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Antibacterial activity of crude extract of leaves of *Pterospermum acerifolium*

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ABSTRACT

Pterospermum acerifolium is one of the most popular medicinal plants in middle regions of India, commonly known as kanakchampa. Traditionally the leaves of the plant have been used as Haemostatic and for its wound healing properties, but to date there is no documented evidence corroborating its antimicrobial activity. A study was conducted to determine the antibacterial activities of *Pterospermum acerifolium*. Seven extract were obtained in extraction and fractionation process. And then carried out Phytochemical screening of hydroalchohalic extract. Antimicrobial activity carried by seven extract against pathogenic bacteria using the well diffusion and disc diffusion method. In this study antibacterial activity of extract of Leaves *Pterospermum acerifolium* was tested *Staphylococcus faecalis*(072), *Escherichia Coli, P. Vesicularis*(088), *S. Typhi* (109), *Aeromonas hydrophilia*(104), *Stphylococcus cohni(*121), *Serratia ficaria* (076) at the concentrations 0f 50mg/ml, 37.5mg/ml, 25mg/ml, 12.5mg/ml. Overall, the findings of this study indicate that the strongest inhibitions were obtained against *Pseudomonas vesicularis* for extract AE 1 and *Stphylococcus cohni* for extract BAE 1.

Key words: Pterospermum acerifolium, Methanol/Acetone extract, Antibacterial activity, Soxhletion, Maceration

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1. INTRODUCTION

Bacterial infections were some of the most serious global health issues of the present century. Numerous biologically active plants have been discovered by evaluation of ethno pharmacological data, and these plants may offer the local population immediately accessible therapeutic products (Ahmed & Beg, 2001). Several herbs were known to possess medicinal value including anti-microbial properties (Cowan, 1999). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen, et al. 1987), as well as in tannin (Saxena et al., 1994). *Pterospermum acerifolium* is used medicinally in at the Bhopal M.P. India. Different parts of these plants have been claimed to be effective in a wide spectrum of diseases. The present work is based on the effect of herbal medicine or natural products on different bacterial infection caused by different pathogenic bacteria. The present investigations were, therefore, proposed in evaluate the efficacy of the crude extract of leaves of *Pterospermum acerifolium* against pathogenic microbes.

2. METHOD & MATERIALS

2.1. Plant material

The leaves of *Pterospermum acerifolium were* collected from Bhopal M.P., India. A herbarium sheet was prepared. The leaves were dried in shade to avoid the deterioration of phytoconstituents and made into a coarse powder by using a grinder.

2.2. Preparation of leaves Extract of *Pterospermum acerifolium*

The powdered leaves of *Pterospermum acerifolium* were subjected to soxhlet extraction (Continuous Hot Extraction) using Methanol/Acetone and Methanol/Water as solvent and cold extraction (Maceration) using Petroleum ether as solvent.

2.3. Fractionation

Solvent-solvent fractionation (Cokatte et al., 2007) was performed for hydro alcoholic extract of Pterospermum acerifolium.

Procedure- Volume of Hydro alcoholic extract reduced to 100 ml. Acidified with sulphuric acid 0.5 molar (5 to 10ml). Add chloroform (25ml). Kept for 20 min. separated the layer: lower- Chloroform, upper- Aqueous. In aqueous layer added 25 ml chloroform. This procedure i.e. addition of chloroform repeated 3 times. Chloroform Evaporated (Obtain Sterpinoids, glycosides, phenolic compounds). Basified NH_4OH drop-wise (Maintained-pH- 10). Add 25 ml (Chloroform 75%, Methanol-25%) 3 times. Separated 2 layers (lower – Chloroform basic) (Upper- aqueous alkaloids).

2.4. Phytochemical Screening of extract

Phytochemical Screening (Khandelwall, 2009) was performed on methanol: acetone extract.

2.5. Bacterial Strains

The various organisms used in the present study include *Staphylococcus faecalis* (072), *Escherichia Coli, P. Vesicularis* (088), *S. Typhi* (109), *Aeromonas hydrophilia* (104), *Stphylococcus cohni* (121), *Serratia ficaria* (076) were collected from MPCST, Bhopal M.P. India. These organisms were maintained on nutrient agar slopes and the organisms were confirmed by biochemical test.

2.6. Antimicrobial Activity of Extracts

2.6.1. Well diffusion method

The agar well diffusion method technique (Bauer et al., 1966) was used to determine the antibacterial activity of the plant extracts. The test solution was prepared in Di methyl sulfoxide (DMSO).

Procedure - Inoculate the different culture on Nutrient agar plate. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labelled accordingly; 50mg/ml, 37.5mg/ml, 25mg/ml, 12.5mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48hours in upright condition. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimetres along two axis i.e. 90° to each other and the mean of the four reading were then calculated included 5mm well.

2.6.2. Disc diffusion method

The disc diffusion method (Bauer et al., 1966) was used to test antimicrobial activity of the extractives against 7 bacteria.

Procedure- Solutions of known concentration (µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (5mm diameter) were then impregnated with known amount of the test substances using micropipette and the residual solvent were completely evaporated. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30ug/disc) and blank discs (impregnated with solvent followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4˚C) for 24 h to allow maximum diffusion. These were a gradual change of test material concentration in the media surrounding the discs. The plates were then incubated at 37˚C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition included 5mm disc expressed in millimetre.

3. RESULTS

3.1. Extraction yield

leaves have higher extraction yield both for Hydroalchohalic and Alcohol+ Acetone extraction .Yield was not much more significant for PE extraction is 0.52%. The extraction yields were close for both methods that are soxhletion & maceration. The total % of yield for each extraction procedure is mentioned in Table 1. The total yield for different fraction of Hydro alcoholic extract was highest in BAE 1fraction and least in BCE fraction. The % yield for different fraction is mention in Table 2.

3.2. Qualitative Phytochemical Screening

Results shows that Alcoholic extract is having contain carbohydrate, Flavonoids, Alkaloids, oxalic acid, malic acid, Sulphate, chloride, Tannic and phenolic compound(Lead acetate solution, Dilute iodine solution).

3.3. Antibacterial Activity

All the extracts and fractions from *Pterospermum acerifolium* show antibacterial activity against all tested strains. Zone of inhibition were test for concentration ranging from 12.5mg/ml to 50mg/ml. (12.5mg/ml, 25mg/ml, 37.5mg/ml, 50mg/ml). Antibacterial activity tested for two methods such as well diffusion method and Disc diffusion method.

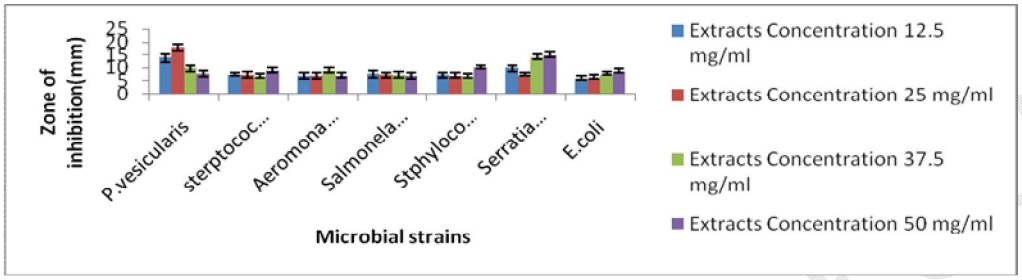
The strongest inhibitions were obtained against *Pseudomonas vesicularis* for extract AE 1 and *Stphylococcus cohni* for extract BAE 1. Extract 1 AE 1 was having highest zone of inhibition for *Pseudomonas vesicularis* (18.12mm) at 25mg/ml and for *Serratia ficaria* (15.02mm) at 50mg/ml (Graph 1). Extract 2 AE 2 was having highest zone of inhibition for *E.coli* (12.05mm) at 37.5mg/ml and *Pseudomonas vesicularis* (8.9mm) at 50mg/ml. Extract 3 PEE was highest zone of inhibition against *Pseudomonas vesicularis* (12.6mm) at 12.5mg/ml and for *E.coli* (12.05mm) at 37.5mg/ml. Extract 4 BAE 1 was having highest zone of inhibition for *Stphylococcus cohni* (17.02mm) at 37.5mg/ml and *Salmonela typhae* (11.35mm) at 50mg/ml (Graph 2). Extract 5 BAE2 was having highest zone of inhibition for *E.coli* (16.52mm) at 50mg/ml and *Pseudomonas vesicularis* (13.02mm) at 50mg/ml. Extract 6 BCE was having highest zone of inhibiton for *Salmonela typhae* (16.05mm) at 37.5mg/ml

Table 1
Total % of yield for each extraction procedure

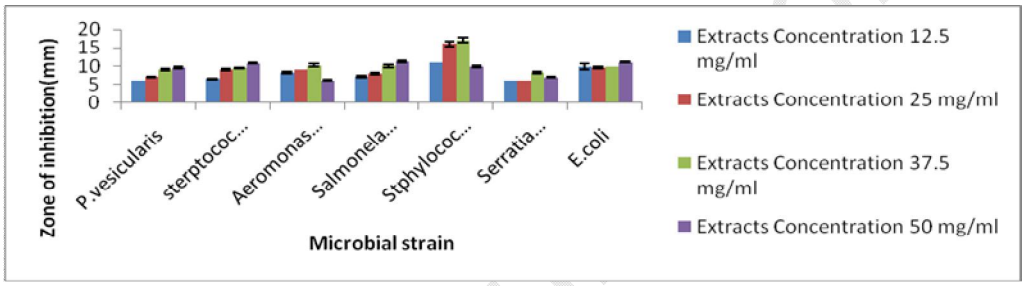
S.No.	Extraction Process	% yield
1.	Soxhletion(Alcohol+ Acetone) AE 1	0.87%
	Soxhletion(Alcohol+ Acetone) AE 2	0.73%
2.	Maceration(Petroleum ether) PEE	0.52%
3.	Soxhletion(Hydroalchohalic)	-

Table 2
% yield for different fraction

S.No.	Fractions	% yield
1.	Basified Aqueous Layer (BAE1)	0.55%
2.	Basified Aqueous Layer (BAE2)	0.51%
3.	Basified Chloroform layer (BCE)	0.37%
4.	Acidify Chloroform layer(ACE)	0.46%



Graph 1
The graph shows zone of inhibition Extract AE1



Graph 2
The graph shows zone of inhibition Extract BAE1

and for *Pseudomonas vesicularis* (15.97mm) at 50mg/ml. And the Extract 7 ACE was having highest zone of inhibition for *Streptococcus faecalis* (15.05mm) at 50mg/ml and for *E.coli* (14.92mm) at 50mg/ml.

4. DISCUSSION

Alkaloids have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoids, and diarrhea, and as antidote in heavy metal poisoning. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti inflammatory, antiallegic, antithrombitic and vasoprotective effects. Flavonoids are reported to possess antimicrobial activity (Finnermore, 1988). The observed antimicrobial activity against the tested organisms could be due to the presence of Alkaloids and Flavonoids in the extract as these have previously been reported to possess antimicrobial activities (Finnermore, 1988; Erach, 1996). These could explain the rationale for the use the plant in the treatment of the various conditions in traditional medical practice.

Pterospermum acerifolium is being used traditionally for treatment of inflammation, antiseptics, ulcer, and some fungal infection like candiasis. The antibacterial activity has been attributed to the presence of some active constituents in the extract. Study suggested that *Pterospermum acerifolium* was found to contain all the bioactive compounds screened namely, alkaloids, anthraxquinone and Flavonoids. There have been several reports on the natural occurring plant chemicals found in these plants. These include steroids, sap phenols, flavonols, xanthones and alkaloids (Rizvi & Tajwar, 1972).

The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Due to variable diffusability in agar medium, the antibacterial properties demonstrate to ZOI commensurate to its efficiency (Cheesbrough, 1993). The plant extracts exhibited varying degrees of activity against *P. vesicularis, E.coli, S. typae, Styplococcus cohni, Serratia ficaria, A. Hydrophilia*, and *Streptococcus faecillus.* The leaf alcoholic extracts was active against all the bacterial isolates and the water extract. According to (Thatal, 2008) *Pterospemum acerifollium* leaves show ZOI against *Styplococcus aureus. Shigella flexneri*, and *Bacillus licheniformis.* These data validated the traditional uses of this plant to as an antibacterial activity and phytochemical investigation show that Flavonoids & alkaloids are may be antimicrobial agent.

5. CONCLUSION

Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of Phytochemical which have inhibitory effects on all types of microorganisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal micro biota. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated. Also, alternative mechanisms of infection, prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened for currently. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles. *Pterospermum acerifolium* leaves possess antimicrobial activity. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper substitute for conventional drugs

since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or soxhletion. From the above results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. *Pseudomonas vesicularis* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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REFERENCE

1. Ahmed I, Beg AZ. Antimicrobial and Phytochemical studies on 45 Indian medicinal Plants against multi-drug resistant human pathogens, *J. Ethnopharmacol*, 2001, 74, 113-123
2. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. *J. Clin. Pathol*. 1966, 45, 493-496
3. Cheesbrough M. Medicinal laboratory manual for tropical countries, ELBS reprinted Edition, 1993
4. Cohen ML. Epidemiology of drug resistance: implications for a postantimicrobial era. *Science 1992,* 257, 1050-1055
5. Cokatte CK, Prruhit AP, Gokhale SB. Test Book of Pharmacognosis. 39th Edition. 2007, 107
6. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev,*1999, 12(4), 564-582
7. Ellof JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol*. 1998, 60, 1-6
8. Erah PO, Osuide GE, Omogbai EKI. Hypoglycaemic effect of the extract of *Solenostemon monostachys* (P. Beauv) leaves". *J. West Afr Pharm.* 1996, 10(2), 21-7
9. Finnermore H, Cooper JM, Stanley MB, Cobcroft JH, Harris LJ. *J. Soc. Chem. Ind.* 1988, 57, 162-9
10. Jansen AM, Cheffer JJC, Svendsen AB. Antimicrobial activity of essencial oils: a 1976-1986 literature review. Aspects of test methods. *Planta Med*. 1987, 40, 395-398
11. Khandelwall KR. Practical Book of Pharmacognosis. 2009, 19th Edition:149-156
12. Rizvi SAI, Tajwar S. Phytochemical studies of the flowers of *Pterospermum acerifolium. J.Phytochemistry.* 1971, 11(2), 856-858
13. Santos PRV, Oliveira ACX, Tomassini TCB. Controle microbiógico de produtos fitoterápicos. *Rev. Farm. Bioquím*. 1995, 31, 35-38
14. Saxena G, McCutcheon AR, Farmer S, Towers GHN, Hancock REW. Antimicrobial constituents of *Rhus glabra*. *J. Ethnopharmacol*. 1994, 42, 95-99
15. Thatal HN, Panda SK, Rath SK, Dutta SK. Antimicrobial Activity and Ethnomedicinal uses of some Medicinal plants from Similipal Biosphere Reserve. *Asian J. of Plant Sciences.* 2008, 7(3), 260-267