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Antimicrobial Activity of Medicinal Plant: *Parthenium hysterophorus* L.

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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* antimicrobial activity of medicinal plant *Parthenium hysterophorus*, was evaluated by using chloroform, methanol, acetone, ethyl acetate, petroleum ether and distilled water. The extracts were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Staphylococcus aureus* by using agar well diffusion method. Ciprofloxacin and Amphotericin were used as standard antibiotics. Some of the solvent extracts of the plant showed the highest activity against some pathogenic microorganisms than standard antibiotics used. The findings provide support for the use of this plant in producing new bioactivity compounds having antimicrobial activity.

Key words: Bioactive, diverse, extracts, pathogenic, solvent

INTRODUCTION

Antibiotics have been called miracle drugs, but more than 60 years of use, the efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials (DiazGranados *et al.*, 2008; Overbye and Barrett, 2005). Due to under use and over use, the rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances' (Wise, 2008). Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe (Clark, 1996). The World Health Organization (WHO) estimates that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare. These evidences contribute to support and quantify the importance of screening natural plants. 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.*, 2006). A wide variety of medicinal plants used traditionally have not yet been systematically investigated against various microbial pathogens (Aqil and Ahmad, 2003). 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). Literature search reveals the conducting of a few studies on antibacterial and antifungal activity of parthenium plant. However, antimicrobial studies of parthenium against pathogens causing human diseases are lacking. The present communication therefore has been designed to assess the potency of parthenium leaves extracts against diseases causing bacteria and yeast of clinical origin.

Parthenium hysterophorus is a rich source of terpenoids, volatile oils and flavonoids as well as amino acid, sugars and phenolic derivatives. Parthenolide (the major sesquiterpene lactone), parthenin and different solvent extracts showed significant analgesic, anti-inflammatory and antipyretic activities, which confirmed the use for treatment of migraine headache, fever, externally leaf paste application of *P. hysterophorus* showed wound healing activity (Kumar *et al.*, 2012). The present study was carried out to validate the antimicrobial potential of *Parthenium hysterophorus* against the microbial pathogens with a view of searching a novel extract as a remedy for treating microbial diseases.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Parthenium hysterophorus* L., were collected from different parts of Kurukshetra and its adjoining area during, 2015. The plants were identified taxonomically by the Department of Botany, Kurukshetra University, Kurukshetra, India. Fresh leaves were washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade-drying (Aneja *et al.*, 2009).

Extraction of plant material: The samples were air dried at room temperature (40°C) for 4-5 days and then homogenized to a fine powder using a sterilized mixer grinder and stored in air tight bottles. Five different solvents namely ethyl acetate, methanol, acetone, petroleum ether, chloroform and distilled water were used for extraction. A 10 g amount of homogenized leaves were soaked in conical flasks each containing 100 mL of ethyl acetate, acetone, petroleum ether, chloroform, methanol (95%) and sterile distilled water. Each preparation was filtered through a sterilized Whatman No. 1 filter paper and finally concentrated to dryness under vacuum at 40°C using rota evaporator. The dried extract thus obtained was sterilized by overnight UV-irradiation and checked for sterility on nutrient agar plates and stored at 4°C in labelled sterile bottles until further use (Kumar *et al.*, 2006; Okeke *et al.*, 2001).

Test microorganisms: Clinical strains of Human pathogenic bacteria such as *Escherichia coli* (MTCC1652), *Pseudomonas aeruginosa* (MTCC741), *Saccharomyces cerevisiae* (MTCC170), *Candida albicans* (MTCC3017), *Bacillus subtilis* (MTCC121) and *Staphylococcus aureus* (MTCC96), were procured from Microbial Type Culture Collection IMTECH, Chandigarh. The microorganisms were subculture on nutrient agar and incubated aerobically at 37°C.

Screening for antimicrobial activity: The acetone, methanol, chloroform, ethyl acetate, petroleum ether and distilled water parthenium leaves extracts were used for evaluation of the antimicrobial activity by the agar well diffusion method. In this method, pure isolate of each microbe was subculture on the agar media plates at 37°C for 24 h. One plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^6 CFU mL⁻¹ (standardized by 0.5 McFarland standard) and used as the inoculum for performing agar well diffusion assay. One hundred microliter of inoculum of each test organism was spread onto the agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 8 mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The dried extracts were

reconstituted in 20% dimethylsulphoxide (DMSO) for the bioassay analysis. A 100 μL volume of each extract was propelled directly into the wells (in triplicates) of the inoculated agar plates for each test organism. The plates were allowed to stand for 1hr for diffusion of the extract into the agar and incubated at 37°C for 24 h (Rios *et al.*, 1988; Okeke *et al.*, 2001). The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract measured in mm.

Data analysis: Each treatment consists of three replicates and repeated twice. The data were analyzed by one way ANOVA followed by Dunnett's *t* test compared to positive control at 5% significant level.

Determination of Minimum Inhibitory Concentration (MIC): The MIC for each test organism was determined by following the modified agar well diffusion method. A two fold serial dilution of each extract was prepared by first reconstituting the dried extract (100 mg mL⁻¹) in 20% DMSO followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50 mg mL⁻¹ to 0.39 mg mL⁻¹. A 100 μL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 μL of standardized inoculum (10⁶ CFU MI⁻¹) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for the inhibition zones. The MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition (>8 mm), was recorded for each test organism (Okeke *et al.*, 2001; Thongson *et al.*, 2004; Nkere and Iroegbu, 2005; Aneja *et al.*, 2009).

RESULTS

The results of antimicrobial potency of methanol, acetone, petroleum ether, ethyl acetate, chloroform and aqueous extracts of parthenium leaves and the positive control ciprofloxacin (for bacteria) and amphotericin-B (for fungi) are presented in Table 1 and values of MIC of these extracts against the test pathogens are presented in Table 2. The antimicrobial activity of parthenium extracts on the agar plates varied in different solvents (Fig. 1). Both the positive controls produced significantly sized inhibition zones against the test bacteria (ciprofloxacin) and yeasts (amphotericin-B). Of the six plant extracts of parthenium, screened for antimicrobial activity, ethyl acetate, acetone and chloroform showed antimicrobial activity against all the tested pathogens. However, water extracts, petroleum ether and methanol showed least activity against the test strains (Table 1). A perusal of the data (Table 1) reveals that the ethyl acetate extract of parthenium was the most effective against all the four tested bacteria and the two pathogenic yeasts. It showed the highest zone of inhibition against *Bacillus subtilis* (30 mm) followed by *Staphylococcus aureus* (28 mm), *Pseudomonas aeruginosa* (26 mm), *E. coli* (10 mm). Among the tested yeasts, ethyl acetate extract showed the maximum zone of inhibition against *Saccharomyces cerevisiae* (18 mm), followed by *C. albicans* (10 mm). *Bacillus subtilis*, *S. aureus* and *P. aeruginosa* were found to be most sensitive pathogens which survived upto 6.25, 12.5 and 12.5 mg mL⁻¹ thus having an MIC of 3.12, 6.25 and 6.25 mg mL⁻¹, respectively (Table 2). *Saccharomyces cerevisiae*, *C. albicans* and *E. coli* were found to be comparatively less sensitive as they survived up to 25 mg mL⁻¹ (ethyl acetate extracts), 100 mg mL⁻¹ (ethyl acetate extract) and 50 (acetic extract) thus having an MIC of 12.5, 50 and 25 mg mL⁻¹, respectively.

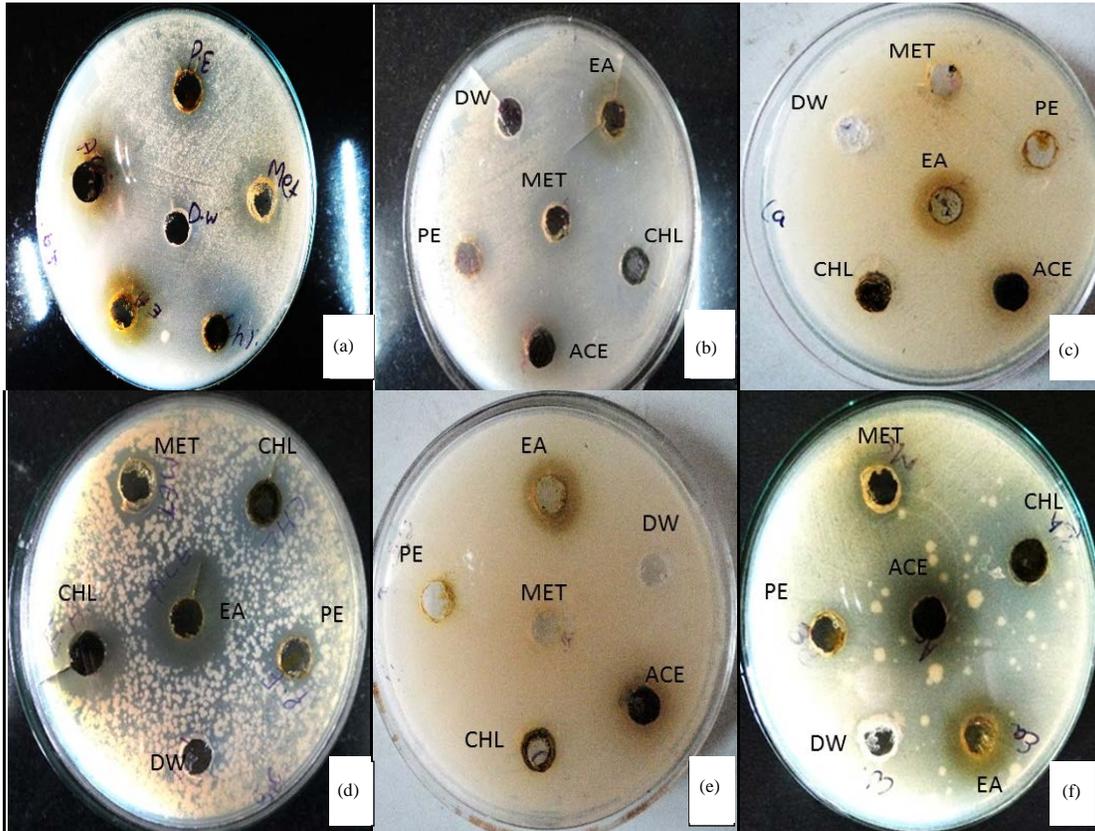


Fig. 1 (a-f): Zone of antimicrobial inhibition of parthenium leaves extracts against (a) *S. aureus* (b) *B. subtilis* (c) *C. albicans* (d) *S. cerevisiae* (e) *E. coli* and (f) *P. aeruginosa*

Table 1: Antimicrobial activity of Parthenium leaves extract on pathogenic microorganisms determined by agar well diffusion method

Parthenium leaves extract	Diameter of growth of inhibition zone (mm)					
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
Petroleum ether	-	13.3±0.57	-	-	-	6.3±0.57
Methanol	10.3±0.57	16.6±0.57	-	8.6±0.57	-	10.6±1.54
Distilled water	-	4.3±0.57	-	-	-	-
Chloroform	26.6±0.57	26.3±0.57	-	22.3±0.57	-	12.3±0.57
Acetone	26.3±0.57	24.6±0.57	12.6±1.54	18.6±0.57	-	20.3±0.57
Ethyl acetate	30.6±0.57	28.3±0.57	10.3±0.57	26.3±0.57	10.3±1.54	18.6±1.54
Ciprofloxacin	24.0±0.57	26.6±0.57	25.0±1.54	22.3±1.54	-	-
Amphotericin-B	-	-	-	-	16.6±1.54	19.3± 0.57

-: No activity. The data was analyzed by one way ANOVA followed by Dunnett's t test compared to positive control at 5% significant level, nt: not tested

This study reveals that organic extracts of parthenium have broad spectrum antibacterial activity against the human pathogens as visualized by the formation of inhibition zones of both gram positive and gram negative bacteria. Interestingly, some of the organic extracts of this plant showed much more potent activity against the tested organisms than that of standard antibiotics thus having a great potential to be developed as an herbal medicine to control the bacterial and fungal infections.

Table 2: Minimum inhibitory concentration of parthenium leaves extract on pathogenic microorganisms determined by modified agar well diffusion method

Parthenium Leaves extract	Minimum inhibitory concentration (mg MI ⁻¹)					
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
Petroleum ether	-	12.5	-	-	-	nt
Methanol	50	12.5	-	nt	-	50
Distilled water	-	nt	-	-	-	-
Chloroform	6.25	6.25	-	12.5	-	25
Acetone	6.25	12.5	25	12.5	-	12.5
Ethyl acetate	3.125	6.25	50	6.25	50	12.5
Ciprofloxacin	6.25	6.25	6.25	12.5	-	-
Amphotericin-B	-	-	-	-	12.5	12.5

nt- not tested, - : no activity

DISCUSSION

There are so many investigations reporting the antiviral, antifungal, antibacterial, antihelmintic, antimolluscal and antiinflammatory properties of parthenium (Durga *et al.*, 2013; Sukanya *et al.*, 2009). The Antimicrobial efficacy of *P. hysterophorus* has been reported by various scientists as: *Escherichia coli* (Madan *et al.*, 2011), *Bacillus subtilis*, *Enterococcus* spp. (Fazal *et al.*, 2011), *Staphylococcus aureus* (Barsagade and Wagh, 2010), *Pseudomonas aeruginosa* (Madan *et al.*, 2011), *Bacillus cereus* (Kumar *et al.*, 2013), *Enterobacter aerogenes* (Khan *et al.*, 2011), *Aspergillus niger* (Madan *et al.*, 2011; Bajwa *et al.*, 2003), *Candida albicans* (Malarkodi and Manoharan, 2013), *Aspergillus flavus* (Kumar *et al.*, 2013). The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Cowan, 1999). In parthenium the antimicrobial efficacy may be due to the presence of five terpenoids, volatile oils and flavonoids as well as amino acid, sugars and phenolic derivatives. The plant leaves extracts tested for antimicrobial potential showed varying degree of antimicrobial activities against the test bacterial and yeasts species (Table 1). The antibacterial activities of the ethyl acetate, acetone and chloroform extracts compared favourably with these of two standard antibiotics (Amphotericin B and Ciprofloxacin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The inhibition zone of methanolic extract, petroleum ether and distilled water extracts of *P. hysterophorus* was found nil for *Candida albicans* and *E. coli* and minor zone exhibited by the other four tested pathogens. The traditional practitioners use water and methanol as a primary solvent but according to our results depicted in Table 1 showed that ethyl acetate and acetone were better solvent for extracting antimicrobial substance from this weed compared to water and methanol. This may be due to better solubility of the active compounds in organic solvents (De Boer *et al.*, 2005; Salama and Marraiki, 2010). The different components diffusing at different rates may have been responsible for the varying zone of inhibition obtained in our assays against microorganisms. Of the organic extracts of parthenium screened, the ethyl acetate and acetonic extract has been found to have a better antimicrobial activity than the corresponding methanolic extracts (Table 1) thus substituting the findings of earlier workers (Cowan, 1999; Eloff, 1998; Aneja *et al.*, 2009; Nair *et al.*, 2005) who rated acetone as the best solvent.

It is interesting to note that even crude extracts of parthenium showed good activity against pathogenic microorganisms where modern antibiotic therapy has failed. It may, therefore, be concluded from the above investigation that the crude extracts obtained from the parthenium may be used to treat the infections caused by *Bacillus subtilis*, *S. aureus*, *P. aeruginosa* and

Saccharomyces cerevisiae which has shown comparable inhibition zone with the standard antibiotic drugs. The present study differs from the previous study since the antimicrobial activity was evaluated using six different organic extracts residues of the *Parthenium hysterophorus*. The finding of this study also paves the way for further research to identify the specific bioactive compounds that is responsible for its claimed antimicrobial activity.

CONCLUSIONS

From this study it is concluded that the crude extracts obtained from the leaves of the *Parthenium hysterophorus* showed the antimicrobial activity, indicating that this plant is a good source of antibiotics for their treatment of certain bacterial diseases. As of now, little work has been done on the antimicrobial activity and plausible medicinal applications of the phytochemical compounds and hence extensive investigations are needed such as *in vivo* studies of this plant necessary to determine toxicity of the active constituents, their side effects, pharmacokinetics properties to exploit the bioactive principles, for therapeutic utility in treating the bacterial infections. The antibacterial activities can be enhanced if the active components are purified and adequate dosage determined for proper administration. At last, the development of an effective phyto compound into an exploitable herbal product, which is devoid of side effects and drug resistance problem. However, isolation of pure compound and their toxicological study and clinical trials in animal model are to be made before their trials on human.

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