mentioned compounds we had found the presence of additional phytochemical constituents of Alkaloids, Cardiac glycoside, Flavonoids, Glycoside, Phenols, Tannins, Terpenoids and Steroids. Most of these compounds will play a major role in various biological activities right from inhibiting the microbial growth to destroying of cancer cells.

Previously different extracts of inflorescence of *Parthenium hysterophorous* were tested against different human pathogenic fungi such as *Microsporum gypseum, Pencillium Chrysogenum, Rhizopus stolonifer, Aspergillus niger, Mucor* at various concentrations. The results were found to be less effective while organic extract of the same displayed no activity[18]. But in this study methanol extract of *Parthenium hysterophorus* L. leaves had inhibited the growth of *Candida albicans* and *Candida glabrata* with the inhibition zone of 17mm and 19mm respectively. From the results obtained in this study, it is evident that the methanol extract of *Parthenium hysterophorus* L. leaves is having good antifungal potential. These results were supported by previous findings in which it is reported that the aqueous and organic extracts of *P. hysterophorus* have potential to obstruct dreadful effect of pathogenic fungi by suppressing their growth[19]. It has to be further explored against different fungi to know its wide range of activities various fungal strains.

Parthenium hysterophorus L. leaves extract exhibited significant activity against all the human pathogens studied. Against *Staphylococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *E. coli, Enterobacter* sp., *Streptococcus* sp., and *Citrobacter* sp. it showed a considerable activity with inhibition zone of 14mm, 12mm, 20mm, 10mm, 13mm 12mm, 10mm and 9mm respectively. Against, *E. coli, Pseudomonas* sp., *Klebsiella* sp., *Citrobacter* sp., *Streptococcus* sp., and *Enterobacter* sp., the plant extract has shown considerably higher activity than that of the standard drug used. The previous study showed that the ethanol extract of *P. hysterophorus* having antibacterial activity over different bacteria[20]. The present investigation proved that the methanol extract of *Parthenium hysterophorus* L. leaves posses promising antibacterial potential against both the Gram positive and Gram negative bacteria. The activity of plant extract against both gram positive and gram negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds[21]. Figure 1 and Figure 2 has clearly shown the efficiency of the methanol leaf extract of *Parthenium hysterophorus* L. in inhibiting bacterial and fungal cultures along with the standard drug respectively. From this it is clear evidence that the methanol extract of *Parthenium hysterophorus* L. leaves having broad spectrum of antimicrobial property. Based on these studies we can strongly suggest that this plant is having all the qualities of being an effective drug in the near future. On further analysis we can able to know many more of its diverse activities in detail.

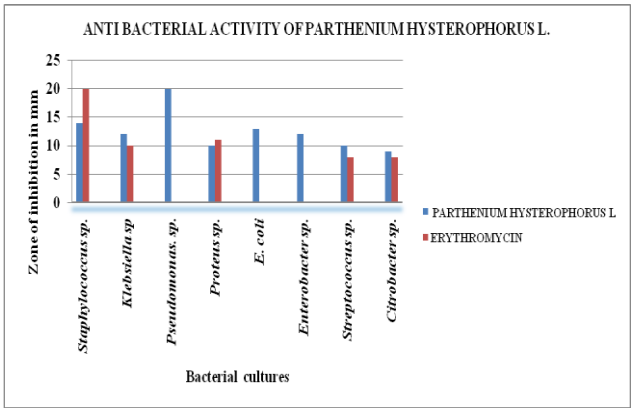

Figure1: Antibacterial activity of methanol extract of *Parthenium hysterophorus* L. against different pathogenic bacteria

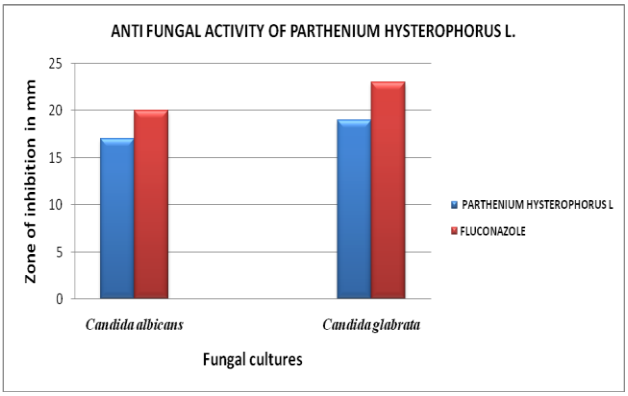

Figure 2: Antifungal activity of methanol extract of *Parthenium hysterophorus* L. against different pathogenic fungi

ACKNOWLEDGMENT

We thank Dr. K. Mahadevan, Head, Dept. of STD, Coimbatore Medical College and Hospital, Dr. Thangiah, Ms. Maya and DR. Sarmad Moin for their support for the study.

REFERENCES

1. Zakaria, M. Isolation and characterization of active compounds from medicinal plants. Journal of Natural Products 1991; 50 : 1025-1040. (URL - http://scialert.net/fulltext/?doi=rjphyto.2012.54.60&org=10)
2. Swati Gupta and Sunirmal Chanda. Aerobiology and some chemical parameters of *Parthenium hysterophorus* pollen. Grana 1991; 30 : 497-503. (URL - http://www.tandfonline.com/doi/abs/10.1080/00173139109432017#preview)
3. Sharma GL, and Bhutani KK. Plant based antiamoebic drugs, Part II. Amoebicidal activity of parthenin isolated from *Parthenium hysterophorus.* Planta Medica 1988; 54 : 20-22. (URL - http://www.hort.purdue.edu/newcrop/cropfactsheets/parthenium.html)
4. Unphof JC. Dictionary of economic plants. Englemann Weinheim, 1959. (URL - http://www.hort.purdue.edu/newcrop/cropfactsheets/parthenium.html)
5. Dominguez XA, Sierra A. Isolation of a new diterpene alcohol and parthenin from *Parthenium hysterophorus.* Plant Medica 1970; 18 : 275-277. (URL - http://www.hort.purdue.edu/newcrop/cropfactsheets/parthenium.html)
6. Morton JF. The puzzling whitetop. *Parthenium hysterophorus:* Noxious weed, health hazard, folk-remedy, flea repellent, Unpublished report. Univ. of Miami, Florida, 1981. (URL - http://www.hort.purdue.edu/newcrop/cropfactsheets/parthenium.html)
7. Abubacker MN, and Ramanathan R. Antibacterial Activities of *Argemone mexicana* L. (Papavaraceae) Leaf Extract on Pathogenic Bacterial Strains. Drug Invention Today Trease GE, Evans WC. Text book of Pharmocognosy. 13th (eds). Alden Press; Oxford 19892012; 4 (6) : 385-387. (URL - http://ditonline.info/index.php/ditonline/article/view/13146)
8. Krishnavignesh Lakshmanan, Mahalakshmipriya Arumugam and Ramesh Mani. Phytochemical Screening and *In Vitro* Antimicrobial activity of *Vitex negundo* L. var. *purpurascens* Sivar. and Mold. against pathogenic microorganisms. Drug Invention Today 2012; 4(12) : 667-670. (URL - http://ditonline.info/index.php/ditonline/article/view/15656)
9. Trease GE, Evans WC. Text book of Pharmocognosy. 13th (eds). Alden Press; Oxford 1989; 512-513.
10. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur. Phytochemical screening and Extraction: A Review. IPS 2011; 1 : 98 -106. (URL - http://www.ipharmsciencia.com/Dacuments/1/11.pdf)

11. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol 2005; 4(7) : 685-688. (URL - http://www.ajol.info/index.php/ajb/article/view/15167)
12. Yadav RNS and Munin Agarwala. Phytochemical analysis of some medicinal plants. Journal of Phytology 2011; 3(12) : 10-14. (URL - http://journal-phytology.com/index.php/phyto/article/viewfile/9530/5167)
13. Krishnavignesh Lakshmanan, Mahalakshmipriya Arumugam and Maya Madhusudanan. Evaluation of antimicrobial activity and phytochemical screening of *Tridax procumbens* L. And *Aerva lanata* (L.) Juss. Ex Schult. Against Uropathogens. Journal of Pharmacy Reaearch 2012; 5(4) : 2377-2379. (URL - http://jpronline.info/index.php/jpr/article/view/12875)
14. Ramesh K. Satdive, Raghuram Chimata, Ajay Namdeo and Devanand P. Fulzele. *In vitro* screening for phytochemical and antimicrobial activity of poisonous plant *Ficus tseila* roxb. IJPBS 2012; 3(2) : 213-221. (URL - http://www.ijpbs.net/vol-3/issue-2/pharma/24.pdf)
15. Sharma B, Kumar P. Extraction and Pharmacological Evaluation of Some Extracts of *Tridax procumbens* and *Capparis decidua.* International Journal of Applied Research in Natural Products 2009; 1(4) : 5-12. (URL - www.ijarnp.org/index.php/ijarnp/article/download/58/59)
16. Jigna Parekh, Sumitra Chanda. In vitro antifungal activity of methanol extracts os some Indian medicinal plants against pathogenic yeast and moulds. Afr. J. Biotechnol 2008; 7(23) : 4349-4353. (URL - http://www.academicjournals.org/ajb/PDF/pdf2008/3Dec/Parekh%20an%20Chanda.pdf)
17. Gupta RK, Dutta TR, Patil BD. Chemical investigation of *Parthenium hysterophorus.* Indian Journal of Pharmacy 1977; 39(3) : 64-66. (URL - http://www.ansfoundation.org/Uploaded%20Pdf/41/137-143.pdf)
18. Sunita Sharma, Nidhi Gupta. Antimicrobial potential of a weed plant Parthenium hysterophorus: An Invitro study. IJPRD 2012; 4(04) : 112-118. (URL - http://www.ijprd.com/June_12_Issue.html)
19. Zunera Zaheer, Shazia Shafique, Sobiya Shafique and Tahir Mehmood. Antifungal potential of *Parthenium hysterophorus* L. Plant extracts against *Fusarium solani.* Sci. Res. Essays 2012; 7(22) : 2049-2054. (URL - http://www.academicjournals.org/sre/PDF/pdf2012/14Jun/Zaheer%20et%20al.pdf)
20. Nair R, Chanda S. Activity of some mecicinal plants against certain pathogenic bacterial strains. Indian J Pharmacol 2006; 38(2) : 142-144. (URL - https://tspace.library.utoronto.ca/bitstream/1807/8784/1/ph06037.pdf)
21. Nagarsenker, KS, Nagarsenker, MS Kulkarni, SR. Evaluation of composition and antimicrobial activity of supercritical fluid extract of leaves of *Vitex negundo*. Indian J. Pharm. Sci 2010; 73(5) : 641-643. (URL - http://www.ijpsonline.com/article.asp?issn=0250-474X;year=2010;volume=72;issue=5;spage=641;epage=643;aulast=Nagarsekar