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RESEARCH ARTICLE

PHYTOCHEMICAL EVALUATION OF LEAF AND STEM OF IPOMOEA PES-CAPRAE (L) R. BR.

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Abstract

The present study was to investigate various medicinal value phytochemicals in leaf and stem portion of *Ipomoea pes-caprae* were determined by various physic chemical characteristics like moisture content, total ash content acid insoluble ash content, water insoluble ash content and solvent extractive values. The qualitative phytochemical screening and quantitative analysis by GC-MSD with nonpolar solvent n-hexane, suggested the presence of alkaloid, sugar, glycoside, saponins, steroids, terpenoids and flavonoids. The study provides evidence that the leaf and stem has potential source of antimicrobial, anti-inflammatory, antioxidant and cytotoxic agents.

Introduction: -

Ipomoea pes-caprae (Convolvulacea) is a valuable medicinal plant, distributed in the tropics and subtropics regions and uses in folk and tribal medicines. *I. pes-caprae* (L.) R. Br.-bayhops 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 inches 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. [1,2] *I. pes-caprae* has the potential in scavenging free radicals and can be a vital source of antioxidant phytochemicals [3]and good antinociceptive property due to the presences of compounds, such as glochidone, betulinic acid, alpha and beta-amyrin acetate, isoquercitrin in the writhing test and formalin test in mice, and to treat dolorous processes. [4]. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves has the astringent, diuretic and laxative properties. It has biological activity like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic [5]. It is also used in inhibition of platelet aggregation, diarrhea, vomiting, and piles [6]. The antioxidant compounds in a typical diet are mostly derived from plant sources and polyphenolic components of higher plants act as antioxidant or other mechanisms contributing to anti-carcinogenic action [7].

The therapeutic ability of the plants is not part specific. It varies from plant to plant but all parts are endowed with medicinal properties. It may be root, rhizome, stem, flower, leaf, fruit or seed. Different parts of the plant show different biological activities. Hence it is very essential and important to perform pharmacognostic studies of medicinal plants [8]. Before the plant can be taken up as drug alone or in formulation with other compounds, it is of utmost importance to lay down standardization parameters which will enable to maintain the authenticity and quality of the drug and prevent it from being adulterated and/ or substituted. Pharmacognostic studies of different parts is reported for many medicinal plants. Some of the examples are pharmacognostic studies are *Tephrosiapurpurea* root [9]; *Ferulasumbul* root [10]; rhizome of *Smilax domingensis* [11]; *Mangiferaindica* leaf [12]*; DiplaziumEsculentum*

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leaf [13]; *Cissusquadrangularis* stem [14]; *Argyreiapilosa* stem [15]; fruit of *Helicteresisora* [16]; flowers of *Woodfordiafruticosa* [17] and *Aervalanata* [18].

The objective of the present study is to evaluate and screening of various phytochemical and physicochemical parameters of leaves and stems of *I. pes-caprae* like moisture content, ash contents, extractive values, preliminary qualitative and quantitative phytochemical finger print analysis by GC-MSD Chromatography.

Materials And Methods: -

Plant collection

The fresh leaves and stems of *I. pes-caprae* were collected from the sandy beaches of Mannakudi coastal area, Kanyakumari district (8°5'41"N 77°29'7"E), Tamilnadu, India, in the month of September 2017. These plants were identified using standard keys and voucher specimen of these plants was deposited at herbarium of VOC College, Tuticorin (voucher no. SCCN: 3352 and 3353). The leaves and stems were separated, washed thoroughly with water, shade dried and homogenized to fine powder and stored in closed container for further studies.

Pharmacognostic studies

Physicochemical analysis

The physicochemical parameters like moisture content, total ash content, acid-insoluble ash, water-soluble ash, and extractive values were determined as per WHO guidelines in dried powder of leaves and stem parts.

Determination of Moisture Content

Ten grams of plant parts were weighed separately in pre-weighed glass petri dishes and dried in hot air oven (80-95^{o}C) for 3 hours. The dishes were then transferred to desiccator, cooled to room temperature and recorded the weight. The drying was continued till the final weight became stable. The weight differences were noted and the moisture content was expressed in percentage weight of fresh plant parts.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight} \times 100}{\text{Initial weight}}$$

Determination of Total Ash Content

Two grams of air dried powder taken in a silica crucible and ignited gradually up to 500-600^{o}C until it was white indicating the absence of carbon, allowed to cool and weighed to determine the percentage of ash with reference to air-dried respective samples.

Determination of Acid Insoluble Ash Content

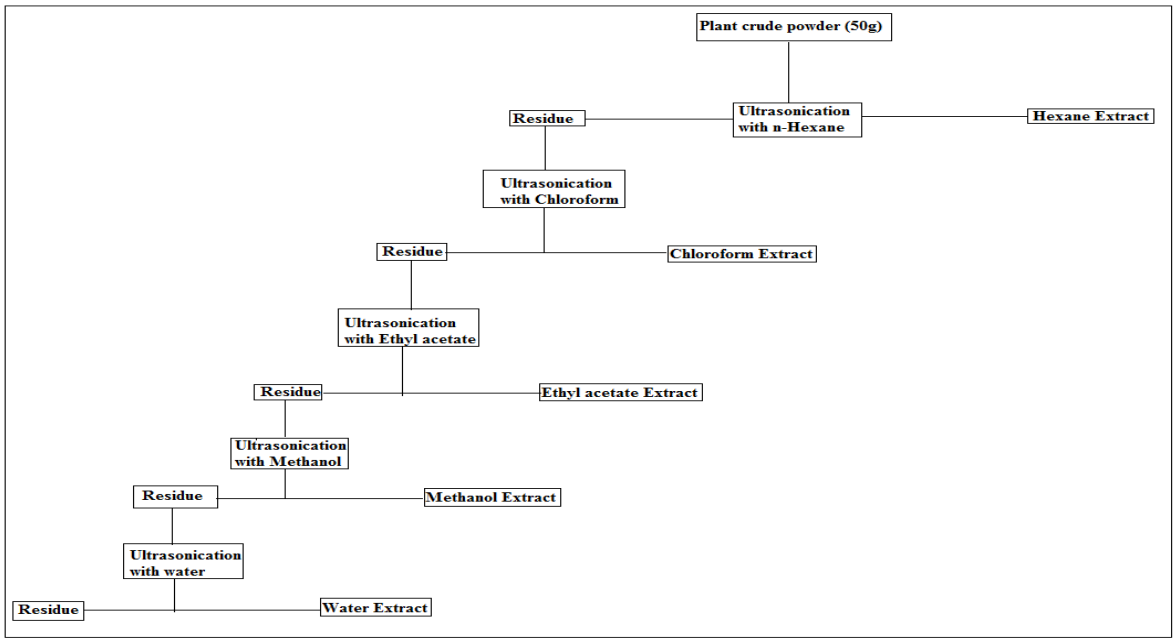
The ash was digested with dilute HCL for 5 minutes and insoluble matter was collected in a sintered glass crucible washed, ignited, and cooled finally it was weighed to estimate the percentage of acid-insoluble ash with reference to the bone dried material.

Determination of Water Insoluble Ash Content

The ash was boiled with water for 5 minutes and insoluble ash was collected in a sintered glass crucible washed ignited at a temperature not exceeding 450^{o}C. Cool and weighed for the determination of water soluble ash with reference to the bone dried drug.

Determination of Solvent Extractive Values

The plants were washed and air dried over a period of one month. The dried samples were milled into a fine powder by pounding manually with a clean, sterile mortar, stored in sterile cellophane bags in a cool dry place till further use. 50 g of leaves and stem parts of the plant was extracted in a ultrasonication at 150 hertz for 1.0 hour at 60^{0}C sequentially with 250 ml of hexane, ethyl acetate, methanol and water. After which the sample was filtered using Whatman filter paper and 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. The schematically representation of the plant extraction is presented in scheme 1.



Scheme 1:-Extraction of plant crude powder by increasing order of solvent polarity

Qualitative phytochemical Analysis

Test for alkaloids by Mayer's test

Solvent free extract (50 mg) was stirred with 2 ml of dilute hydrochloric acid (1 ml HCL + 1 ml water) and filtered. The filtrate was tested carefully with various alkaloid reagents. To 2 ml of filtrate, a drop or two of Meyers's reagent was added by the sides of the test tube. A white creamy precipitate indicated the presence of alkaloids**.**

Test for glycosides by Bontrager's test

Fifty milligrams of extract were hydrolyzed with 5 ml of concentrated hydrochloric acid for two hours on a water bath and filtered. To 2 ml of filtrate hydroxylate, 3 ml of chloroform was added and shaken. The chloroform layer was separated and 10 % ammonia solution was added to it. Appearance of a pink, red or violet color in the ammonia layer indicated the presence of glycosides.

Test for phenolic compounds by ferric chloride test

The extract 50 mg was dissolved in 1 ml of distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green color indicated the presence of phenol.

Test for flavonoids by sodium hydroxide test

0.5 g of extract was dissolved in 5 ml of distilled water and filtered. To 2 ml of filtrate a little quantity of the each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produces a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

Test for tannins by neutral ferric chloride

0.5 g of the extract was boiled in 10 ml of water in test tube and then filtered. A few drops of 1 % ferric chloride was added and observed. Blue-green, green or brownish green precipitate indicates the presence of tannins.

Test for reducing sugars by Fehling's test

The extract 100 mg was dissolved in 5 ml of water and filtered. 1 ml of filtrate was boiled on water bath with 1 ml each of Fehling's solution I and II. A red precipitate indicates the presence of sugars.

Test for saponin by foam test

The extract 50 mg was diluted with 5 ml of distilled water. The suspension was shaken in a graduated cylinder for 15 mins. A 2-cm layer thick of foam indicates the presence of saponin.

Test for proteins by biuret test

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through Whatman (No.1) filter paper and the filtrate was collected. To 2 ml of filtrate, one drop 2 % of copper sulphate solution and 1ml of ethanol (95 %) was added, followed by excess of potassium hydroxide pellets (1pellet). Pink color in the ethanolic layer indicates the presence of proteins.

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow color precipitate.

Test for Terpenoids

Liebermann – Burchard test: Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green color.

Test for Steroids

Liebermann – Burchard test: Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red color.

Test for Amino acids

Biuret test- Equal volume of 5 % sodium was added. Appearance of pink or purple color shows the presence of free amino acids.

Quantitative determinations by GC-MSD

Required quantity of powder was weighed and transferred to scot Duran bottle (50.0 ml), treated with n-hexane until the powder was fully immersed. The bottle was placed in ultrasonication for 1 hour at 80^{o}C at frequency of 20 MHz Then the extract was filtered through Whatman (No.1) filter paper and evaporated to dryness by using a roto evaporator. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a fused silica capillary column (30 mm X 0.25 mm ID X 0.25 µ, composed of 5% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 1 µl was employed (split ratio of 10: 1); Injector temperature 290^{o}C; Ion-source temperature 230^{o}C. The oven temperature was programmed from 50^{o}C (isothermal for 1min.), with an increase of 30^{o}C/min, to 180^{o}C, then 15^{o}C/min to 260^{o}C (isothermal for 3 min.), with an increase of 25 ^{o}C/min to 270^{o}C (isothermal for 4 min.), ending with an increase of 10 ^{o}C/min to 300^{o}C, 5-min isothermal at 300^{o}C.Mass spectra were taken at 70 ev; a scan interval of 0.5 seconds and fragments from 45 to 550 Da. Total GC running time was 26 minutes. The relative % 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 Turbo mass.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown compound was compared with the spectrum of the known compound stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion:-

Physicochemical analysis

The result of quantitative determinations such as moisture content, total ash, acid insoluble ash, and water insoluble ash in stem and leaves of *I. pes-caprae* are presented in Table 1 and Figure 1. Moisture contents of the leaf parts of

I. pes-caprae contain 48 % and stem was found to be 55 %. The moisture content of the drug was not too high; thus, it could discourage bacteria, fungi or yeast growth. Another equally important parameter in the evaluation of crude drugs is the ash value and acid insoluble ash value determination. *I. pes-caprae* contained 16.85 % in leaf and 14.25 % in stem portions of total ash. Leaf parts of *I. pes-caprae* contain 5.10 % of acid insoluble ash and 4.09% of water insoluble ash. Stem parts of *I. pes-caprae* was found to be 8.25 % of acid insoluble ash and 5.74 % of water insoluble ash. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.

Table 1:-Physico-chemical characters of crude powder of each plant

S. No	Name of the parameters	Leaf portion (%)	Stem portion (%)
1	Moisture Content	48	55
2	Total ash	16.85	14.25
3	Acid insoluble ash	6.10	8.25
4	Water insoluble ash	4.09	5.74

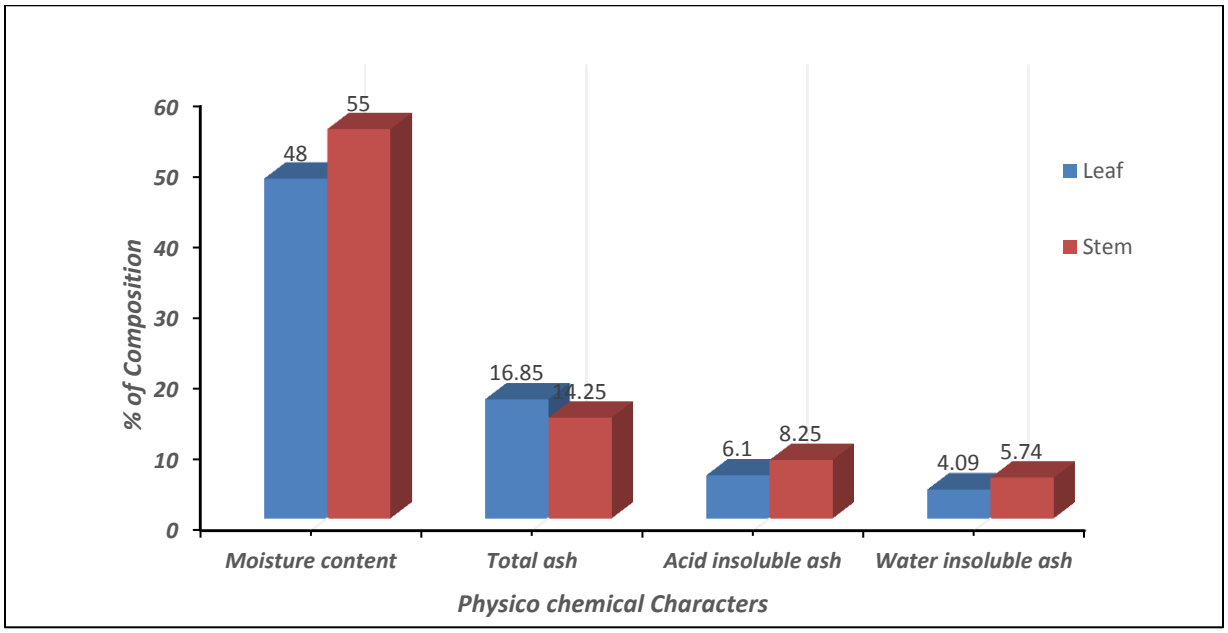


Figure 1:-Physico-chemical characters of *I. pes-caprae leaves and stems*

Extractive values

Sequential extraction of 50 g of dried powder of leaf and stem parts of *I. pes-caprae* was carried out using hexane, chloroform, ethyl acetate, methanol and water and are presented in Table 2 and Figure 2.

Table 2:-Solvent Extractive yield of crude powder of *I.pes-caprae*

S. No	Name of the parameters	Leaf portion	Stem portion
1	Hexane	1.5	3
2	Chloroform	7.9	5.1
3	Ethyl acetate	1.1	1.7
4	Methanol	8.7	8.5
5	Water	4.5	6.5

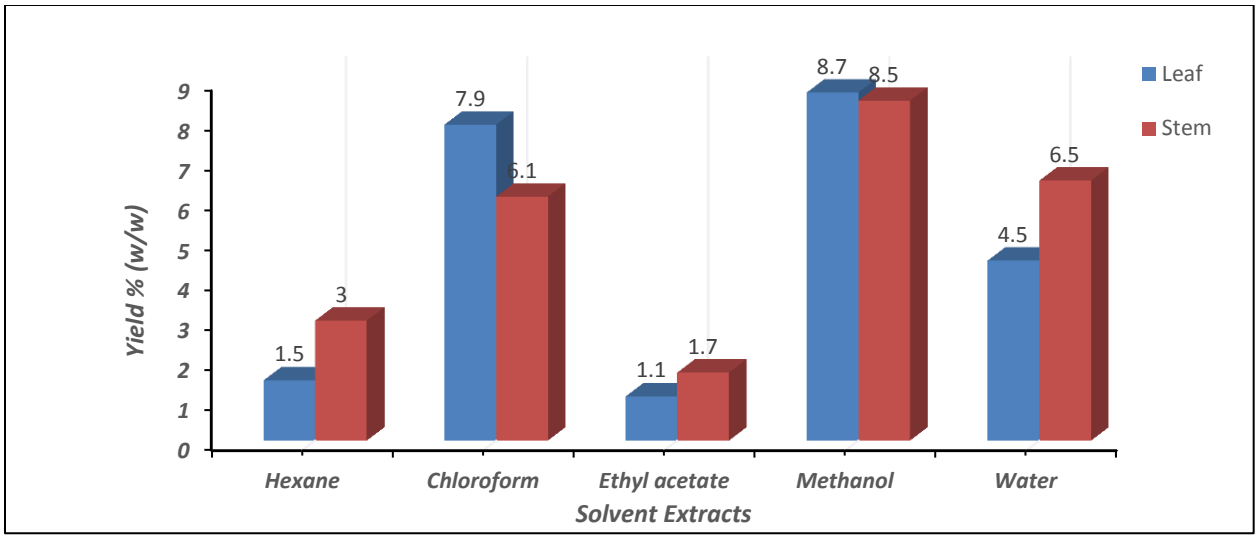


Figure 2:-Solvent Extractive yield of crude leaves and stem of *I.pes-caprae*

Leaf parts of *I. pes-caprae* yielded highest methanol extract (8.7 g) followed by chloroform (7.9 g) and water extracts (4.5 g). Increase in yield of the solvent extracts followed the order, Ethyl acetate < Hexane <Water<Chloroform< Methanol.

Almost Similarly, stem of the *I. pes-caprae* yielded the highest methanol extract (8.5 g) followed by water (6.5 g) and chloroform (5.1 g). Increase in yield of the solvent extracts followed the order, Ethyl acetate < Hexane < Chloroform <Water<Methanol.

Qualitative phytochemical analysis

The results of qualitative phytochemical screening of the crude powder of *I. pes-caprae* leaf and stem are given in Table 3. In leaf, alkaloids, steroids and triterpenes were present in maximum amount followed by flavonoids, phenols and leucoanthocyanins (Table 3); tannins, saponins, cardiac glycosides, anthocyanins were present in trace amount; other phytoconstituents were absent. In stem, phenols were present in maximum followed by moderate amount of other all phytoconstituents except anthocyanins, coumarin and quinones which were absent (Table 3).

Table 3:-Preliminary Qualitative phytochemical analysis of crude extracts of I. pes-caprae

Phytochemicals	Stem			Leaf		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
Alkaloids	-	-	+	-	++	++
Flavonoids	+	-	++	+	-	++
Phenols	+	++	+++	++	+	++
Tannins	+	+	++	-	+	++
Glycosides	++	-	-	++	-	-
Reducing sugars	-	-	+	-	-	+
Proteins	-	-	+	+	+	+
Saponins	++	+	-	+	+	-
Quinones	-	+	+	++	++	++
Steroids	+	+	+	+	+	++
Amino acids	+	+	+	+	+	+
Terpenoids	++	++	+	+	++	++

+ Present in minor quantity; ++ present in moderate quantity; +++ present in higher quantity; - Not detected

The presence of glycosides moieties like saponins, glycosides and flavonoids, which are known to inhibit tumor growth and serve to protect against gastro-intestinal infections. Amino acids, glycosides, reducing sugars and steroids were present in lower amount. Phenols, flavonoids and tannins were present in higher concentrations than other phytochemicals.

Quantitative determinations by GC-MSD

Twenty compounds were identified in *I.pes-caprae* stem and leaves by GC-MS analysis. The active principles in stem portions with their retention time (RT), molecular formula (MF), molecular weight (MW), and concentration (%) were presented in Table 4. The GC-MS chromatogram was showed in Figure 4. Table 5 listed the various phytochemical constituents which contribute to the medicinal activity of n-hexane extract of *I.pes-caprae* leaves and stem.

Among the identified phytochemicals, Vitamin E is detected in *I. pes-caprae* whole plant which was found to be effective antioxidant and belongs to the class of compounds identified to enhance sperm quality and prevent sperm agglutination, thus making more motile with forward progression and hence promote male fertility. The compound stigmasterol was identified in both stem and leaf of *I.pes-caprae* was found to possess anticervical cancer property. Squalene has antioxidant, antibacterial, antitumor, immunostimulant and lipoxygenase inhibitor activity.

Table 4:-Components identified in the Hexane extract of Stem of *I. Pes-caprae*

S.NO	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1	8.63	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol (S: FARNESOL)	$C_{15}H_{26}O$	222	0.80
2	10.32	methyl ester Octadecanoic acid (S: Methyl stearate)	$C_{19}H_{38}O_2$	298	0.96
3	11.32	1-Docosanol	$C_{22}H_{46}O$	326	3.39
4	12.688	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	312	1.18
5	12.898	n-Eicosanol	$C_{20}H_{42}O$	298	0.82
6	14.897	9,19-Cyclo-9β-lanost-24-en-3β-ol, acetate	$C_{32}H_{52}O_2$	468	2.38
7	15.265	2,6,10,15,19,23- Hexamethyl 2,6,10,14,18,22-tetracosahexaene (S: SQUALENE)	$C_{30}H_{50}$	410	2.08
8	18.158	(6E,10E)-3,7,11,15-Tetramethyl-1,6,10,14-hexadecatetraen-3-ol	$C_{20}H_{34}O$	290	8.49
9	19.053	Geranylgeraniol	$C_{20}H_{34}O$	290	4.35
10	19.789	Dimethyl(bis{[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl] oxy}) silane	$C_{32}H_{56}O_2Si$	500	2.76
11	20.631	n-Heptacosane	$C_{27}H_{56}$	380	10.9
12	20.788	Vitamin E	$C_{29}H_{50}O_2$	430	2.01
13	21.63	(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol	$C_{30}H_{50}O$	426	7.73
14	21.893	Campesterol	$C_{28}H_{48}O$	400	3.46
15	22.209	Stigmasterol	$C_{29}H_{48}O$	412	5.54
16	22.998	β-Sitosterol	$C_{29}H_{50}O$	414	21.2
17	23.576	α-Amyrin	$C_{30}H_{50}O$	426	1.01

18	24.313	Urs-12-ene	$C_{30}H_{50}$	410	3.32
19	24.966	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.85
20	25.522	Lanosterol	$C_{30}H_{50}O$	426	5.93

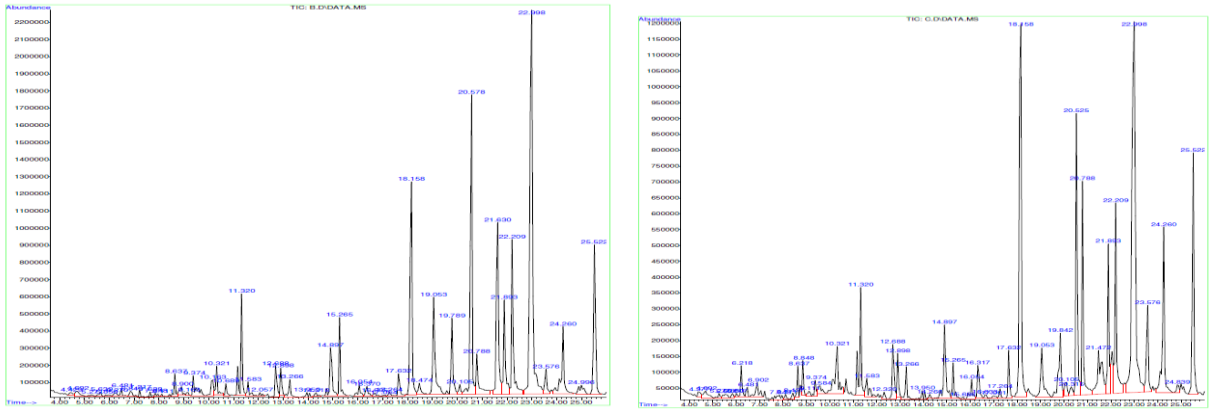


Figure4:-GC-MS Chromatogram of Hexane Extract of Stem and leaves of *I. Pes-caprae*

Table 5:-Activity of Components in the n-Hexane extract of Stem and leaves of I. Pes-caprae

S. No	Name of the compound	Molecular Formula	Nature of compound	Activity
1	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol (S: FARNESOL)	$C_{15}H_{26}O$	Sesquiterpene	Anti-tumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
2	methyl ester Octadecanoic acid (S: Methyl stearate)	$C_{19}H_{38}O_2$	Fragrance Agents	Antifoaming agent and fermentation nutrient, Food additives, Flavoring Agents
3	2-Hexadecanol	$C_{16}H_{34}O$	Aliphatic alcohol	Anti-acne agents, antidepressants
4	1-	$C_{22}H_{46}$	Aliphat	Antiviral activity

	Docosanol	O	ic alcohol	
5	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	Polyethylene Glycol	Antioxidant
6	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-Tetracosahexaene, (SQUALENE)	$C_{30}H_{50}$		Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase inhibitor, pesticide
7	Heptacosane	$C_{27}H_{56}$		
8	(6E,10E)-3,7,11,15-Tetramethyl-1,6,10,14-hexadecatetraen-3-ol	$C_{20}H_{34}O$		
9	Geranylgeraniol	$C_{20}H_{34}O$	Diterpene alcohol	potent inhibitor of *Mycobacterium tuberculosis*
10	n-Heptacosane	$C_{27}H_{56}$		
11	Vitamin E	$C_{29}H_{50}O_2$	Vitamin	Antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, anticancer, hepatoprotective, hypocholesterolemia, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
12	Campesterol	$C_{28}H_{48}O$	Phytosterol	Anti-hypercholesterolemia
13	Stigmasterol	$C_{29}H_{48}O$	Phytosterol	prevention of certain cancer including ovarian, prostate, breast, and colon cancers, potent antioxidant, hypoglycemic and thyroid inhibiting

				properties.
14	β-Sitosterol	$C_{29}H_{50}O$	Steroid	Antimicrobial, anticancer, antiarthritic, antiasthma diuretic
15	α-Amyrin	$C_{30}H_{50}O$	Triterpene	Cytotoxicity, Antitrypanosomal activity
16	Lanosterol	$C_{30}H_{50}O$	tetracyclic Triterpenoid	Prevention of cataract

Conclusions:-

The results show the *I. pes-caprae* has broad spectrum of pharmacological activity. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. The phytochemical present in *I.pes-caprae* have several activities such as antioxidant, analgesic and anti-inflammatory, 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 effects. Thorough research work was needed to be done on this potential plant which may yield many bio-active compounds.

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