Research Article

In-Vitro Antimicrobial Activity of *Parthenium hysteroporus* Comparison with Ofloxacin

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ABSTRACT

The plant produces different types of bioactive compounds that have several medicinal activities in the human body. Most of them are established as natural medicines for human consumption. In the present study, seen the in-vitro antimicrobial study on *Parthenium hysteroporus* comparison with Ofloxacin. The plant produces different types of bioactive compounds that have several medicinal activities in the human body. Most of them are established as natural medicines for human consumption. In the present study, seen the in-vitro antimicrobial study on *Parthenium hysteroporus*. The fractionation of the plant extract was done by using Pet ether, chloroform, ethanol, and water. Extracts show its antimicrobial activity against different microorganism strain like *S.aureus* (ATCC-29737) and (ATCC-25157), *S.dysentri* (QCM-4714), S.typhi (NCTC-74). In the case of antimicrobial study used two types of media MHB (Mularhington broth) and MHA (Mularhington agar) were prepared in varying proportions. All among the fraction the direct-ethanol fraction of this plant was given better antimicrobial activity in lower concentration (2mg/ml, 1mg/ml) than the other fraction against those mentioned organisms. The plant extracts were compared with standard antibiotic ofloxacin and a comparison study showed in this work against the same mentioned microorganism. The plant direct-ethanol fraction was given almost related antimicrobial activity as compared to ofloxacin. After completion of this research work that if we reduce the concentration of the drug solution, the antimicrobial activity was increased as there are less contained steroids that reduce antimicrobial activity.

Keywords: In-vitro, *P.hysteroporus*, *S.aureus*, MHA, MHB, antibiotic, fraction, ofloxacin

INTRODUCTION

Antimicrobial study now a day a popular and developed study in the whole world. Different types of synthetic or chemically synthesis potent antibiotics are available in the market those give better result in case of antimicrobial study as well as antifungal study. Nature gives several types of plants with numerous chemical constituents those have different types of activity which compete with the chemical synthetically prepared potent drugs and several cases observed that natural resources give much better action than the synthetic drug those are available in the market with the different brand name and different types of formulation. Nature gives this type of plants that give potent antimicrobial activity against the different marketed drug.[1,2] One of them *Parthenium hysteroporus* is a common plant easily available in India, in several places. This plant introduced in Asia in 1950 and then its seed distributed by air in the whole country. Its stem consists of some tentacles which any time touch with our skin causes some skin irritation. But the main chemical constituents which give the antimicrobial or antibacterial properties against different types of microbes present in plant leaves. This plant has different types of species and identified the species depend on the structural design of the leaves. Most of them *P.hysteroporus* have much more anti-microbial properties compare to the other species of these plants. It consists several chemical constituents like camphene, b-pinene, sabinene, b-myrcene, b-terpene, limolene, b-ocimene, ocimene, p-cymene, linalool, caryophyllene, humulene, terpinene-4-ol.Thetoxic and inhibitory constituents contained by all parts (stem, leaves, leaf hair, flower, pollen grain) of *P.hysterophorus.* It consists of steroids also. According to the ayurvedic medicines that identify the potent constituent that gives the antibacterial activity. Several times failure will happen to identify the potent compound so one of the most important processes which can help the separate the nearer mixture of the compound with some similarity with the potent compound and as well as its give the wanted result, this process is called fractionation of the group of the compound using different grades of solvent. By the solvent fractionation process get the group of the compound will give the better result and compare that compound with already established standard potent compound already available in the market. This plant local name is Parthenium weeds belong to the Asteeraceae family. In this study, Ofloxacin was selected for the comparison study with several fractionation obtained by the different method by using different solvent and this was used as a standard drug in this study which is a potent antibacterial agent available in market. [3 4]

MATERIALS AND METHODOLOGY

Plant collection and identification

The young tender leaves of *Parthenium hysterophorus* were collected from BANDEL nearby rail

line side and roadside at Sugandha, Hooghly. Generally, *the Parthenium hysterophorus* grows in July to August; hence the collection was made in September. The identification and authentication were done by a Taxonomist of the Botanical Survey of India, Shibpur, Howrah, and voucher specimen (*Herbarium No. CNH/71b/2013/Tech.II/15*) has been deposited at the Herbarium and the host institution for future references.

Extraction and Fractionation

Extraction by Maceration technique

Collected samples were washed thoroughly, shed dried for a single day and dried in tray dryer (at 400C), then pulverized by a mechanical grinder. A portion of 200gm of powder material in a ratio of course: fines of (3:2) is directly fractionated by soaking in 300ml of 99.9 % ethanol (Merck, Germany) at room temperature for overnight. The solvent was filtered by vacuum filtration and the residues are soaked in 600ml of distilled water overnight, filter it and collect the solvent. Another portion of 200gm of powder material in the same ratio of course: fines are indirectly fractionated with petroleum ether, chloroform, ethanol and water (600ml of each solvent as previously) according to the increasing grade of polarity. After extraction solvents were removed by rotary vacuum evaporator (Eyela Rotary Evaporator, Japan) to get sticky mass and stored in vacuumed desiccators for further use. [5 6 7]

Solvents used

Pet ether, Chloroform, Ethanol, Water.

Table 1: (Extractive fraction value)

EXTRACTIVE FRACTION						
	Pet ether	**chloroform**	**Direct ethanol**	**Indirect ethanol**	**Direct water**	**Indirect water**
%Yield	0.55	1.65	7.5	1.25	3.24	1.74

Phytochemical Screening

According to the survey, those parts of the plant consist higher percentage of flavonoids and phenolic compounds in their chemical constituents, that plant extractive fraction gives better antimicrobial activity. In the presence study determination of antimicrobial activity so phytochemical screening is the most important part of research work to identify what type of compound is present in this plant which responsible for the antimicrobial activity. In this screening process, several chemical tests were proceeding to identify the content of alkaloids, flavonoids, glycoside, phenolic compound, tannins, steroids, and terpenoids. The flavonoids and phenolic content determination process done separately by determination absorbance of the plant fraction using UV spectroscopy method (**Flavonoids content(mg/g) =**[{(absorbance (A)-0.0132)÷0.0067}/sample used (g)]×10^{-3} and **Phenolics content (mg/g)=**[{(absorbance (A)+0.058)÷0.0034}/sample used(g)}]×10^{-3}) **.** [8]

Determination of Antimicrobial Activity of *P.Hysteroporus*

Determination of the antimicrobial activity of P.hysteroporus is occurred by the preparation of different media, used sterilized apparatus, and different strains of microorganism. In this study using Mularhington Agar and Mularhington Broth as a media to determine the antimicrobial study. The preparation of media done by using different types of media constituents- Beef extract, casein, starch, agar, distilled water. During media, preparation used a clean and sterilized glass apparatus. The plant extractive fractions showed antimicrobial activity against different types of stains used in this study- S. *aureus* (ATCC-29737), S. *aureus* (ATCC-25157), S. *dysentery* (QCM-4714), S.*typhi* (NCTC-74). [9 10 11]

Preparation of media

MHA (Mularhington agar)

The constituents are weighed according to requirements. Take a chronical flask and take some quantity of water in the flask. Then add beef extract, casein, starch, MHA and stirred continuously with a glass rod. Then add some quantity of water and PH 7.2-7.4 measured with the help of 0.1(N) sodium hydroxide. After PH adjustments the agar is added and volume make up with water. [1213]

MHB (Mularhington broth)

The constituents are weighed according to requirements. Take a chronical flask and take some quantity of water in the flask. Then add beef extract, casein, starch, MHB and stirred continuously with a glass rod. Then add some quantity of water and PH 7.2-7.4 measured with the help of 0.1(N) sodium hydroxide. After PH adjustments volume make up with water. [1213]

Distribution and sterilized

MHA are distributed in the maccokney bottol. Each bottol is consist 9ml of media. Then the media is sterilized in autoclave at 125°C for 15 min.

MHB are distributed 5 ml in test tube and cotton ploughed is necessary. Then the media is sterilized in autoclave at 125°C for 15 min.

Inoculation of Strains in MHB using McFarland Standard
Inoculation is done in Mularhington broth in a laminar airflow bench (LAF). Then incubated at 37oC in the

incubator. After incubation, the turbidity is adjusted by McFarland standard (In microbiology, Macfarlane standards are used as a reference to adjust the turbidity of suspensions so that the number of bacteria will be within a given range to standardize microbial testing. An example of such testing antibiotic susceptibility testing by measurement of minimum inhibitory concentration which is routinely used in medical microbiology and research. If a suspension used is too heavy or too dilute, an erroneous result (either falsely resistant or falsely susceptible) for any given antimicrobial agent could occur.

Preparation of McFarland standards

Original McFarland standards were mixing specified amounts of barium chloride and sulphuric acid together. Mixing the two compounds forms a barium sulphate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dehydrate ($BaCL_2.2H_2O$), with 9.95 ml of 1% sulphuric acid.

Adjustment turbidity

The standard can be compared visually to a suspension of bacteria in sterile normal saline. If the bacterial suspension is too turbid, it can be diluted with more diluents. If the suspension is not turbid enough, more bacteria can be added. [13 14]

Table 2: (McFarland Nephelometer Standards)

McFarland Standard No.	0.5	1	2	3	4
1.0% Barium Chloride(ml)	0.05	0.1	0.2	0.3	0.4
1.0% sulphuric acid	9.95	9.9	9.8	9.7	9.6
Approx. Cell density (1x10^8 CFU/ml)	1.5	3.0	6.0	9.0	12.0
Absorbance*	0.08 to 0.1	0.257	0.451	0.582	0.669
***measure absorbance at 600nm.**					

Preparation of Drug Solution of *P. Hysteroporus* and Standard Drug Ofloxacin

Preparation of drug solution of direct ethanol of P. Hysteroporus

Take 100mg of drug and add 10 ml sterilized water and mixed thoroughly so concentration 10mg/ml is produced. Then take 5ml from the above solution and add 5ml of sterilized water so, concentration 5mg/ml is produced. Then take 2.4 ml from the second drug solution and add 1.6 ml of water so concentration 2mg/ml is produced. Then take 1 ml from the third solution and add 1 ml of water. All solution is prepared in a test tube and mixed thoroughly in the vortex. [16]

Preparation of drug solution of standard drug Ofloxacin

Take 100mg of drug and add 10 ml sterilized water and mixed thoroughly so concentration 10mg/ml is produced. Then take 5ml from above solution and add 5ml of sterilized water so, concentration 5mg/ml is produced. Then take 2.4 ml from second drug solution and add 1.6 ml of water so concentration 2mg/ml is produced. Then take 1 ml from the third solution and add 1 ml of water. The all solutions are prepared in test tube and mixed thoroughly in vortex.

Evaluation of Antimicrobial activity of *P.hysteroporus* by direct inoculation method

Take four sterilized Petri dishes with the upper lid and clean every Petri dish with the help of cotton and acetone. Then take each maccokny bottle and add 1 ml of drug solution in the bottle according to the different concentrations (10, 5, 2, 1 mg/ml). Then each bottle was shaken well and poured into the Petri dish and the plate containing media is allowed to become semisolid without any disturbance. Then four boxes were cut by a marker for four different strains of microorganisms. Then the plates are kept in an incubator for blank incubation at 37^0C. The next day the contaminated plate is rejected and the fresh plate is directly inoculated by the specific culture. The results were taken after overnight incubation at 37^0C. [17]

Comparison activity of direct ethanol fraction of *P.hysteroporus* with standard drug Ofloxacin

Preparation of the plate of standard drug is same as above. Take four drug plate of standard and four drug plate of direct fraction. Then direct inoculation is done in both plate with same four stains culture organisms. After incubation compare the inhibition of organism by standard drug and inhibition by direct ethanol fraction of *P.hysteroporus*

RESULT AND DISCUSSION

Table 3: Phytochemical Screening results

Name of the test	Pet ether	Direct ethanol	Indirect ethanol	Direct water	Indirect water	Chloroform
Test for alkaloid						
a) Dragendroff's Reagent	Positive	Positive	Positive	Positive	Positive	Positive
b) Mayer's Reagent	Positive	Positive	Positive	Positive	Positive	Positive
c) Wagner's Reagent	Positive	Positive	Positive	Positive	Positive	Positive
d) Hager's Reagent	Positive	Positive	Positive	Positive	Positive	Positive
e) Tannic Acid Test	Positive	Positive	Positive	Positive	Positive	Positive
Test for flavonoids						
a) Shinoda Test	Positive	Positive	Positive	Negative	Positive	Positive
b) Alkaline Reagent Test	Positive	Positive	Positive	Positive	Positive	Negative
c) Zinc Hydrochloride Test	Positive			Negative	Negative	Positive
Test for Anthraquinones glycoside						
a) Borntrager's Test	Negative	Negative	Negative	Negative	Negative	Negative
b) Modified Borntrager's Test	Positive	Positive	Positive	Positive	Positive	Positive
Test for cardiac glycoside						
a) Keller Killiani Test (for deoxy sugar)	Positive	Negative	Negative	Negative	Negative	Negative
b) Raymond's Test	Negative	Negative		Negative	Negative	Negative
c) Legal's Test	Positive	Negative	Positive	Positive	Positive	Positive
d) Baljet's Test	Positive	Negative	Negative	Negative	Negative	Negative
Test for Tannins						
a) Ferric Chloride Test	Positive (condensed tannins)	Negative	Positive (condensed tannins)	Positive (condensed tannins)	Positive (condensed tannins)	Negative
Test for steroids						
a) Liberman Burchard Test	Positive	Positive	Positive	Brown color	Brown color	Positive
b) Solkowski Test	Negative	Positive	Positive	Positive	Positive	Negative
c) Sulfur Powder Test	Positive	Positive	Positive	Positive	Positive	Positive
Test for Amino acid						
a) Ninhydrin Test	Negative	Positive	Positive	Positive	Positive	Positive
Test for Carbohydrate						
a) Molisch's Test	Positive	Negative	Negative	Negative	Negative	Positive
c) Osazone Formation Test	Positive	Negative	Positive	Positive	Positive	Positive
Test for volatile oil						
a) Sudan III Test	Positive	Positive				

Table 4: Total Flavonoids Content

SL.NO	Extractive fraction	Absorbance	Flavonoids Content (mg/g)
1	Direct ethanol	0.877	128.92
2	Indirect ethanol	0.376	27.07
3	Chloroform	0.505	36.70
4	Direct water	0.078	9.67
5	Indirect water	0.052	5.79
Flavonoids content(mg/g) = [{(absorbance (A)-0.0132) ÷ 0.0067}/sample used (g)] × 10^{-3}			

Table 5: Total Phenolic Content

SL. NO	Extractive Fraction	Absorbance	Phenolics Content (mg/g)
1	Direct ethanol	0.033	53.82
2	Indirect ethanol	0.028	50.58
Phenolics content (mg/g) = [{(absorbance (A)+0.058) ÷ 0.0034}/sample used(g)}] × 10^{-3}			

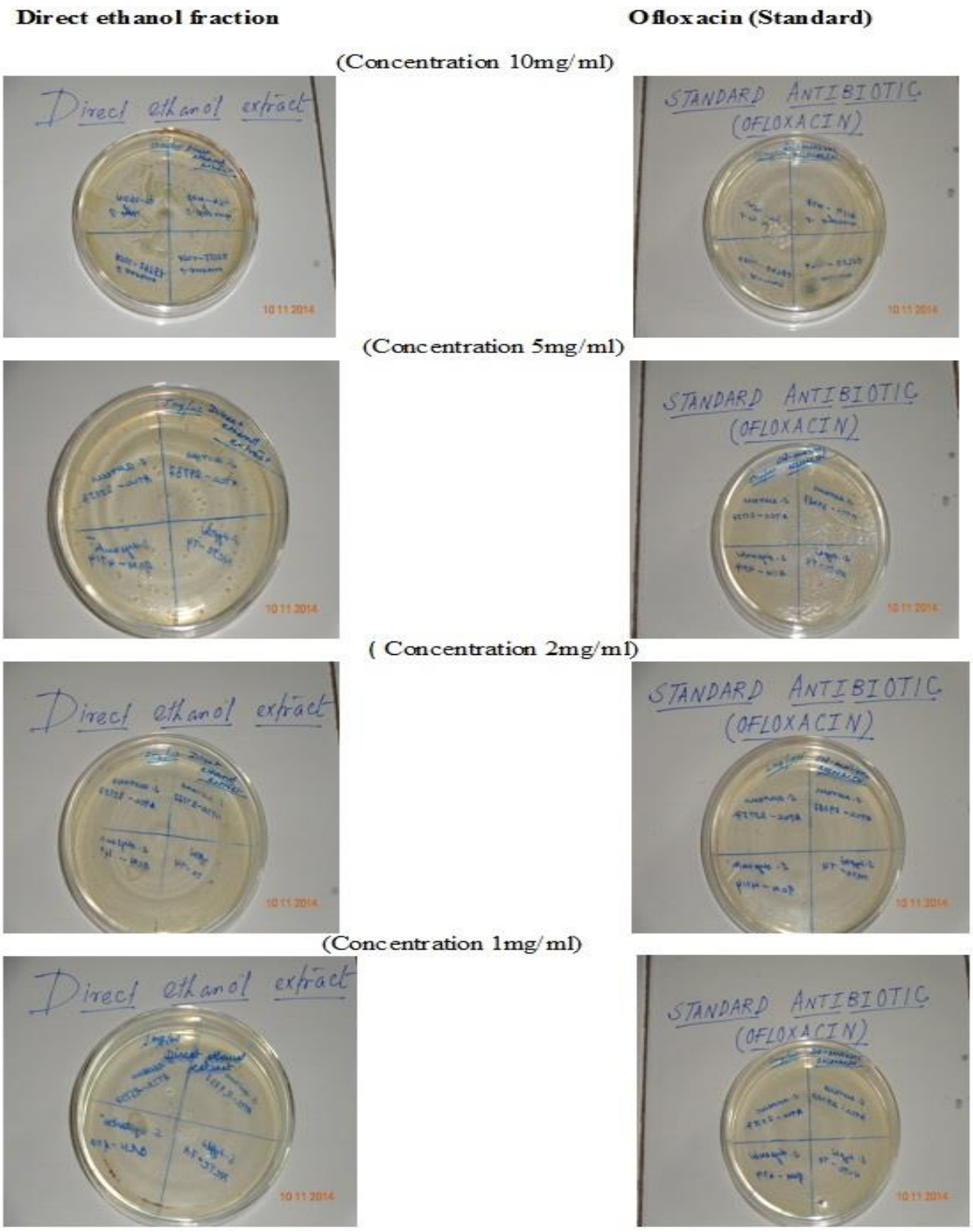

Figure 4: Comparison figure between Ofloxacin and direct ethanol fraction of *P.hysteroporu*

Table 7: (A&B) Comparison result between standard drugs Ofloxacin and direct ethanol fraction of *P.hysteroporus*.

(A)

Organisms	Drug	Concentration 10 mg/ml	Concentration 5 mg/ml	Concentration 2mg/ml	Concentration 1mg/ml
S. *aureus* ATCC-29737	Direct ethanol fraction	++	+	+	++
S. *aureus* ATCC-25157	Direct ethanol fraction	-	+	++	++
S. *typhi* NCTC-74	Direct ethanol fraction	++	++	++	+
S. *dysentri* QCM-4717	Direct ethanol fraction	+	+	+	+
_ No inhibition+ Inhibition++ Almost inhibition					

(B)

Organisms	Standard Drug	Concentration 10 mg/ml	Concentration 5 mg/ml	Concentration 2mg/ml	Concentration 1mg/ml
S. *aureus* ATCC-29737	Ofloxacin	++	++	+	++
S. *aureus* ATCC-25157	Ofloxacin	++	++	++	++
S. *typhi* NCTC-74	Ofloxacin	_	++	++	++
S. *dysentri* QCM-4717	Ofloxacin	++	_	++	++
_ No inhibition+ Inhibition++ Almost inhibition					

DISCUSSION

Plant compounds have had great therapeutic potentials than synthetic compounds. *P.hysteroporus* have antimicrobial activity but better activity in direct-ethanol fraction than other fractions. The maceration technique is used for the extraction of all fractions like direct-ethanol, pet ether, chloroform, indirect ethanol. The drug consisting of polyphenolic compounds or flavonoids are responsible for better antimicrobial activity. So, using phytochemical analysis get conformation of the presence of flavonoids and polyphenolic compounds and those compounds responsible for antimicrobial activity, done in this presence study. In the case of antimicrobial study used two types of media MHB and MHA. Those media are prepared in varying proportions. Four different types of organisms are used for anti-microbial like- *S.aureus* (ATCC-29737) and (ATCC-25157), *S.dysentri* (QCM-4714), *S.typhi* (NCTC-74). The activity of directethanolic fraction is efficient in lower concentrations because higher concentration consists of more steroid which reduces the antimicrobial activity. After the inoculation of MHB with organisms, the turbidity is adjusted by McFarland standard by added sterile normal saline in the culture broth and stirred with a glass rod. The same four organisms are used in the antimicrobial study of standard drug Ofloxacin because the comparison study of this extract concerning a standard antibiotic and the comparison results is seeing in above in the chart.

CONCLUSION

The direct-ethanol extract of *P.hysteroporus* have prominent Antimicrobial activity than other fraction at very low concentration (2mg/ml,1mg/ml) and its antimicrobial activity is maybe comparable if we compare the result with a standard antibiotic which have done in this project work, by taking ofloxacin. Conclude that after performing this total work that if reduce the concentration of the drug solution prepared from the extract of *P.hysteroporus*, the antimicrobial activity was increased as per the results of this work. So, concentration reduces as well as the percentage of steroids also reduces because presence high percentage of steroids reduces the antimicrobial activity.

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