World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

In vitro studies of antibacterial and antioxidant activity of *Ipomoea pes-caprae* root extracts

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Received: 18-01-2015 / Revised: 27-02-2015 / Accepted: 28-02-2015

ABSTRACT

To evaluate the antibacterial and antioxidant activity of *Ipomoea pes-caprae* root (belonging to the family of *Convolvulaceae)* was studied in five different solvents. Antibacterial activity was determined using well diffusion assay for eight strains of bacteria. Minimum inhibitory concentrations were determined in eight bacterial strains by agar well diffusion assay. Other focuses included the determination of antioxidant activity using DPPH assay and IC_{50} (Inhibitory concentration) values were also determined using broth dilution assay. Preliminary phytochemical screening of the crude extracts revealed the presence of alkaloids, flavonoids, tannins, saponins and phenolics. The presence of these bioactive constituents is associated with the antibacterial activity of the plant. Methanol extracts of *I.pes-caprae* exhibited highest inhibition zone of 17mm, 18mm and 21mm against *Klebsiella pnemoniae, Escherichia coli, and Bacillus subtills*. During DPPH assay of methanol extract of *I.pes-caprae* root shows highest antioxidant activity of 92.04% in 200(µg/ml). The results confirm that *I.pes-capra* roots can be used as source of drugs to fight infections caused by susceptible bacteria.

Keywords*: I.pes-caprae*, phytochemical, antibacterial, bacterial strains, antioxidant, DPPH assay.

INTRODUCTION

Medicinal plants represent a rich source from which antimicrobial agents are obtained. They are a source of many potent and powerful drugs [1]. The use of medicinal plants to treat human diseases has its pre-historical roots. Medicinal plants are used by 80% of the world population as the only available source of medicines especially in developing countries [2]. Most part of the medicinal plants including leaves, roots, stems, flowers, fruits and twigs are used for extract as raw drugs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local uses, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [3]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronically infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites [4, 5]. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehydes and phenolic [6]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenolics, flavonoids, steroids, resins, and fatty acids, which are capable of producing definite physiological action [7].

Ipomoea pes-caprae (L.) R. Br. *Convolvulaceae* (morning glory Family). This specie is known as salsa-da-praia or batateira-da-praia in Brazil[8] and Railroad vine, bay hops or beach morning-glory in North America[9]. *Ipomoea pes-caprae* Linn is commonly used as a first aid to treat jelly-fish stings and in ritual baths to alleviate evil spirits [10]. The leaves were used against pain, inflammation, and rheumatism [11]. These species are used in different parts of the world for the treatment of several diseases, such as, diabetes, hypertension, dysentery, constipation, fatigue, arthritis, hydrocephaly, meningitis, and kidney ailments [12]. Some of these species showed antimicrobial, analgesic, spasmolitic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic and anticancer

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activities [13]. Alkaloids, phenolics compounds and glycolipids are the most common biologically active constituents from these plant extracts. This papers present preliminary phytochemical investigations of *I.pes-caprae*, which are responsible for the antibacterial and antioxidant activity of the extracts of roots on selected assay.

MATERIAL AND METHODS

Plant materials and Chemicals: The root part of *I.pes-caprae* was collected from sandy beaches of Kanyakumari, Tamilnadu, India, in the month of March 2012. This plant was identified and authenticated by Dr. S. Jeeva, Department of Botany, Scott Christian College (Autonomous), Kanyakumari, Tamilnadu, India. Voucher specimen of this plant was deposited at herbarium of this institute (voucher no.SCCN 3352). All chemicals and solvents were of analytical grade (RANKEM). The roots were washed and air dried over a period of one month. The dried samples were milled into fine powder by pounding manually with a clean sterile mortar, stored in sterile cellophane bags in a cool dry place till further use.

Extraction: 100 gram of root of *I.pes-caprae* was extracted in soxhlet sequentially in 1000ml of hexane, chloroform, ethyl acetate, methanol and water. The process was run for 24h after which the sample was concentrated using reduced pressure distillation under vacuum pump and freeze dried to powdered form. The dried extracts were weighed and kept in labeled sterile specimen bottles. A plant powder was extracted by increasing order of their solvent polarity (scheme 1).

Preliminary phytochemical investigations: The major secondary metabolites of tannins, saponins, terpenoids, flavonoids, alkaloids and glycosides were screened according to the common phytochemical methods [14].

Antimicrobial activity: Bacterial strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, and Chandigarh, India. Four Gram negative strains MTCC 443 (*Escherichia coli*), MTCC 109 (*Klebsiella pneumoniae*), MTCC 450 *(Schigella flexneri)*, MTCC 441 (*Proteus vulgaris*)**,** Four Gram positive strains MTCC 441 (*Bacillus subtilis*), MTCC 96 (*Staphylococcus aureus*), MTCC 1457 *(Clostridium perfringens)*, MTCC 1538 (*Micrococcus luteus*) were used in the present study as testing organisms for investigating antimicrobial activity.

Well diffusion assay (Eloff, 1998)[15]: Nutrient agar was prepared and poured in the sterile petri dishes and allowed to solidify. 24 h growing bacterial cultures were swabbed on it. Then, five wells (8mm diameter) were made by using a sterile cork borer. The four different concentrations (250µg, 500µg, 750µg and 1000µg) of the plant extracts were loaded in the wells. DMSO served as negative control. The plates were then incubated at 37ºC for 24h. After incubation the inhibition diameter was measured.

Antioxidant activity assays

DPPH assay: (2, 2-diphenyl-1-picrylhydrazyl): The Radical Scavenging Activity of different extracts was determined by using DPPH assay according to Chang *et al* (2008)[16] with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2.960 µl of 0.1mm ethanol DPPH solution mixed with 20 to 200µg/ml of plant extract and vortexes thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was used as references. Absorbance values were corrected for radicals decay using blank solutions. IC_{50} values have done by Broth dilution assay [17].

RESULTS

The crude extracts of *I.pes-caprae* root revealed the presence of tannins, saponins, flavonoids, alkaloids, glycosides and phenolics (Table 1). In the present study, the different solvents of *I.pes-caprae* roots were selected for antibacterial activity on eight – different organisms in five different solvents given in Table 2a and 2b. MIC test was done on selectively against eight organisms by agar diffusion assay in Table 3. The antioxidant activity of the methanol extract was found to be significant activity and is shown in Table 4a. IC_{50} values of the plant extracts are given in table 4b.

DISCUSSION

The presence of tannins, saponins, flavonoids, alkaloids, glycosides and phenolic compounds has potentially significant application against human pathogens, including those that cause enteric infections [18]. Phenols, tannins, quinones, saponins, steroids and flavanoids were present in the higher concentrations. Proteins and glycosodes were absent in methanol extract of *I.pes-caprae* roots. Alkaloids, aminoacids and terpenoids were also present in the methanol extract. The presences of alkaloids interesting as significant quantities are used as antimalarial, analgesics and stimulants [19]. The presences of glycosides moieties like saponins, glycosides and flavonoids are serve to protect against gastro-intestinal infections. Tannins are widely used in traditional medicine in treating wounds and arrest bleeding [20]. Some of these bioactive compounds which are synthesized as

secondary metabolites as the plant grows also serve to protect the plant against microbial attacks and predation by animals. Among the five different extracts, the methanol extract showed the highest antibacterial activity against *Bacillus subtilis* (21mm-1000ug) and *E.coli* (18mm-1000ug). These results clearly indicate, the sample at different concentration gave different inhibition activities towards tested organisms when compared with negative control. A Gram positive strains have most effective in all extracts than Gram negative bacteria. It is revealed the methanol extract shows highest activity than other. So, further work concentrated only in this extract. MIC test was done on selectively against eight organisms that were found susceptible in agar diffusion assay. MIC values also showed that extracts were able to inhibit the bacterial strains at lower concentrations. The lowest MIC for methanol extract was reduced against the six organisms. The MIC technique is used to evaluate the efficacies of antimicrobial agents. The antibacterial screening shows clearly that the methanol extract was more potent than other solvents. This antioxidant potential of root of *I.pes-caprae* could be attributed to the presence of flavonoids, alkaloids, quinones, terpenoids, and phenols. The antioxidant potential of these extracts possessed higher activity than standard. It shows remarkable antioxidant activity could be attributed to its different phytochemicals. It had done in ten different concentrations. The inhibitory percentage was increased with increased concentrations. It was evident that root of *I.pes- caprae* shows moderate antioxidant activity when compared to with standard antioxidant L-ascorbic acid whose antioxidant activity at different concentrations like 100 to 200µg were 80%, 82%, 85%, 90% and 92%. *I.pes-caprae* possesses considerably better antioxidant activities at all concentration. DPPH radical scavenging activity of *I.pes-caprae* was 92.04% (200µg) and 75.28 % (20µg) respectively. In vitro antioxidant activity of *I.pes-caprae* showed a radical scavenaging effects that increased with concentration. The IC_{50} values of the plant extracts, which were found to be, vary from 25.84 % to 73.55% for methanol extracts in Table 4b. The inhibitory concentrations are determined to use not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents. In the present investigation, the obtained data show that methanol extract are free radical scavengers and may act as primary antioxidants which can react with free radicals by donating hydrogen.

CONCLUSION

These results are used to evaluate the efficacies of antimicrobial agents in this plant. The result obtained in the anti-bacterial screening by agar diffusion showing clearly that the methanol extracts were more potent than either chloroform, hexane, ethyl acetate and water extract. In conclusion, all these extracts exhibited antimicrobial activities, though to a varied extent. Some extracts exhibit least activity to tested microorganisms (as the zone of inhibition is much less compared to others) under the conditions. The results of the present investigation also indicate that the solvents and extraction procedure may modify the final results to get maximum antimicrobial activity. From the results of antioxidant activity; it indicates that the methanol extract of *I.pes-caprae* root could be a potential source of natural antioxidant that they have great importance as therapeutic agents. In the current study, this plant is selected on their relevant ethno-medical use and they provide when their active constituents were extracted with different solvents. Further work is needed to isolate the active components from the plant extracts and to carry out pharmaceutical studies.

Table 1: Phytochemical screening of crude extracts of roots of *I.pes-caprae*

Phytochemical compounds	methanol extract
Alkaloids	+
Flavonoids	+
Phenols	++
Tannins	++
Glycosides	_
Reducing sugars	+
Proteins	_
Saponins	++
Quinones	++
Steroids	++
Amino acids	+
Terpenoids	+

+ → Present in minor amounts

++ →Present in moderate ; -→ not detected

Table 2a: Antibacterial activity of crude extracts of roots of *I.pes-caprae*

Name of the extract	Conc. of extract (µg)	Zone of Inhibition (mm)			
		Gram negative bacteria			
		E.coli	*K.p*	*P.v*	S.f
Hexane	250	-	12	-	-
	500	-	15	-	-
	750	-	16	-	-
	1000	-	17	-	-
Chloroform	250	-	-	-	-
	500	-	-	-	-
	750	-	-	-	-
	1000	-	-	-	-
Ethylacetate	250	-	-	-	-
	500	-	-	-	-
	750	-	-	-	-
	1000	-	-	-	-
Methanol	250	12	10	11	10
	500	14	12	13	12
	750	16	14	14	14
	1000	18	16	16	15
Water	250	-	-	-	-
	500	-	-	-	11
	750	-	14	-	12
	1000	-	16	-	13

Table 2b: Antibacterial activity of crude extracts of roots of *I.pes-caprae*

Name of the extract	Conc. of extract (µg)	Zone of Inhibition (mm)			
		Gram positive bacteria			
		M.l	*B.s*	*S.a*	*C.p*
Hexane	250	10	-	-	10
	500	11	14	-	11
	750	1	16	-	12
	1000	13	18	11	13
Chloroform	250	-	10	-	-
	500	-	11	-	-
	750	-	13	-	-
	1000	-	15	-	-
Ethylacetate	250	-	-	-	-
	500	10	-	-	10
	750	13	10	-	12
	1000	15	11	-	13
Methanol	250	11	13	10	11
	500	13	15	12	13
	750	14	18	14	14
	1000	15	21	15	15
Water	250	-	-	-	-
	500	-	-	11	-
	750	-	10	13	-
	1000	-	11	14	-

Table 3: Minimum inhibitory concentration of *I.pes-caprae*

	Minimum Inhibitory Concentration (mg/ml)				
Name of the Organism	Water	Methanol	Ethyl acetate	Chloroform	Hexane
E.coli	-	0.25	-	-	-
K.p	-	0.25	-	-	0.25
P.v	-	0.25	-	-	-
M.l	-	0.25	0.5	-	0.25
B.s	-	0.25	-	0.5	0.25
S.a	0.5	0.25	-	-	-
S.f	0.5	0.25	-	-	-
C.p	-	0.25	0.5	-	0.25

Table 4a: Radical Scavenging Activity (RSA) of the methanol root extract

Concentration (µg/ml)	**RSA (%)**	
	Methanol extract	Standard
20	75.28	18.54
40	78.43	35.28
60	81.59	47.76
80	84.68	50.19
100	86.17	55.04
120	88.34	59.06
140	89.96	66.37
160	90.15	72.80
180	91.66	74.46
200	92.04	84.51

Table 4b: IC_{50} Determination – broth dilution Assay

Concentration (µg)	**Inhibition (%)**
	Methanol extract
100	25.84
200	31.13
300	39.17
400	43.52
500	49.30
600	**52.45**
700	59.45
800	63.44
900	68.08
1000	73.55

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