In vitro antibacterial activity of crude methanol extracts of various parts of *Parthenium hysterophorus* against pathogenic bacterial strains

Yimer Muktar, Muzeyen Mohammadnur, Anteneh Wondimu and Adem Hiko*

College of Veterinary Medicine, Haramaya University, P.O. Box 138 Dire Dawa, Ethiopia

**Corresponding author: Yimer Muktar, e-mail: yimermktr21@gmail.com.*

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Abstract

Parthenium hysterophorus is an aggressive and exotic weed plant traditionally reported to be used as remedy for various diseases. In the present study *in vitro* antibacterial activities of *P. hysterophorus* leaf, flower, bark and root crude methanol extracts were evaluated against five reference strains of pathogenic bacterial species. The *P. hysterophorus* was collected from Haramaya University campus from December 2015 to April 2016. Each leaf, flower, bark and root of *P. hysterophorus* was separately collected, dried, grinded and crude methanol soluble was extracted. The extracts were tested against *Shigella flexneri, Pseudomonas aeruginosa*, *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli* using standard agar disc diffusion assay at three different concentrations (1000mg/ml, 500mg/ml and 250 mg/ml) from each parts. Standard antibiotic (Chloramphenicol 30µg/disc) was used as positive control and DMSO (0.5%) was used as negative control. Among the *P. hysterophorus* parts tested: leaf, flower, bark and root showed promising inhibitory activities against the tested bacterial strains at all concentration except bark extract at 250mg/ml, root 500mg/ml, root 250mg/ml did not show antibacterial activity against *Streptococcus agalactiae,* S*taphylococcus aureus* and *Streptococcus agalactiae,* respectively. The crude extract from all parts of the plant showed a concentration dependent mean zone of inhibition on all the tested bacterial strains. Methanol crude extract of *P. hysterophorus* parts were active against the tested bacterial strains and promising for refined medicinal drug source. *In vivo* toxicity and efficacy test should be done to validate the importance of this plant for future development of lead compounds.

Keywords: Antibacterial activity; Bacterial pathogen; Concentration; *Parthenium hysterophorus*

Introduction

Since ancient times, plants have been indispensable sources of both preventive and curative traditional medicine preparations for human and livestock (Areaya Hailamariam *et al.,* 2015), whereby the majority of farmers and pastoralists in the developing countries rely on medicinal plants or ethno-veterinary medicine practices (EVM) (Kaaya, 2003; Matlebyane *et al*., 2010). Numerous biochemical compounds obtained from such plants possess important antimicrobial properties (Abdallah, 2011) and preferred over synthetic drugs and considered safe to humans (Rakholiya *et al*., 2013). New and effective antimicrobials identified from plants for development of new drugs to combat health problems associated with drug resistance (Bonjar, 2004).

Ethiopia is the home of remarkably diverse flora, including numerous endemic species used for different traditional medical practices (Dawit Abebe, 1986). The vast majority of the rural populations and the practitioners depend on traditional medicine (TM) (Shiferaw Messeret, 1996). Nowadays many bacterial pathogens have developed resistance against many antimicrobials due to the indiscriminate use of commercial antimicrobials, leading to the continuing emergence of drug resistant organisms (Overbye and Barrett, 2005; Diaz *et al*., 2008). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host causing hypersensitivity, immune-suppression and allergic reactions which increased the demand for development of antimicrobial substances. Hence, development of alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases is one of the priority research areas (Agrawal *et al.,* 1996; Parekh *et al.,* 2005)

Traditionally, *P. hysterophorus* is used for the treatment of wounds, diabetes, ulcerated sores, fever, diarrhea, anemia, heart troubles, malaria, headache, inflammatory urinary infections, skin rashes, neurological disorder, migraine headaches, rheumatoid arthritis, stomachaches, toothaches, insect bites, infertility, and problems with menstruation and labor during childbirth (Veena and Shivani, 2012; Shashank and Abhay, 2014). Its multiple pharmacologic properties, such as anticancer, anti-inflammatory, cardiotonic, antispasmodic, an emmenagogue, and an enema for worms have been reported (Dipankar and Munan, 2013). On the other hand, *P. hysterophorus* is also mostly considered as dangerous weed and toxic plant that affect livestock, humans and other plants species (Holm, *et al.* 1977; Robert, 2014).

In Ethiopia, many of the research with regard to *P. hysterophorus* focused on the controlling and eradication from agricultural field (Robert, 2014; Zuberi *et al*., 2014) rather than investigating it for its possible medicinal value. All parts of the plant are reported to be used as traditional medicine for treatment of wounds, ulcerated sores, anemia, fever, and heart troubles (Kumar *et al*., 2013). Although parts of *P. hysterophorus* are rich source of useful bioactive ingredients (Shashank and Abhay, 2014), limited study have been documented regarding its antibacterial activities (Parekh *et al.,* 2006). Therefore, the objective of the present study was to evaluate *in vitro* antibacterial activities of crude methanol extract of *P. hysterophorus* against selected pathogenic bacterial species.

Materials and Methods

Study area

The study was conducted at Haramaya University from December 2015 to April 2016 located 507 Km East of Addis Ababa, the capital city, Ethiopia, at 09.°N and 42.°E at an altitude of 1950 meters above sea level. The area receives bimodal rain fall, long rainy season (July to September) and short rainy season (March to June) with average annual rainfall about 790mm, the mean maximum and minimum temperature of 23.6°C and 10.1°C respectively (HADB, 2014).

Collection and preparation of plant materials

Whole fresh plant of *P. hysterophorus* was collected from Haramaya University (HU) campus. Plant specimens were identified and authenticated by experts at the Department of Plant Sciences, Haramaya University (HU), Ethiopia and Voucher specimens were deposited at HU. Leaf, flower, bark and root parts were separately collected in to separate containers and then labeled. The collected plant parts were washed to remove dirt and soil particles, cut into small pieces, spread out on paper sheets, dried in shaded area at room temperature for two weeks and were grinded separately using electrical mill (GM1001Retsh, Germany). The fine powder was kept in different labeled bottles.

Preparations of crude extract

Preparation of crude extracts was conducted according to the method described by Hailu Tadeg *et al.* (2005) with little modification. Each powder part of the

plant was weighed, put into separate flask and mixed with methanol at ratio of 1:5 (i.e. 150gm) of each part of the plant was mixed with 750 ml of 100% methanol) shaking with an orbital shaker (GFL 3006, Germany) at 80 rpm for 24hours at room temperature. The maceration was done three times for each part. After 24 hours, the macerates of each part were filtered in separate flasks using a qualitative folding filter paper (Whatmann No. 1) (New Delhi, India). The residues after filtration process were discarded while the filtrate part was concentrated using a rotary evaporator at (RE-5299 Henan, China) at 40°C. All the crude extracts were dissolved in dimethyl sulfoxide (DMSO) (EINECS No. 200-664-3Anhui, China), which did not influence the microbial growth, to achieve the final working concentration. Finally, the percentage yields of each parts of the crude extract were calculated using amount and yield obtained from maceration. The resulting concentrated extracts of each plant material was transferred to bottle which had tight fitting cups and then labeled and kept in refrigerator at 4°C until tested for antibacterial activity (Okeke *et al*., 2001; Kumar *et al*., 2006).

Phytochemical screening

The methanol extract of *P. hysterophorus* subjected to phytochemical screening using a standard screening procedure for the detection of the saponins, tannins, phenolics, alkaloids, steroids, flavonoids, glycosides and phlobatannins (Harborne, 1973) as shown in Table 1.

Table1. Methods used in phytochemical screening of the study plant

Chemicals analyzed	Amount in ml	Methods used	Time	Result
Saponin	1.0 ml	Frothing test	3 min	Frothing remains for 10-15 min
Tannins	2.0 ml	Ferric chloride test	10 min	Blue-black coloration
Phenolics	0.2 ml	Ferric chloride test	10 min	Bluish-green coloration
Alkaloids	1.0 ml	Mayer's test	5 min.	Brown or white precipitate
Steroids	1.0 ml	Libermann Burchardt test	3 min	Ring of blue-green coloration
Flavonoids	3.0 ml	Shinoda test	3 min	Pink or red coloration
Glycosides	0.5 ml	Ferric chloride test	15 min	Green to black precipitate
Phlobatannins	0.2 ml	Hydrochloric acid test	5 min	Red precipitate

Preparation of selected test bacterial strains

Five bacterial strains consisting of American Type Culture Collection (ATCC) standard strains obtained from Ethiopian Public Health Institute (EPHI) namely *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Streptococcus agalactiae* (ATCC 12386) and *Escherichia coli* (ATCC 25922) were used as test strains. The strains were sub-cultured in Brain Heart Infusion broth (Oxoid, United Kingdom) prior to test.

Experimental design and layout of the study

In vitro antibacterial activities of crude methanol extract of leaf, flower, barks and roots of *P. hysterophorus* against selected pathogenic bacterial species were conducted. The crude extracts from each of the *P. hysterophorus* parts were tested at 1000mg/ml, 500mg/ml, and 250mg/ml concentration. Chloramphenicol was used as standard antibacterial drug (positive control) at 30µg/disc. DMSO (0.5%) was used as negative control

Antibacterial activity assay

The disc diffusion technique followed by the National Committee for Clinical Laboratory Standards (Bauer *et al*., 1966; CLSI, 2012) protocol was used to evaluate antibacterial activities. For susceptibility testing, three concentrations (1000 mg/ml, 500 mg/ml and 250 mg/ml) crude extracts prepared in dimethyl sulfoxide (DMSO 0.5%) were used. Sterile antibiotic assay discs (Whatman, No.1 filter paper with 6 mm diameter) were impregnated with triplicate of each 1000mg/ml, 500mg/ml and 250mg/ml of the reconstituted extract obtained from each part of *P. hysterophorus*. For inoculums standardization, the BHI growth turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard (10^8 CFU/ml) using spectrophotometric device (Shimadzu, Japan) at 660 nm of wave length. The bacterial lawn was surface spread on Muller Hinton agar (Oxoid, United Kingdom) using sterile cotton swab. Each disc was gently placed on and pressed down on plate agar to ensure complete contact with inoculated bacteria. In Parallel, chloramphenicol 30µg discs (Oxoid, United Kingdom) were used as control drug. Then plates were incubated at 37°C for 24 h. Each of the extracts was tested in triplicate. Zones of inhibition were recorded as the diameter of growth free zones (Das *et al*., 2010) and results were recorded.

Data analysis

Data was analyzed using statistical package for social sciences (SPSS Version 20.0., Armonk, NY: IBM Corp). The results of the study were expressed as a mean zone of inhibition ± standard error (Mean ± SE) and difference among means was compared using one way ANOVA.

Results

The physical characteristic features and percentage yield of the extracts are shown in Table 2. From the macerates of different parts of *P. hysterophorus,* leaf part yielded highest followed by flower, root and bark. The crude extract from leaf, flower, bark and root has dark green, green, green and yellow color respectively. The plant was also examined for phytochemical composition qualitatively and the presence of different secondary metabolites were confirmed (Table 3).

Table 2. *P. hysterophorus* parts and extract yielded using 100% concentrated methanol.

P. hysterophorus parts	Amount macerated (g)	Yield (g)	Yield (%)	Extract color
Leaf	150	17.88	11.92	Dark green
Flower	150	16.55	11.0	Green
Bark	150	15.41	10.1	Green
Root	150	15.97	10.65	Yellow

Table.3 Preliminary phytochemical screening of methanolic extract of *Parthenium hysterophoru*

Phytochemical	Secondary Metabolite Test and Reagents	Result
Steroids	Libermann Burchardt test	+
Flavonoids	Shinoda test	-
Alkaloids	Mayer's test	+
Phenol	Ferric chloride test	+
Tannins	Ferric chloride test	+
Saponins	Frothing test	+
Glycosides	Ferric chloride test	+
Phlobatannins	Hydrochloric acid test	-

The mean±SE clear zone of inhibition of extracts of test plant parts are shown in Table 4. Whereby all of four parts of *P. hysterophorus* showed antibacterial activity at all concentrations except lower concentrations of bark and root extracts that did not show any antibacterial effect against *Streptococcus agalactiae*. The mean±SE zone of inhibition of the leaf (21.7±0.58) and flower (20.5±0.49) at a concentration of 1000 mg/ml was comparable with that of chloramphinecol 30 µg (23±0.27) on *Shigella*. The mean±SE zone of inhibition of crude extract at 1000 mg/ml and 500 mg/ml on *P. aeruginosa* (10.7±1.23 to 21.7±1.0) from all parts of *P. hysterophorus* was almost higher than that for chloramphinecol (10.7±1.23). Mean±SE zone of inhibition of crude extract from all parts *P. hysterophorus* were lower (0.0 ±0.0 to 21.7±0.58) than chloramphinecol (16.2±0.68 to 23.8±0.27) on *Shigella, E. coli, P. aeruginosa* and *S. agalactiae*. Crude extract from leaf and flower has higher mean±SE zone of inhibition on all of the tested strains than the extract from bark and root at all concentrations. No effect was observed on *S. agalactiae* by root extract both at 500 mg/ml and 250 mg/ml and bark extract at 250mg/ml (Table 4).

Table 4. Mean ±SE zone of inhibition of *P. hysterophorus* parts against bacterial pathogens at different concentrations.

Plant parts	Conc. (mg/ml)	Mean zone of inhibition (mm) (±SE)				
		S. flexneri	**PSA**	*E. coli*	**SA**	*Str.aga.*
Leaf	1000	21.7(0.58)	21.7(1.00)	17.0(0.00)	13.5(0.28)	12.0(0.29)
	500	21.0 (0.00)	18.5(0.29)	11.0 (0.58)	10.5 (0.29)	9.5(0.29)
	250	20.0 (0.58)	14.0 (1.00)	10.0 (0.00)	7.5 (0.29)	6.5 (0.29)
Flower	1000	20.5 (0.49)	20.5 (1.22)	15.5 (1.10)	11.5 (0.66)	9.5 (0.78)
	500	18.0 (0.58)	16.0 (0.58)	9.5 (0.29)	9.5 (0.29)	7.5 (0.87)
	250	17.8 (0.43)	12.5 (0.87)	8.5 (0.29)	7.0 (0.00)	6.5 (0.29)
Bark	1000	17.3 (0.44)	15.5 (0.29)	9.0 (0.58)	8.5 (0.87)	7.5 (0.87)
	500	16.0 (0.29)	14.0 (0.58)	8.0 (0.58)	7.0 (0.00)	6.5 (0.87)
	250	14.5 (0.29)	10.5 (0.87)	3.5 (2.02)	6.5 (0.29)	0.00 (0.00)
Root	1000	10.3 (0.14)	11.0 (1.15)	8.5 (0.29)	9.0 (0.58)	0.0(0.00)
	500	10.5 (0.29)	10.0 (0.00)	7.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	250	9.0 (0.58)	8.2 (0.44)	7.0 (0.00)	7.0 (0.00)	0.0 (0.00)
Chloramp.	(30µg)	23.8 (0.27)	10.7 (1.23)	21.5 (0.39)	18.2 (1.49)	16.2 (0.68)
DMSO	(0.5%)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)

* PSA= *Pseudomonas aeruginosa;* SA= *Staphylococcus aureus;* Str.aga= *Streptococcus agalactiae, S. flexneri= Shigella flexneri*

Discussion

Methanol crude extraction of *Parthenium hysterophorus* against certain gram negative and gram positive pathogenic bacteria was found promising. This result was in agreement with the pervious finding of Sukanya et al. (2009) and Kamarapu et al. (2015) from India. Rastogi and Mehrotra (1991) also described *P. hysterophorus* as a medicinal plant. This could be attributed to presence of antimicrobial activities in various secondary metabolites of this plant. Bioactive compounds such as alkaloids, tannins, steroids, glycosides, saponins and phenolic compounds were detected to be present in the *P. hysterophorus* plant. Since this plant had been used in the treatment of different ailment such as malaria, cancer and skin burn etc., the medicinal roles of these plants could be related to these bioactive compounds. The extract from all parts of plant have shown to be effective against all bacterial strains at a high concentration except for 500 mg/ml of root which did not show effect against S. aureus and Str. agalactiae. The leaf and flower extract of *P. hysterophorus* showed a better inhibitory effect on all tested organisms compared to the other parts which is in agreement with previous study (Fazal *et al*., 2011). This could be attributed to accumulation of effective metabolites at high concentration in the leaves and flower than the other parts.

It was clear from the present results, that methanol extract of leaves and flowers of *p. hysterophorus* exhibited pronounced activity against *E. coli*, *P. aeroginosa*, *Shigella* species, *Str. agalactiae* and *S. aureus.* The antibacterial efficacy of *P. hysterophorus* showed comparable result with the previous reports of (Fazal *et al.,* 2011; Madan *et al*., 2011), against *E. coli,* (Barsagade and Wagh, 2010; Kamarapu *et a*l., 2015) *S. aureus*, (Madan *et al*., 2011; Kaur, *et al.,* 2016) against *p. aeruginosa*. Leaf and flower extracts showed highest inhibition zone against *Shigella* species and *P. aeruginosa* even better than chloramphinecol. Similar results were recorded by Malarkodi and Manoharan (2013) for *P. hysterophorus* leaf and flower against *P. aeruginosa*.

Even though extraction was not done in this study chloroform in leaf and flower part as extracting solvent presented a better inhibitory effect on the test organisms. Motamedi *et al* (2010) reported that solubility of active principles in plant materials varies according to extraction solvent used, which may relate to differences in antimicrobial effect of plant extracts (El Astal *et al.,* 2010). Hence, the extraction solvent used in this study could have caused variation in the antimicrobial activity.

Methanol extract of *P. hysterophorus* leaf and flower was presented as better inhibitory effect on all of the pathogenic bacteria tested. Kamarapu *et al* (2015) recorded the same result for the methanol extract of flower against *P. aeruginosa* and *S. aureus*. Kamarapu *et al.* (2015) also recorded the result of flower extraction against *E. coli* with high zone of inhibition than other bacterial strains which was different from the current study. In general, the disparities between our findings and others may result from differences in chemical composition of extracts, effects of secondary metabolites (Cowan, 1999), and could also be associated with geographic variation or methodological considerations and solvent used.

Regarding the bark and root parts, crude extract of *P. hysterophorus* as far as our literature search is concerned, no study was reported against the present tested bacterial pathogens. Extract at 1000 mg/ml and 500 mg/ml showed inhibitory effect against all bacterial strains whereas at 250 mg/ml concentration showed an effect against all bacteria except *Streptococcus agalactiae.* This can be attributed to the presence of different inhibitory molecules in roots and bark parts of *P. hysterophorus* (Afsharypuor *et al.*, 1995) but not as effective as those in the leaf and flower.

Conclusion

The crude extracts obtained from the leaves, flowers, barks and roots of *P. hysterophorus* showed antibacterial activity indicating the potential of this plant as a source of antibiotics for the treatment of certain bacterial diseases. The overall results of the current study revealed that there was a promising antibacterial effect that suggests the need of further in-depth study on this plant. In vivo toxicity and drug efficacy test should be done to validate the importance of this plant for future development of lead compounds towards producing alternative medicine.

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