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Original Article

ANTIBACTERIAL AND PHYTOCHEMICAL ASSESSMENT ON VARIOUS EXTRACTS OF *IPOMOEA PES-CAPRAE* (L.) R. BRTHROUGH FTIRAND GC- MS SPECTROSCOPIC ANALYSIS

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ABSTRACT

Objective: *Ipomoea pes-caprae (L.) R.Br* (IP) is a valuable medicinal plant, distributed in the tropics and subtropics regions.The present investigation was carried out to determine the antimicrobials as well as possible chemical components in IPby FTIR and GC-MS technique. **Methods:** The different solvent extracts (hexane, di-chloromethane, ethyl acetate and methanol) of the plant were tested for antibacterial activity against human pathogens. FTIR method was used to detect the characteristic peak values and their functional groups.GC-MS technique was used in this study to identify the components present in the extract.

Results: The results highlighted that the methanol extracts exhibited remarkable antibacterial activity compared to other extracts against four out of the five human pathogens. FTIR method showed the specific peak for phenol, ester, alcohol etc. A total of nineteen biological compounds were isolated from the leaves of IP by GC-MS analysis among whichstigmasterol, 1-(+)-ascorbic acid, 2-6-dihexadecanoate and Phytol (3, 7, 11, 15-tetramethylhexadec-2-en-1-ol) are the major compounds.

Conclusion: The study encourages this plant useas an alternative medicine for the treatment of diseases.

Keywords: *Ipomoea Pes-caprae,* phytochemicals, antibacterial, GC-MS, FTIR

INTRODUCTION

Pathogenic bacteria can invade in the body through inhalation into nose and lungs, ingestion in food or through sexual contact. General symptoms of bacterial diseases include fever, chills, headache, nausea and vomiting. Commonly occurring pathogenic bacteria are *Salmonella typhi, Klebsiellapneumoniae, Escherichia coli*, *Listeria monocytogene, Vibrio parahemlyticus, Proteus mirabilis*[1].

The use of medicinal plants is very important for our health. All drugs of the past were extracted from medicinal plants [2]. The medicinal plants have been screened for their antimicrobial activities. Medicinal plants were used in traditional medicines to treat infectious diseases. It contains new bioactive secondary metabolites [3]. The phenolic compounds showed antibacterial and antiphytoviral activities [4]. The increased quantity of phenolic in Chilli may be attributed to resistance to viral infection [5].Phenolics inhibit diseases development through inhibition of exteracellular enzymes and antioxidant activity in plant tissue [6].

Ipomoeapes-caprae(L.) R. Br(Convolvulacea) is a valuable medicinal plant, distributed in the tropics and subtropics regions and uses in folk and tribal medicines. *Ipomoea pes-caprae* is a pan tropical, trailing vine that routinely colonizes on sand dunes. It grows just above the high tide line along coastal beaches, forming large mats that assist in stabilizing sands. This is an evergreen perennial with a large, thick root that can be 10 ft long and 2 inch in diameter. The entire plant is glabrous and somewhat fleshy. The stem runs along the ground rooting at the nodes with only the flowers being erect [7, 8]. *I.pes-caprae*has the potential in scavenging free radicals and can be a vital source of antioxidant phytochemicals [9] and goodantinociceptive property due to the presences of compounds, such as glochidone, betulinic acid, alphaand beta-amyrin acetate, isoquercitrin in the writhing test and formalin test in mice, and to treat dolorous processes [10]. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves have the astringent, diuretic and laxative properties. It has biological activity like antioxidant, analgesic and antiinflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic [11]. It is also used in inhibition of platelet aggregation, diarrhea, vomiting, and piles [12].The present investigation was deals with the identification of bioactive compounds and to screen the antibacterial assay of *Ipomoea pes-caprae.*

MATERIALS AND METHODS

Plant material and Collection

Fresh leaves of *Ipomoea pes-caprae*were collected from Parangipettai costal area near Annankovil landing centre during January, 2014. The collected specimens were identified based on the manual by Kathiresan [13]. Withered leaves of *Ipomoea pes-caprae*were rinsed under running tap water to eliminate dust. After that samples were washed several times with distilled water and air-dried at 25-30^{0}C for about 3-5 days. The dried samples were ground to fine powder using mortar and pestle. The powder was passed through a sieve of 22 mm mesh size. The powder sample was kept in a clean, dried, air tight amber glass container to protect it from sunlight.

Preparation of extracts

A100 gram of ground *Ipomoea pes-caprae* was extracted using three fold volumes of solvents of different polarity in order of increasing hydrophilic property (i.e. hexane, dichloromethane (DCM), ethyl acetate and methanol respectively) for 48 h on an orbital shaker to make the extracts [14, 15].This procedure was repeated for two more times. Finally, the extracts were concentrated using a rota-evaporator (IKA- RV 10, USA) at a reduced pressure at <40°C. The resulting extracts were then dissolved in dimethylsulfoxide (DMSO) and kept at 4^{0}C until further use.

Antibacterial activity

Test microorganisms used

The inhibitory effects of extracts were carried out on five species of human pathogenic bacteria as following

S. No.	Bacterial strains name	Type of bacteria	Causing disease
1.	*Escherichia coli*	Gram negative	Urinary track, Infection, Pneumonia, Toxic shock
2.	*Salmonella typhi*	Gram negative	Typhoid /Enteric fever
3.	*Klebsiellapneumoniae*	Gram negative	Diabetes, alcoholism, malignancy, liver disease etc.
4.	*Vibrio parahemlyticus*	Gram negative	Wound infections
5.	*Proteus mirabilis*	Gram negative	Urine more alkaline, kidney stones

Antibacterial activity test

The crude extracts were dissolved in the corresponding solvent for the antibacterial activity test. Antibacterial activity was assayed using a paper disc diffusion assay.

Paper disc diffusion assay

Nutrient Agar Medium (Himedia, M001-500G) was prepared and sterilized by autoclaving at 121˚C or 15 lbs pressure for 15 minutes. 20 ml of the sterilized media was poured into a sterilized petri dish and allowed to solidify at room temperature in UV light. 50 mg of each extract was dissolved in 1 ml of corresponding solvent and 5 mg was applied to sterile filter paper discs (6mm). Absorption of extracts per paper disc was 20µl. The discs were placed on to the agar plates inoculated with an 18 hour culture of the test pathogen (10^6 bacteria/ml) in nutrient broth. A disc load with a commercial antibiotic, such ampicillin was prepared as a positive control, and a disc load with only corresponding solvent was similarly prepared as a negative control. The plates were incubated for 24 hours at 37˚c.

The zone of inhibition of bacteria around the disc was measured and the assay was scored positive (+) if the zone was< 2 mm, doubly positive (++) if ≥ 2 mm, triple positive (+++) if ≥ 7 mm, and negative (–) if no zone was visible.

Fourier transform infrared spectrophotometer (FT-IR) spectroscopy

Fourier transform infrared spectrophotometer analysis was used to predict functional groups present in a molecule based on their frequencies of vibration between bonds of the atoms. All crude extracts (10 mg/ml) were characterized using Fourier transform infrared spectrophotometer (FT-IR; IR Affinity-1, Shimadzu, Tokyo, Japan) for FT-IR spectra measurement in the frequency range of 400 to 4,000 cm^{-1}.

GC-MS Analysis

GC-MS technique was used in this study to identify the components present in the extract. GC-MS technique was carried out at VIT University, Vellore, Tamil Nadu. GC-MS analysis of this extract was performed using a Perkin Elmer GC Claurus680 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-5MS column (30.0m, 0.25mmID, 250µm df). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 1µl was employed (Split ratio of 10:1). Injector temperature was 250ºC. The oven temperature was programmed from Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 50 to 600 Da. Total GC running time was 32 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbomass Ver5.4.2.

Identification of Components

Interpretation of mass spectrum GC-MS was conducted using data base of National Institute Standard and Technology (NIST) and Wiley spectra Libraries. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST and Wiley spectra Libraries were recorded.

RESULTS AND DISCUSSION

As a wide range of extract holds a better chance for the extraction and isolation of biologically active molecules for general screening of bioactivity [16], four different solvents (with different polarity) were used in the present study.

Antibacterial activity

Four extracts from of *Ipomoea pes-caprae*, were tested for antibacterial activity against the five human pathogens. The antibacterial activity of extracts of the *Ipomoea pes-caprae*is given in the table 1. The methanolic extracts of the *I.pescaprae* showed a strong inhibition in the growth of tested bacteria. The maximum zone of inhibition was observed against *Klebsiellapneumoniae* and minimum was in *Proteus mirabilis.* The extracts were ineffective against the *Salmonella typhi.*

Table 1.Antimicrobial activity of *Ipomoea pes-caprae*against human

S. No.	Name of the human pathogen	Zone of inhibition (mm)			
		Hexane	DCM	Ethyl acetate	Methanol
1.	*Escherichia coli*	-	-	-	25.6
2.	*Salmonella typhi*	-	-	-	-
3.	*Klebsiellapneumoniae*	-	-	-	29.6
4.	*Vibrio parahemlyticus*	-	-	-	28.3
5.	*Proteus mirabilis*	-	-	-	19.6

The discs were impregnated with methanolic extracts showed promising inhibitions zones. Similarother studies also reported that methanolic extracts exhibit stronger antibacterial activity [17, 18, 19, 20].

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines, halogen and phenolic compound (Figure 1, and table-2).

Table 2: FTIR peak values and functional groups of different extracts of *Ipomoea*

(a). Hexane

S.No.	Peak values	Functional Groups	S.No.	Peak values	Functional Groups
1.	518.85	Alkyl halide	16.	1963.53	Unknown
2.	617.22	Alkyl halide	17.	2065.76	Unknown
3.	667.37	Alkyl halide	18.	2250.93	Noncnjugated, Alkyne
4.	713.66	Alkyl halide, Alkene	19.	2347.37	Unknown

5.	831.32	Alkene	20.	2860.43	CH2, Alkane
6.	920.05	Alkene	21.	2924.09	Alkane
7.	1062.78	Alcohol, Alkyl halide	22.	3406.29	Amide, Alcohol
8.	1139.93	Alcohol, Alkyl halide, Amine, Ether	23.	3720.69	Unknown
9.	1257.59	Amine, Ether	24.	3772.76	Unknown
10.	1384.89	Alkyl halide	25.	3790.12	Unknown
11.	1452.40	CH2, Alkane, Aromatic, Nitro	26.	3803.63	Unknown
12.	1519.91	Aromatic, Nitro	27.	3838.34	Unknown
13.	1627.92	Nonconjugated, Alkene, Amide	28.	3874.99	Unknown
14.	1726.29	Nonconjugated, Carbonyl	29.	3892.35	Unknown
15.	1911.46	Unknown	30.	3938.64	Unknown

(b). Dichloromethane

S.No.	Peak values	Functional Groups	S.No.	Peak values	Functional Groups
1.	522.71	Alkyl halide	18.	1610.56	Conjugated
2.	567.07	Alkyl halide	19.	1635.64	Alkene, Amide
3.	619.15	Alkyl halide	20.	1730.15	Carbonyl
4.	678.94	Alkyl halide, Alkene	21.	2065.76	Unknown
5.	723.31	Alkyl halide, Alkene	22.	2333.87	Unknown
6.	810.1	Alkyl halide, Alkene	23.	2358.94	Unknown
7.	837.11	Alkene	24.	2735.06	Acid
8.	916.19	Alkene	25.	2852.72	Acid, Alkane
9.	987.55	Alkene	26	2922.16	Acid, Alkane
10.	1064.71	Alkyl halide, Ether	27.	3402.43	Amine
11.	1138	Alcohol, Alkyl halide, Ether	28.	3724.54	Unknown
12.	1166.93	Alkyl halide, Ether	29.	3768.91	Unknown
13.	1259.52	Alkyl halide, Ether	30.	3788.19	Unknown
14.	1379.1	Alkyl halide, Alkane, Nitro	31.	3869.2	Unknown

(c). Ethyl acetate

S.No.	Peak values	Functional Groups	S.No.	Peak values	Functional Groups
1.	472.56	Unknown	17.	1631.78	Nonconjugated, Amide
2.	507.28	Alkyl halide	18.	1726.29	Carbonyl, Conjugated, Nonconjugated
3.	615.29	Alkyl halide	19.	1859.38	Nonconjugated
4.	721.38	Alkyl halide, Alkene	20.	2069.62	Unknown
5.	792.74	Alkyl halide, Alkene	21.	2266.36	Unknown
6.	833.25	Alkene	22.	2231.94	Unknown
7.	920.05	Alkene	23.	2360.87	Unknown
8.	987.55	Alkene	24.	2852.72	Acid, CH2
9.	1060.85	Ether, Alkyl halide	25.	2922.16	Acid, CH2
10.	1132.21	Amine, Ether, Alkyl halide, Alcohol, Ester	26.	3431.36	Free NH, Amide
11.	1163.08	Alkyl halide, Ether, Ester	27.	3766.98	Unknown
12.	1261.45	Ether, Acid, Ester, Alkyl halide	28.	3840.27	
13.	1330.88	Alkyl halide, Amine	29.	3855.7	Unknown
14.	1381.03	CH3, Alkyl halide, Alkane	30.	3871.13	
15.	1460.11	Aromatic, Alkane, CH3	31.	3890.42	Unknown
16.	1514.12	Aromatic	32.	3919.35	Unknown

(d). Methanol

S.No.	Peak values	Functional Groups	S.No.	Peak values	Functional Groups
1.	518.85	Alkyl halide	15.	1726.29	Carbonyl
2.	567.07	Alkyl halide	16.	2065.76	Unknown
3.	615.29	Alkyl halide	17.	2355.08	Unknown
4.	715.59	Alkyl halide	18.	2856.58	Alkane
5.	813.96	Alkene	19.	2924.09	Alkane
6.	918.12	Alkene	20.	3390.86	Phenol
7.	1060.85	Alcohol, Alkyl halide	21.	3788.19	Unknown
8.	1126.43	Alcohol, Alkyl halide, Amine	22.	3852.56	Unknown
9.	1163.08	Ester, Ether, Alkyl halide	23.	3892.35	Unknown
10.	1261.45	Alkyl halide, Amine, Ester, Ether, Acid	24.	3925.14	Unknown
11.	1394.53	Alkane, CH3	25.	3934.78	Unknown
12.	1454.33	Alkane, Aromatic	26.	3967.57	Unknown
13.	1517.98	Aromatic, Nitro	27.	3983.01	Unknown
14.	1631.78	Amide, Alkene			

GC-MS Analysis

The compounds present in the methanolic extract of *Ipomoea pes-capraewere* identified by GC-MS analysis (Figure 2). The active principle Molecular Weight (MW), concentration (%), molecular Formula (MF), and retention time (RT) is presented in Table 3. Nineteen compounds were identified in the extract. Same numbers of phytochemicals were registered in methanolic extract of *Tageteserecta*L. leaves which has high therapeutic value in the field of medicine [21].

Table 3. Components identified in *Ipomoea pes-capraeplant extract* (GC- MS

S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	15.934	1H-3A,7-METHANOAZULENE-6-METHANOL, 2,3,4,7,8,8A-HEXAHYDRO-3,8,8-TRIMETHYL-,	$C_{15}H_{24}O$	220	0.808
2.	16.794	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	$C_{37}H_{70}O$	530	1.601
3.	18.300	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	$C_{38}H_{68}O_8$	652	6.775
4.	19.470	PHYTOL	$C_{20}H_{40}O$	296	3.269
5.	20.000	ETHYL 9.CIS.,11.TRANS.-OCTADECADIENOATE	$C_{20}H_{36}O_2$	308	4.286
6.	20.080	3-METHYL-2-(2-OXOPROPYL)FURAN	$C_8H_{10}O_2$	138	3.542
7.	21.056	CIS-1-CHLORO-9-OCTADECENE	$C_{18}H_{35}Cl$	286	0.896
8.	22.141	PHOSPHINE, CYCLOHEXYL(1,1-DIMETHYLETHYL)-	$C_{10}H_{21}P$	172	1.366
9.	26.583	1,3,3-TRIMETHYL-2-HYDROXYMETHYL-3,3-DIMETHYL-4-(3-METHYLBUT-2-ENYL)-CYCLOHEXENE	$C_{15}H_{26}O$	222	1.032
10.	26.778	1-HEPTADECEN-7,10-DIONE	$C_{17}H_{30}O_2$	266	1.939
11.	27.078	1,2-CYCLOHEXANEDICARBOXYLIC ACID, FURFURYL PENTADECYL ESTER	$C_{28}H_{50}O_5$	466	15.815
12.	27.533	4-NITROPHENYL LAURATE	$C_{18}H_{27}O_4N$	321	35.338
13.	27.613	OCTADECANOIC ACID, 1-[[(1-OXOHEXADECYL)OXY]METHYL]-1,2-ETHANEDIYL ESTER	$C_{55}H_{106}O_6$	862	3.427
14.	28.429	EICOSANOIC ACID, 2-[(1-OXOHEXADECYL)OXY]-1-[[(1-OXOHEXADECYL)OXY]METHYL]ETHYL ESTER	$C_{55}H_{106}O_6$	862	3.916
15.	28.659	STIGMASTEROL	$C_{29}H_{48}O$	412	1.346
16.	29.299	STIGMASTEROL	$C_{29}H_{48}O$	412	5.380
17.	29.799	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-OCTADECAHYDRO-2	$C_{30}H_{48}O$	424	1.702
18.	30.409	URS-12-EN-24-OIC ACID, 3-OXO-, METHYL ESTER, (+)-	$C_{31}H_{48}O_3$	468	3.156
19.	31.495	FERN-7-EN-3.BETA.-OL	$C_{30}H_{50}O$	426	2.519

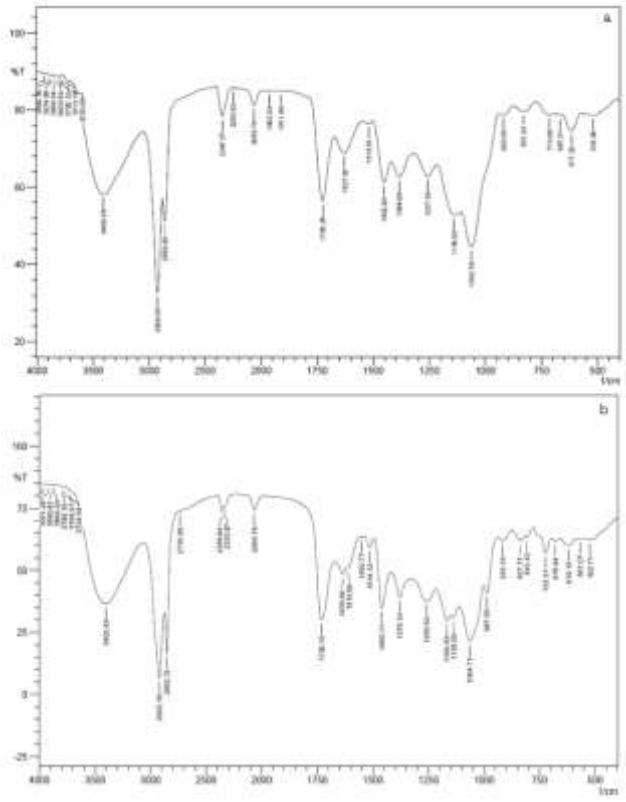

Fig.1: FTIR Spectrum of a. hexane, b. dichloromethane, c. ethyl acetate and d. methanol of *Ipomoea pes-caprae*

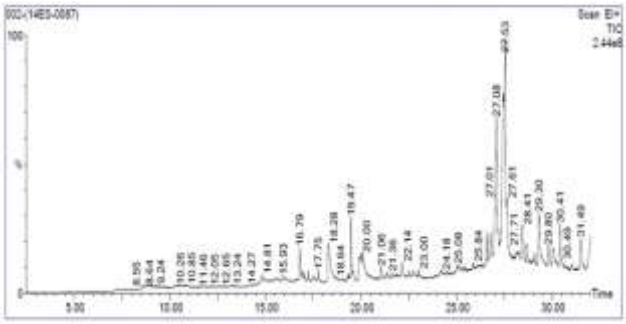

Fig.2: GC-MS pattern of Phytoconstituents obtained from *Ipomoea*

Among the nineteen compounds identified after GC-MS, one of compounds Stigmasterol is found to possess anticervical cancer property (NIST, 2005). Other compound 1-(+)-ascorbic acid, 2-6-dihexadecanoate which is a derivative of ascorbic acid, vitamin C, is present in the essential oil. Vitamin C is an antioxidant and belongs to the class of compounds identified to enhance sperm quality and prevent sperm agglutination, thus making them more motile with forward progression and hence promote male fertility[22, 23].Phytol (3, 7, 11, 15-tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols[24, 25]was also identified which is the product of chlorophyll metabolism in plants. It is known to inhibit the growth of *Staphylococcus aureus*[26].

CONCLUSION

The present study has been found useful in the identification of several constituents present in the methanolic extract of the leaves of *Ipomoea pes-caprae*(IP). The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. The present work also explored the

potential antibacterial effect of methanolic extracts from the leaves of IP.FT-IR and GC-MSrevealed that IP extracts constitute a wide range of bioactive phytochemicals with high therapeutic values. The phytochemicals have several activities such as antioxidant, analgesic and antiinflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic.Further investigation on these phytochemicals will pave a way for the synthesis of cost effective drug with less side effect.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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