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Phytochemical Screening and GCMS Studies of the Medicinal Plant *Pavetta indica Linn.*

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ABSTRACT

Objective: The plant *Pavetta indica Linn.*is variable shrub (or) small tree belonging to the family of Rubiaceae, reported to have medicinal properties. The leaves and roots of this plant are used in poultices for boils and itches, to cure hemorrhoidal pain, constipation, jaundice etc. The present work is aimed at the phytochemical screening and GCMS Studies for the presence of secondary metabolites like alkaloids, flavonoids, terpenoids, steroids, tannins, etc.
Methods: The Phytochemical screening of the leaf extracts were carried out applying the standard methods and tests. It shows the presence of metabolites like alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavonoids, etc. The ethanolic extract was subjected to GCMS studies.
Results: The phytochemical screening reveals that the both ethanolic and methanolic extracts of *Pavetta indica Linn.* contains the phytoconstituents - alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavonoids, etc. The GCMS analysis of ethanolic extracts indicates the presence of 36 phytoconstituents belonging to the types of acids, alkanes, amines, esters and phenolic compounds.
Conclusion: The phytochemical screening and GCMS analysis of the extracts are in good agreement with the presence of alkaloids; four alkaloids are reported to be present by the GCMS studies. The medicinal properties of *Pavetta indica Linn.* may are attributed to the presence of alkaloids.

Keywords- Medicinal values, GCMS studies, *Pavetta indica Linn.*, Phytochemical screening.

INTRODUCTION

Pavetta indica Linn[1,2] (Tamil: Kattu thirani, Panna pavadai, Sirukonnai, Pavattai) is a shrub or small tree belongs to the family of Rubiaceace. The leaves very variable elliptic – oblong to elliptic – lanceolate and obovate – oblong, glossy – green flowers are white. The roots are said to possess purgative, aperient, diuretic and tonic properties and are prescribed in visceral obstructions, jaundice, headache, urinary diseases and dropsical affections. The phytochemical investigation[3], chemical composition of essential oil[4] and physio – phytochemical screening[5] had been reported to this plant. The plant was studied and found to possess anti – inflammatory potential[6], analgestic[7], antimicrobial[8] , antipyretic activities[9]. The aim of the present study was to identify the phytocomponents of the ethanolic and methanolic extracts of the plant leaves applying the GCMS and the phytochemical screening techniques.

MATERIALS AND METHODS

The leaves of *Pavetta indica Linn.* were collected from Elamanur region (Near Trichy) from the month of July at 5.00pm.They were identified and authenticated by the Rapinet Herbarium, St. Joseph college (Autonomous)Trichy -02, Tamilnadu, India.

Sample preparation

The leaves of *Pavetta indica Linn.* were shade dried and pulverized well. About 20g of the plant leaves were soaked in 100ml of ethanol and methanol. It was left for 24 hours in order to extract the phytoconstituents- alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavanoids, acids and others. The above extracts were filtered using Whatmann No.1filter paper the residue was removed.

Phytochemical Screening[10,11]

The phytochemical screening of the leaf extracts were carried out applying the standard methods and tests as prescribed by J B Harbone[12]. Hence, the presence or absence of various phytoconstituents were determined. The experimental procedures and the results are given in the Table No -1.

Gas Chromatography and Mass spectrometry[13]

The ethanolic extract was subjected to GC-MS analysis of the instrument GCMS (Schimadz U Japan) with Elite – DB – 5M Column and the GCMS solution version 2.53SV3 software. Initially oven temperature was maintained at 30°C for 2 minutes and the temperature was increased gradually up to 200ºC at 10.0/35.0 min and 4.0 µL of sample was injected for analysis. Helium gas 99.9% of purity was used as carrier gas as well a elution. The flow rate of helium gas set to 1.5ml /min. The temperature was maintained at 230ºC. The sample injector with split ratio was 20 throughout the experiment periods. The mass spectroscopic analysis was done at 70 eV. The spectra were recorded for mass range 40 – 1000 m/z for about 35 minutes. The separated compounds were identified by comparing their mass spectra with the mass spectral data of the compounds present in the data bank. The GCMS chromatogram is attached in Figure No. 1.

RESULTS AND DISCUSSION

Phytochemical screening

The results of the phytochemical screening of the plant *Pavetta indica Linn.* and its GCMS profiling are presented here. The plant *Pavetta indica Linn* .was analysed qualitatively for the phytochemically active compounds and the results are given in the Table No: 2. The ethanolic and methanolic extracts of the leaves of *Pavetta indica Linn.* showed the presence of phytochemically

active compounds such as alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavonoids. The following metabolites were analysed to be absent in the ethanolic and methanolic extracts saponins, sapanin glycosides, cumarin, anthocyanin and flavones. The details are given in the Table No: 2.

GCMS Study

GCMS analysis was carried out on the ethanolic extracts of *Pavatta indica Linn*. showed as many as 36 compounds to present. The lists of compounds are given in Table No – 3. The GCMS analysis was done using the instrument GCMS (Schimadz U QP2010 with GCMS solution version 2.53 software. The sample volumes was 4.0µL. The sample of ethanolic extract was run for 35 minutes. The chromatogram (Figure No:1) showed prominent peaks in the retention time ranging 4.0 – 38.0minutes.

Based on the percentage peak area the compounds 1,2- benzene dicarboxylic acid, diethylester(CAS) Ethyl phthalate, 2,4-Imidazolidinedione, 1-[[(5-nitro-2-furanyl) methane]amino]-(CAS)upiol, phalic acid, allyl ethyl ester(CAS) Ethylallylphthalate, 1, 3-dioxoline, tartronic acid, (P-Ethoxyphenyl) diethyl ester were found to be significantly in higher quantities with the peak areas ranging from 59.63 to 60%.

The compounds methane, sulfinyl bis – (CAS) dimethyl sulfoxide, propane, 2-chloro-(CAS) 2-chloropropane, n-butyric D7acid were observed to be in moderate quantities with the peak area ranging from 20 to21%. The following compounds 1, 2-benzenedicarboxylic acid, phthalic acid, butyl ester, di isobutyl benzene – 1, 2 – dicarboxylate, hydrazine, hexadecanoic acid, palmitic acid, octadecanoic acid, strearic acid, 3, 4 – hexanediol, tetradecanoic acid, myristic acid, decanoic acid, capric acid, 1-propanamine, n-propylamine, formamide, nonane, 3-bromodecane, 4-heptane were quatified to be in lower amounts with the peak area ranging from 1 to 5%. The data of GCMS studies are given in the Table No: 3.

CONCLUSION

The results of the phytochemical screening revealed that both ethanolic and methanolic extracts of *Pavetta indica Linn*. contained the phytoconstituents - alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavonoids, etc.

The GCMS analysis of ethanolic extracts indicated the presence of 36 phytoconstituents belonging to the types of acids, alkanes, amines, esters and phenolic compounds. Hence, the medicinal plant *Pavetta indica Linn* had been found to possess significant phytoconstituents that might be attributed to the medicinal characteristics.

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Table 1. Details of Phytochemical Screening of the extracts of *Pavetta indica Linn.*

S. No	Name of the Test	Experimental Procedure	Phytoconstituent
1	a) Mayer's test	0.5 ml of extract was treated with Mayer's reagent (potassiomercuric iodide solution) to gave cream colored precipitate.	Alkaloids
	b) Dragondraff test	0.5 ml of extract was treated with Dragendroff's reagent (potassium bismith iodide). Formation of orange or orange red precipitate was obseved.	Alkaloids
	c) Wagner test	0.5 ml of extract was treated with Wager's reagent (solution of iodine with KI) and it gave an brown or reddish brown precipitate.	Alkaloids
2	a) Molisch test	0.5ml of extract was treated with 1ml of alpha – napthol and con. H_2SO_4, which gave purple coloation.	Carbohydrates
	b) Fehling test	0.5 ml of extract to which equal quantity of Fehling solution – A (copper sulphate) & B (potassium ammonium tartate) were added. The content was heating to give brick red precipitate was obtained.	Carbohydrates
3	Foam test	Dilute 1ml of alcohol in 0.5 ml of extract. The mixture was diluted to 20 ml of distilled water. It was shaken well for 15min. The formation of foam was observed.	Saponins
4	Lead acetate test	0.5 ml of alcoholic or aqueous extracts was treated with lead acetate solution. White precipitate was observed.	Tannins
5	Ferric chloride test	0.5 ml of alcoholic extracts was treated with 2 drops of neutral ferric chloride. brownish green coloration was observed	Pseudo Tannin (Condensed tannin)
6	Ammonia test	0.5 ml of extract was treated with aqueous ammonia solution. It was exposed to air which gradually develops a green color.	Chlorogenic acid
7	NaOH test	0.5 ml of extract was treated with aqueous sodium hydroxide solution formation of blue violet coloration	Anthocyanin
8	Libermann's Burchard test	0.5ml of extract was dissolved in 2ml chloroform. The mixture was treated with acetic acid, acetic anhydride and conc. H_2SO_4 gave bluish green coloration.	Steroidal glycosides
9	H_2SO_4 test	0.5 ml of extract was treated with 80% H_2SO_4, gave deep yellow color.	Saponins glycosides
10	Ammonia test	0.5 ml of extract was mixed 2ml of ammonia. The mixture was observed under UV and visible lights - formation of fluorescence.	Flavonoids
11	Shinoda's test	0.5 ml of alcoholic extract was treated with magnesium foil and conc. HCl. It gave intense cherry red coloration	Flavones
12	NaOH test	0.5 ml of alcoholic extract was treated with 10% Sodium hydroxide solution, yellow coloration was observed.	Coumarin
13	Salkowaski test	0.5 ml of extract was dissolved in 1ml of chloroform. The mixture was treated with conc. H_2SO_4. It gave red coloration.	Steroids

Table 2. Details of Phytochemical Screening of the extracts of *Pavetta indica Linn.*

S. No	Phytochemical constituents	Name of the test	Methanol Extract	Ethanol Extract
1	Alkaloids	Mayer's test Dragondraff test Wagner test	+ + +	+ + +
2	Carbohydrates	Molish test Fehling test Benedicts test	+ - -	+ + -
3	Saponins	Foam test	-	-
4	Tannins	Lead Acetate test	+	+
5	Pseudo tannins	Ferric chloride.	Condensed Tannin	Condensed Tannin
6	Chlorogenic acid	Ammonia test	+	+
7	Anthocyanin	NaOH test	-	-
8	Steroidal Glycosides	Liebermann's Burchard test	+	+
9	Saponins glycosides	H_2SO_4 test	-	-
10	Flavonoids	Ammonia test	+	+
11	Flavones	Shinoda's test	-	-
12	Coumarin	Sodium chloride test	-	-
13	Anthracene glycoside	Ammonia test	-	-
14	Steroids	Salkowaski test	+	+

Note: + = Present - = Absent

Table 3. Phytoconstituents of *Pavetta indica Linn.* by GCMS Study.

S. No	RT	Name of the compound	Molecular Formula	Molecular Weight	% Peak area	Compound Nature
1	6.418	Methane, sulfinylbis- (CAS) Dimethyl sulfoxide	C_2H_6OS	78	20.52	Organo sulphur
2	6.418	Propane, 2-chloro- (CAS) 2-Chloropropane	C_3H_7Cl	78	20.52	Haloalkane
3	6.418	n-Butyric-D7 acid	C_7H_8	92	20.52	Fatty acid
4	24.191	1,2-Benzenedicarboxylic acid, dimethyl ester (CAS) Methyl phthalate	$C_{10}H_{10}O_4$	194	1.84	Aromatic di carboxylic acid
5	24.191	Methyl o-(Bromochloroacetyl)benzoate	$C_{10}H_8BrClO_3$	296	1.84	Ester
6	25.210	Docosane (CAS) n-Docosane -	$C_{22}H_{46}$	310	1.18	Alkane
7	25.210	Nonane, 5-methyl-5-propyl	$C_{13}H_{28}$	184	1.18	Alkane
8	25.210	3-Bromodecane	$C_{10}H_{21}Br$	220	1.18	Haloalkanes
9	25.210	4-Heptanone, 3-methyl- (CAS) 3-Methyl-4-heptanone	$C_8H_{16}O$	128	1.18	Ketone
10	25.210	Hexadecane (CAS) n-Hexadecane	$C_{16}H_{34}$	226	1.18	Alkane hydrocarbon
11	28.250	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate	$C_{12}H_{14}O_4$	222	59.63	Phthalate ester
12	28.250	2,4-Imidazolidinedione, 1-[[(5-nitro-2-furanyl)methylene]amino]- (CAS) Upiol	$C_8H_6N_4O_5$	238	59.63	Hetero cyclic compound
13	28.250	Phthalic acid, allyl ethyl ester (CAS) Ethylallylphthalate	$C_{13}H_{14}O_4$	234	59.63	Phthalate ester
14	28.250	1,3-dioxolane, 2-phenyL-2-(phenylmethyl)-	$C_{16}H_{16}O_2$	240	59.63	Dioxy ether
15	28.250	tartronic acid, (p-ethoxyphenyl)-, diethyl ester	$C_{15}H_{20}O_6$	296	59.63	Ester
16	34.38	Phthalic acid , butyl ester with ester butyl glycolate (CAS) 1,2-Benzenedicarboxylicacid , 2- butoxy – 2- oxoethyl butyl ester (CAS) butyl (butoxycarbonyl)methyl phthalate	$C_{18}H_{24}O_6$	336	3.52	Ester
17	35.41	Hydrazine, (phenylmethyl) - (CAS) Benzylhydrazine - 95%	$C_8H_{10}O$	122	3.52	Amino Compound

18	35.41	Headecanoic acid (CAS) Palmitic acid	$C_{16}H_{32}O_2$	256	1.19	Fatty acids
19	35.41	Octadecanoic acid (CAS) Stearic acid , n-OCtadecanoic acid	$C_{18}H_{36}O_2$	284	1.19	Fatty acids
20	35.410	10-bromo-7-hydroxy-11-iodolaurene	$C_{15}H_{18}BrIO$	420	1.19	Alcohol
21	35.410	3,6,9-trimethyl-7-nitro-2,3-dihydronaphtho[1,8-bc]pyran	$C_{15}H_{15}NO_3$	257	1.19	Hetro cyclic compound
22	35.410	3,4-Hexanediol, 2,5-dimethyl- (cas) 2,5-dimethyl-3,4-hexandiol	$C_8H_{18}O_2$	146	1.19	Alcohol -
23	35.410	Tetradecanoic acid (CAS) Myristic acid	$C_{14}H_{28}O_2$	228	1.19	Fatty acids
24	35.410	Decanoic acid (CAS) Capric acid	$C_{10}H_{20}O_2$	172	1.19	Saturated fatty acids
25	35.562	butyl-2-ethylhexyl phthalate	$C_{20}H_{30}O_4$	334.44	1.52	Ester
26	35.562	2-methyl-6-beta-d-ribofuranosylimidazo[1,2-c]pyrimidin-5(6H)-one	$C_{12}H_{15}N_3O_5$	281	1.52	Hetero cyclic compound
27	35.562	3-methylhomoadamantane Tricyclo[4.3.1.13,8]undecane, 3-methyl- (CAS)	$C_{12}H_{20}$	164	1.52	Alkane
28	35.562	(3R*,4S*)-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydroxyphenyl)hexane	$C_{19}H_{23}NO_4$	329	1.52	Hrtro cyclic compound
29	37.97	Butanoic acid, 2-hydroxy-, methyl ester (CAS) methyl 2-hydroxybutyrate	$C_5H_{10}O_3$	118	2.67	Ester
30	37.973	4-p-chorophenyl-2-dimethylamino-5-nitrosothiazole	$C_{12}H_{13}N_3OS$	247	2.67	Hetero cyclic compound
31	37.973	1-Propanamine (CAS) n-Propylamine	C_3H_9N	59	2.67	Amine
32	37.973	Formamide, N-methyl- (CAS) N-methylformamide Methylformamide	C_2H_5NO	59	2.67	Amide
33	37.973	1-germa-2-silabutane (ethylsilyl) germane	$C_2H_{10}GESI$	136	2.67	Alkane
34	37.973	N-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]sulfimide-trimethylamine adduct	$C_6H_9F_7N_2O_2S$	306	2.67	Sulfamide
35	37.973	Ethyl 2-(1'-hydroxy-1'-methylethyl)-5,6,6-trimethyl-3,4-heptadienoate	$C_{15}H_{26}O_3$	254	2.67	Alkane
36	37.973	3-Fluoro-2-methoxy-3-(trifluoromethyl)nonan-4-one	$C_{11}H_{18}F_4O_2$	258	2.67	Haloketone

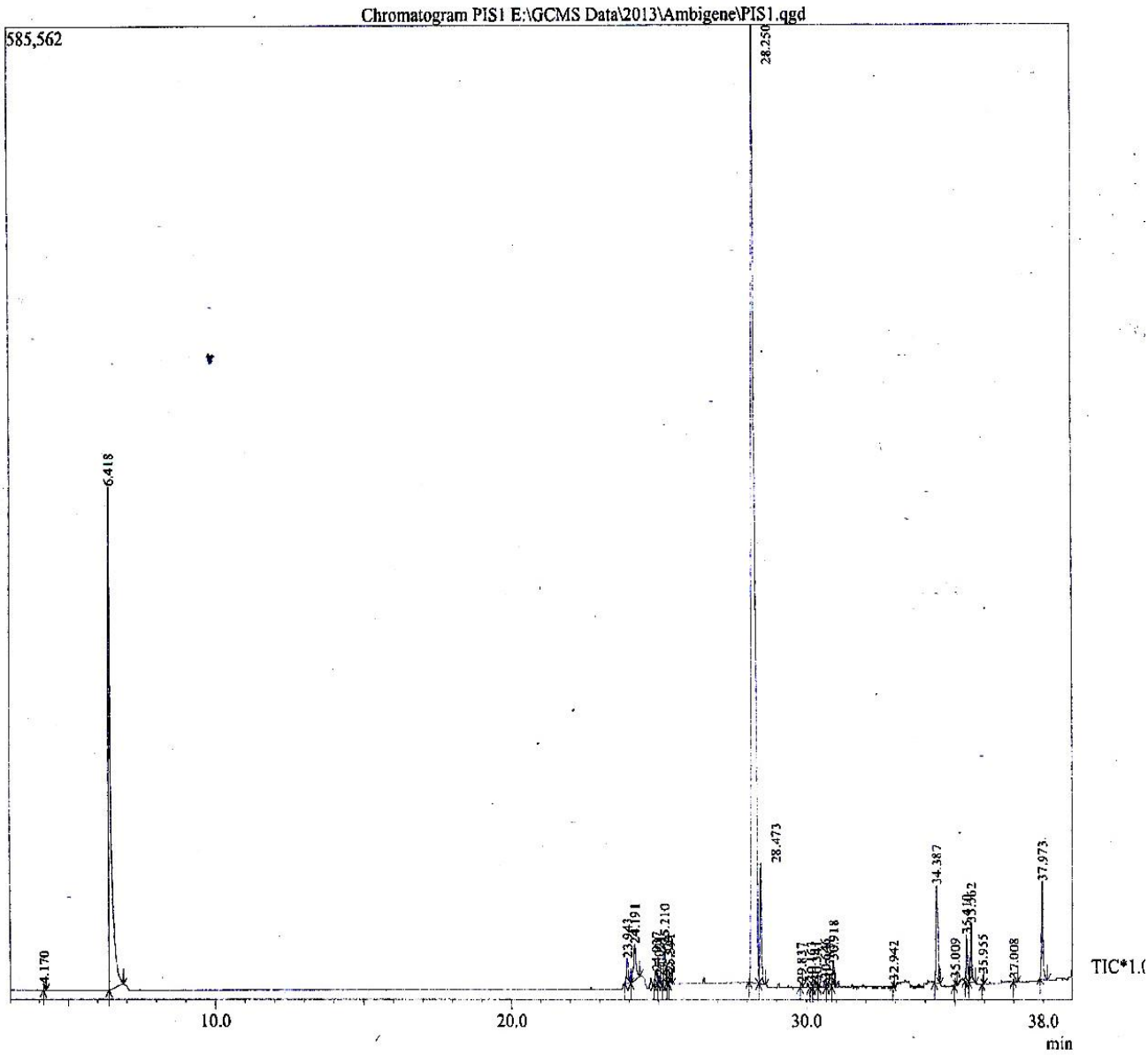

Fig. 1: GCMS Chromatogram of *Pavetta indica Linn.*