WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 946-968. Research Article ISSN 2277– 7105

ISOLATION AND IDENTIFICATION OF CHEMICAL CONSTITUENTS FROM VARIOUS POLAR SOLVENT CRUDE LEAF, STEM AND ROOT EXTRACTS OF ENDEMIC - *POGOSTEMON SPECIOSUS* BENTH OF THE NILGIRI

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Article Received on
30 May 2018,

Revised on 20 June 2018,
Accepted on 10 July 2018

DOI: 10.20959/wjpr201814-12946

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ABSTRACT

Pogostemon speciosus Benth is endemic ethno medicinal plant, belongs to the family Lamiaceae found in Anamalai and Nilgiri hills of South Western Ghats, India. The present study was to analysis the secondary metabolites by preliminary phytochemical screening of the leaf (PSLPE, PSLEA, and PSLE), stem (PSSPE, PSSEA, PSSE) and root (PSRPE, PSREA, PSRE) petroleum ether, ethyl acetate and ethanol extracts by different test. Identification of the phytocompound through FTIR and GCMS from leaf, stem and root ethanol extracts (PSLE, PSSE, PSRE). Among these extracts, the ethanol extracts of leaf, stem and root showed the presence of majority of the metabolites when compared to other extracts. FTIR analysis of PSLE, PSSE and PSRE revealed the presence of 6, 5, 7 major bonds respectively. The results pertaining to GCMS analysis led to the identification of number of compounds from the ethanol leaf, stem and root extracts. GCMS examination revealed 40, 29 and 29 phytocompounds separately, with 7, 7 and 4 bioactive compounds. The important bioactive compounds present in PSLE are Caryophyllene, 1-Naphthalenol, Alpha.-bisabolol, N-Hexadecanoic acid, Phytol,

Hexatriacontane, 2-Tetracosahexaene, Dotriacontane, and Butyl-4(adamantyl-1) benzene, in PSSE extract Caryophyllene, Alpha.-bisabolol, 2H Pyran, Flopropione, Phytol isomer and Dotriacontane. The bioactive compounds present in PSRE extract are Caryophyllene, Heneicosane, 2H Pyran, Dotriacontane with anti-inflammatory, antifungal, antioxidant, antitumor, antibacterial, anti-asthmatics, drugs for skeletal disorders, bronchodilators, antispasmodic, hypocholesterolemic, nematicide, pesticide, anti-inflammatory, cytotoxic bioactivity.

KEYWORDS: *Pogostemon speciosus*, potassium bromide, triterpenoids, alpha bisabolol.

1. INTRODUCTION

Medicinal plants are valuable part of the world flora. Earth has around 250000 plants in which 80000 plants have at least some medicinal value. According to world medicinal situation[1] report around 50000 species have specific therapeutic value bad harvesting management and inadequate cultivation practices may lead to extinction of endangered species or to destruction of natural resources. This di stress situation is raising the question about special efforts which should be paid both to protection of the plant populations and up-to-date knowledge concerning more reasonable and effective utilization of these plants.[2]

The Western Ghats hill ranges in Southern India are one of the unique biological regions in the world. These hills have been recognized as one of the eight hottest hotspots of biodiversity. In India, Tamil Nadu is under strategic geographical location and possesses an invaluable treasure of medicinal plants holding a major share in cultivation and export of more than fifty medicinal plants species.

Pogostemon speciosus, belongs to family Lamiaceae, endemic to Western Ghats, A shrub, reaching 3ft.in height with pilose-hispid brown branches, white flowers tinged with pink and leaves and inflorescence nearly black when dry. The very long stamens give a bottle –brush appearance to the racemes.[3]

Pogostemon speciosus leaf, stem and root used for anti-inflammatory, local anesthetic, antifungal properties[4][5] and cytotoxic activity,[6,7,8],anti-asthmatics, muscle relaxants, neuro degenerative disorder, antibacterial activity[9], dermatological, cosmetic formulations[10], antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic reductase inhibitor[11][12], food additive and antinociceptive[13],treatment of urinary, intestine,

and ophthalmic infections, scalds, ulcerative colitis[14], rheumatoid arthritis, and antispasmodic activity.[15,16] To our knowledge and literature survey there is no report on FTIR and GCMS studies of *Pogostemon speciosus*. Against this background we have reported the preliminary phytochemical, FTIR and GCMS analysis of *Pogostemon speciosus*.

2. MATERIALS AND METHODS

2.1. Plant collection and authentication

A shoot with flower of *Pogostemon speciosus*, Benth. was collected from the natural habitats of Government botanical garden, Udhagamandalam, The Nilgiri, Tamil Nadu, India and this species was identified and certified by Botanical Survey of India (BSI), Coimbatore, India (Certificate No. BSI/SRC/5/23/2014-15/Tech./62). The voucher specimen was deposited in the Department of Botany, Government Arts College, Udhagamandalam.

2.2. Plant extract preparation

The fresh plant part of leaves, stem and root were collected and dried under shade condition, and milled to a coarse powder by mortar and pestle separately. Various poplar solvent such as petroleum ether, ethyl acetate and ethanol were used for successive extraction plant parts of *Pogostemon speciosus*[6] 100 grams of each plant parts leaf, stem and root were extracted by cold maceration for 48 hours. After extraction of each solvent extracts were evaporated the solvents and the extracts of *P. speciosus* leaf petroleum ether, ethyl acetate, ethanol (PSLPE, PSLEA, PSLE); *P. speciosus* stem petroleum ether, ethyl acetate, ethanol (PSSPE, PSSEA, PSSE); *P. speciosus* root petroleum ether, ethyl acetate, ethanol (PSRPE, PSREA, PSRE) stored under 4 °C for further studies.

2.3. Phytochemical tests for secondary metabolites

The extracts PSLPE, PSLEA, PSLE, PSSPE, PSSEA, PSSE and PSRPE, PSREA, PSRE were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material. The preliminary screening of phytochemicals such as detection of alkaloids (mayer's test)[17], flavonoids[18], tannins(ferric chloride test)[19], terpenoids and phytosterols (libermann- burchard's test)[20], saponins, (foam test)[21], glycosides(keller – kiliani test)[22], gum & mucilage's[23], fixed oils[24] and anthroquinones[25] were studied.

2.4. FTIR spectroscopy

FTIR analysis was performed by formation of the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio. About 1 mg of each samples PSLE, PSSE and PSRE with KBr and mixed. The formed pellet was carefully taken on the sample holder and subjected to FTIR analysis. The spectrum was recorded using Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan).

2.5. Gas Chromatogram Mass Spectroscopy (GCMS) analysis

The GC–MS analysis of PSLE, PSSE and PSRE were carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbo mass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 ºC, at the rate of an increase of 50 ºC /min, and maintained for 9 min. The 1 µL of each extract samples injected into the instrument and injection port temperature was ensured as 200 ºC and Helium flow rate as one ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z).Using computer searches on a NIST Version –Year 2011 were used MS data library and comparing the spectrum obtained through GC–MS compounds present in the plants sample were identified.

3. RESULTS

3.1. Preliminary phytochemical screening

The preliminary phytochemical quantitative screening of the PSLPE, PSLEA, PSLE, PSSPE, PSSEA, PSSE and PSRPE, PSREA, PSRE extracts of *P. speciosus* was analyzed which are tabulated in 1, 2 and 3 respectively. More number of secondary metabolites are present in PSLE, PSSE and PSRE extracts compared to other extracts of PSLPE, PSEA, PSSPE, PSSEA, PSRPE and PSREA. *Pogostemon speciosus* leaf, stem and root ethanol extracts have presented same secondary metabolites such as alkaloids, flavonoids, tannins, steroids, triterpenoids and glycosides and some other metabolites of saponins, gum & mucilage, anthraquinones are not present in these extracts.

Table 1: Secondary Metabolite Profiling of Leaf extracts of *Pogostemon speciosus.*

S. No	Chemical Constituent	Tests	PSLPE	PSLEA	PSLE
1	Alkaloids	a. Dragendorff's test	-	-	+
		b. Mayer's test	-	-	+
		c. Wagner's test	-	-	+
		d. Hager's test	-	-	+
2	Flavonoids	a. 10% HCl & 5% NaOH test	+	+	+
3	Tannins	5% $FeCl_3$ test	-	-	+
4	Steroids	Liebermann - Burchard's test	+	+	+
5	Triterpenoids	a. Liebermann - Burchard's test	+	+	+
		b. Salkowski's test	+	+	+
6	Saponins	Foam test	-	-	-
7	Glycosides	Keller - Kiliani test	+	+	+
8	Gum & Mucilage's	Whistler & BeMiller test	+	+	-
9	Fixed oils	Spot test	+	+	-
10	Anthraquinones	NH_4OH test	-	-	-

+ Denotes Presence of compound; - Denotes Absence of compound

Table 2: Secondary Metabolite Profiling of Stem extracts of *Pogostemon speciosus.*

S. No.	Chemical Constituent	Tests	PSSPE	PSSEA	PSSE
1	Alkaloids	a. Dragendorff's test	-	-	+
		b. Mayer's test	-	-	+
		c. Wagner's test	-	-	+
		d. Hager's test	-	-	+
2	Flavonoids	a. 10% HCl & 5% NaOH test	-	+	+
3	Tannins	5% $FeCl_3$ test	-	_	+
4	Steroids	Liebermann - Burchard's test	-	+	+
5	Triterpenoids	a. Liebermann - Burchard's test	+	+	+
		b. Salkowski's test	+	+	+
6	Saponins	Foam test	-	-	-
7	Glycosides	Keller - Kiliani test	-	+	+
8	Gum & Mucilage's	Whistler & BeMiller test	+	+	-
9	Fixed oils	Spot test	-	-	-
10	Anthraquinones	NH_4OH test	-	-	-

+ Denotes Presence of compounds; - Denotes Absence of compounds

Table 3: Secondary Metabolite Profiling of Root extracts of *Pogostemon speciosus.*

S. No	Chemical Constituent	Tests	PSRPE	PSREA	PSRE
1	Alkaloids	a.Dragendorff's test	_	_	+
		b. Mayer's test	_	_	+
		c. Wagner's test	_	_	+
		d. Hager's test	_	_	+
2	Flavonoids	a. 10% HCl & 5% NaOH test	_	+	+
3	Tannins	5% $FeCl_3$ test	-	-	-
4	Steroids	Liebermann - Burchard's test	_	+	+
5	Triterpenoids	a. Liebermann - Burchard's test	+	+	+
		b. Salkowski's test	+	+	+
6	Saponins	Foam test	_	_	_
7	Glycosides	Keller - Kiliani test	_	_	+
8	Gum & Mucilage's	Whistler & BeMiller test	+	+	+
9	Fixed oils	Spot test	+	+	_
10	Anthraquinones	NH_4OH test	_	_	_

+ Denotes Presence of compounds; - Denotes Absence of compounds

3.2. FTIR analysis

In PSLE extract have reported 6 major bands at 3500-500 cm^{-1} which are represented in figure 1. The strong band were observed at 3284.15**,** 2938.93, 1642.33, 1032.33, 483.73, 447.98 cm^{-1}. The functional group are alcohol, phenols, 1^o, 2^o amines, amides, carboxylic acids, alkynes (terminal), alkanes, carboxylic acids, alkenes, 1^o amines, alcohols, carboxylic acids, esters, ethers, aliphatic amines and alkyl halides with assignments are O-H stretch, H-bonded, N-H Stretch,-C≡CH; C-H stretch, C-H stretch, O-H stretch, -C=C-stretch, N-H bend, C-O stretch, C-N stretch respectively (Fig. 1 and Table 4).

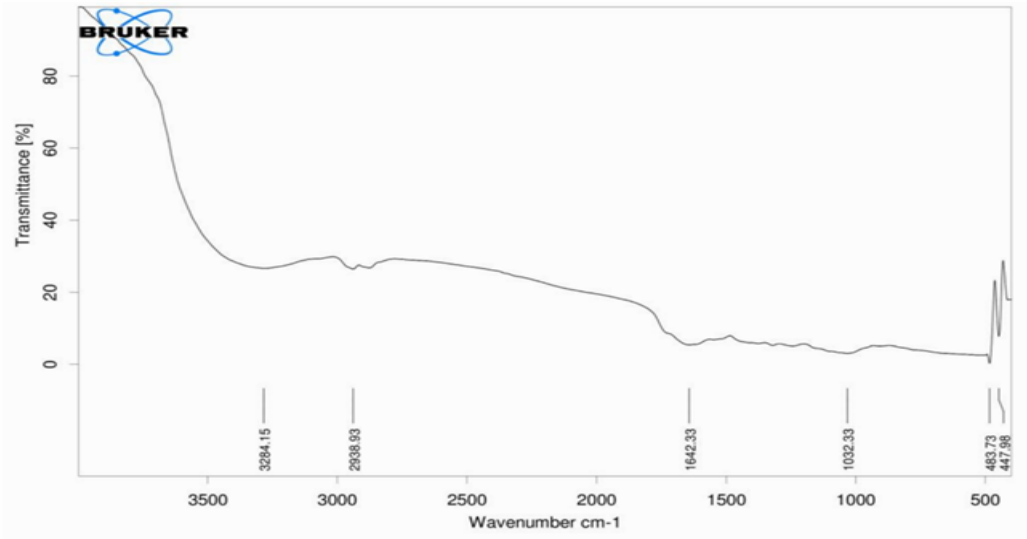

Figure 1: FTIR spectra of PSLE extract.

Table 4: FTIR spectrum analysis of PSLE extract.

Frequency (cm^{-1})	Intensity	Assignment	Characterization
3283.15	Strong, broad, medium, narrow	O-H stretch, Hbonded, N-H Stretch, C≡C-H;CH stretch	Alcohol, phenols, 1 °, 2° amines, amides, carboxylic acids, Alkynes (terminal).
2938.93	medium	C-H stretch, O-H stretch,	Alkanes, carboxylic acids,
1642.33	medium	-C=C-stretch, N-H bend,	Alkenes,1 amines
1032.33	Strong, medium,	C-O stretch, C-N stretch	Alcohols, carboxylic acids, esters, ethers, aliphatic amines,
483.73	Strong	CI stretch	Alkyl halide
447.98	Strong	CI stretch	Alkyl halide

Five major bands were observed in PSSE extract between 3500-500 cm^{-1} which are represented in figure 2. The bands were observed at 3336.61, 2941.46, 2878.93, 1034.23 and 483.82 cm^{-1}. The functional group are Alcohol, phenols, carboxylic acids, alkanes, carboxylic acids, esters, ethers, aliphatic amines and Alkyl halides with assignments are O-H stretch, H Bonded, O-H stretch, C-H stretch, C-O stretch, C-N stretch, C-Br stretch respectively (Fig. 2 and table 5).

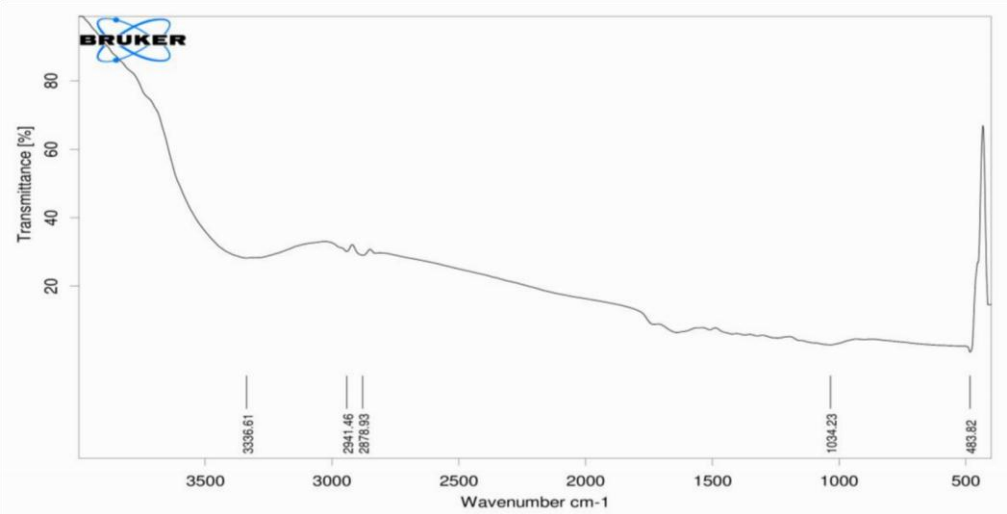

Figure 2: FTIR spectrum analysis of PSSE extract.

Table 5: FTIR spectrum analysis of PSSE extract.

Frequency (cm^{-1})	Intensity	Assignment	Characterization
3336.61	Strong, broad	O-H stretch, H Bonded	Alcohol, phenols
2941.46	medium	O-H stretch, C-H stretch	carboxylic acids, alkanes
2878.93	medium	C-H stretch	alkanes
1034.23	Strong, medium,	C-O stretch, C-N stretch	Alcohols, carboxylic acids, esters, ethers, aliphatic amines,
483.82	Strong	CI stretch	Alkyl halides

Figure 3 represents the FTIR spectra of PSRE extract with 7 major bands between 3500-500 cm^{-1}. The bands are 3355.64, 2934.80, 1643.71, 1033.13, 534.35, 474.72 and 435.97 cm^{-1}. The functional group are alcohol, phenols, carboxylic acids, alkanes, Alkenes, 1^{o}amines, Alcohols, carboxylic acids, esters, ethers, aliphatic amines and alkyl halides with assignments are O-H stretch, H Bonded, O-H stretch, C-H stretch, -C=C-stretch, N-H bend, C-O stretch, C-N stretch and C-Br stretch respectively (Fig. 3 and table 6).

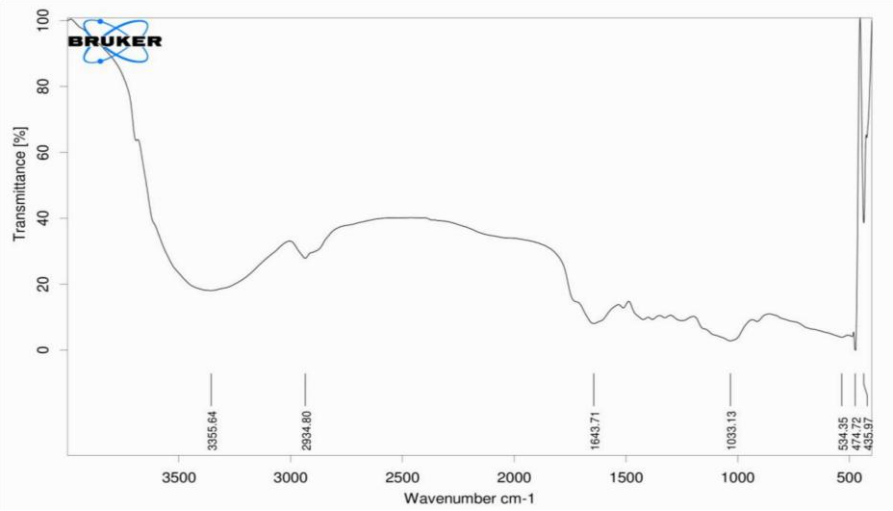

Figure 3: FTIR spectrum analysis of PSRE extract.

Table 6: FTIR spectrum analysis of PSRE extract.

Frequency (cm^{-1})	Intensity	Assignment	Characterization
3355.64	Strong, broad	O-H stretch, H Bonded	Alcohol, phenols
2934.80	medium	O-H stretch, C-H stretch	carboxylic acids, alkanes
1643.71	medium	-C=C-stretch, N-H bend	Alkenes,1^{o}amines
1033.13	strong	C-O stretch, C-N stretch	Alcohols, carboxylic acids, esters, ethers, aliphatic amines,
534.35	Strong	C-Br stretch	Alkyl halide
474.72	Strong	C-I stretch	Alkyl halide
435.97	Strong	C-I stretch	Alkyl halide

3.3. GC-MS analysis

GCMS analysis of PSLE, PSSE and PSRE extracts were carried out by NIST 2011 library. The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanol extract of *Pogostemon speciosus*, leaf, stem and root. These compounds were identified through Mass spectrometry attached with GC.

The chromatogram of PSLE extract shows 40 prominent peak in the retention time range 12.839 – 27.964 (Fig.4). The peak at 17.024 retention time is having the peak area 12.04.

This largest peak is due to the presence of. alpha.-Bisabolol. The second less prominent peak at 17.389 retention time has the peak area 8.94 is due to the presence of 2-butynyl-5-hydroxy-3-oxo-4 Hexanoic Acid. The third less significant peak at 32.120 retention time with the peak area 8.70 is characteristics of Phytol. The fourth less prominent peak at 15.065 retention time with the peak area 5.90 is characteristics of 1-Naphthalenol, 1, 2, 3, 4, 4A, 7, 8,8A. The other less prominent peak at other retention times are given in table 7.

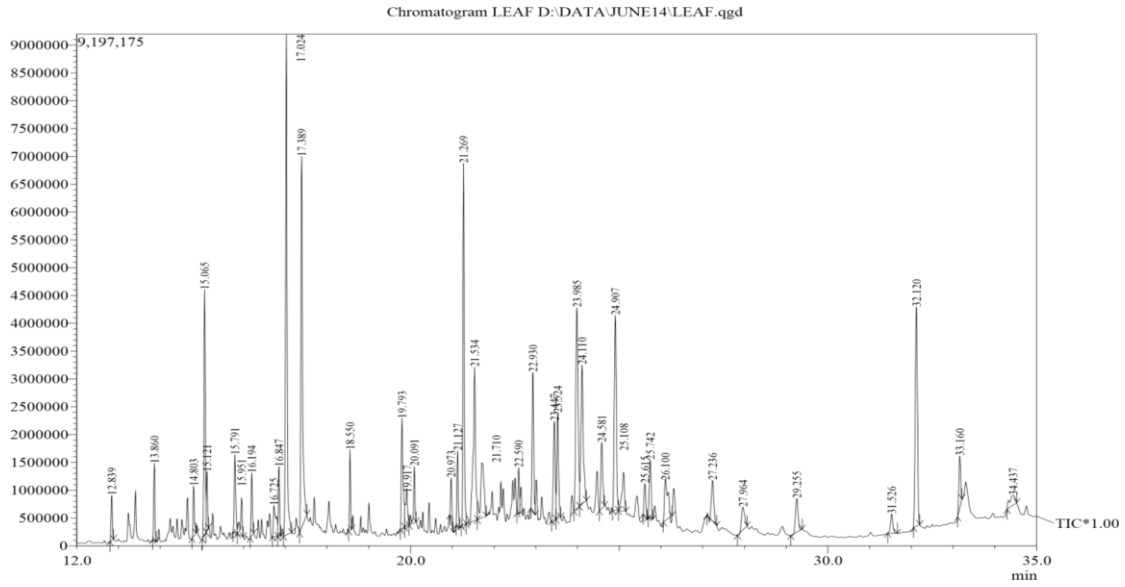

Figure 4: Gas chromatogram of phytochemical constitute of PSLE.

Table 7: Phytocomponents and bio active uses of PSLE.

S. No	RT	Compound Name	Mole. Formula	Mole. Weight	Peak Area	Compound uses
1	12.839	4-Isopropyl-3,7-Dimethyl-3A,3B	C15H24	204	1.09	
2	13.860	Caryophyllene.	C15H24	204	1.86	Caryophyllene, a compound found in spice blends, citrus flavors, soaps, detergents, creams and lotions, and also in a variety of food and beverage products[26,27] is known for its anti-inflammatory, local anesthetic, and antifungal properties.[28,29] In experiments conducted on tumor cell lines, *β*-caryophyllene has been reported to have a potent cytotoxic activity over a wide range of cell lines.[30,31]
3	14.803	Isoledene	C15H24	204	1.21	
4	15.065	1-Naphthalenol, 1,2,3,4,4A,7,8,8A	C15H26O	222	5.90	Anti-asthmatics, muscle relaxants, neuro degenerative disorder and preparation for care of hair. Antibacterial constituents against.[32]
5	15.121	Naphthalene,	C15H24	204	1.42	
6	15.791	1,4-Methanoazulen-9-ol,	C15H26O	222	1.87	
7	15.951	Dodecane,	C15H24O	220	0.92	
8	16.194	1,1,4,7- Tetramethyldecahydr	C15H26O	222	1.52	
9	16.725	Cubenol	C15H26O	222	0.75	
10	16.847	1-Naphthalenol,	C15H26O	222	1.68	
11	17.024	Alpha.-bisabolol3Cyclohexene-1methanol	C15H26O	222	12.04	Bisabolol has been widely used as an ingredient in dermatological and cosmetic formulations such as aftershave creams, hand- and body-lotions, deodorants, lipsticks, sun-care and after-sun products, baby care products and sport creams.[33]
12	17.389	2-butynyl-5-hydroxy- 3-oxo-4-Hexanoic Acid	C10H12O4	196	8.94	
13	18.550	2,6,10-Trimethyl, 14- Ethylene-14Pentadecne	C20H38	278	1.94	
14	19.793	N-Hexadecanoic acid	C16H32O2	256	2.65	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors.[34,35]
15	19.917	Phenanthrene,	C19H28	256	0.81	
16	20.091	Hexadecanoic acid, ethyl ester Palmitic acid	C18H36O2	284	1.36	
17	20.973	Heptafluorobutyrate	C23H27F7O3	484	1.05	

18	20.975	Trans- Dehydroandrosterone, pentafluoropropionate	C22H27F5O3	434	1.81	
19	21.269	Phytol	C20H40O	296	8.70	Used as a food additive and in medicinal field Antinociceptive and Antioxidant Activities.[36]
20	21.534	1-t-Butyl-4(adamantyl-1) benzene.	C20H28	268	3.70	They are also effective for the treatment of urinary, intestine, and ophthalmic infections, scalds, ulcerative colitis[37], rheumatoid arthritis.[38]
21	21.708	1-Heptatriacotanol	C37H76O	536	1.33	
22	22.590	Kaura-5,16-dien18(or 19)-Ol	C20H30O	286	1.10	
23	22.930	Podocarp-7-en- 3.beta.-ol,	C20H32O	288	3.35	
24	23.447	1-Phenanthrenecarboxylic acid,	C21H32O2	316	2.37	
25	23.524	Cholest-14-EN-3-OL	C27H46O	386	2.54	
26	23.985	1,4a,7-trimethyl-, methyl ester	C21H32O2	316	4.84	
27	24.110	Palustric acid	C20H30O2	302	3.35	
28	24.581	ABIETA-8	C20H28O2	300	1.59	
29	24.907	Dibenzo[a,h]cyclotetr adecene	C30H44	404	4.69	
30	25.108	Kaura-9(11),16-dien-18-oic acid, (4.alpha.)	C20H28O2	300	0.89	
31	25.615	2-Propenoic acid,	C18H18O4	298	0.79	
32	25.742	Lycopene	C40H56	536	1.40	
33	26.100	9,19-Cycloergost- 24(28)-en-3-ol,	C32H52O2	468	0.97	
34	27.236	Methandriol	C20H32O2	304	0.98	
35	27.964	15,17,19,21- Hexatriacontatetrayne	C36H58	490	0.55	
36	29.255	Tetrapentacontane	C54H110	758	0.81	
37	31.526	Hexatriacontane	C36H74	506	0.47	
38	32.120	2,6,10,14 Hexatriacontane,18,2 2-Tetracosahexaene,	C30H50	410	5.27	Antioxidant, anticancer, pesticide, sunscreen, perfumery and chemo preventive properties.[39]
39	33.160	Dotriacontane	C32H66	450	1.24	Antimicrobial, antioxidant, Antispasmodic.[40]
40	34.437	Friedelan-3-one	C30H50O	426	0.24	

The chromatogram of stem shows 29 prominent peak in the retention time range 13.860 – 34.749. The peak at 17.424 retention time is having the peak area 35.91 (Fig. 5). This largest peak is due to the presence of 2H-Pyran-2-one, 3-acetyl-4-hydroxy-6-. The second less prominent peak at 34.475 retention time has the peak area 12.94 is due to the presence of Friedelan-3-one $$ Friedelin $$ D: A-Fr. The third less significant peak at 33.303 retention time with the peak area 5.50 is characteristics of 4,4a,6b,8a,11,11,12b,14a-octamet. The fourth less prominent peak at 19.794 retention time with the peak area 5.43 is characteristics of n-Hexadecanoic acid. The other less prominent peak at other retention times are given in table 8.

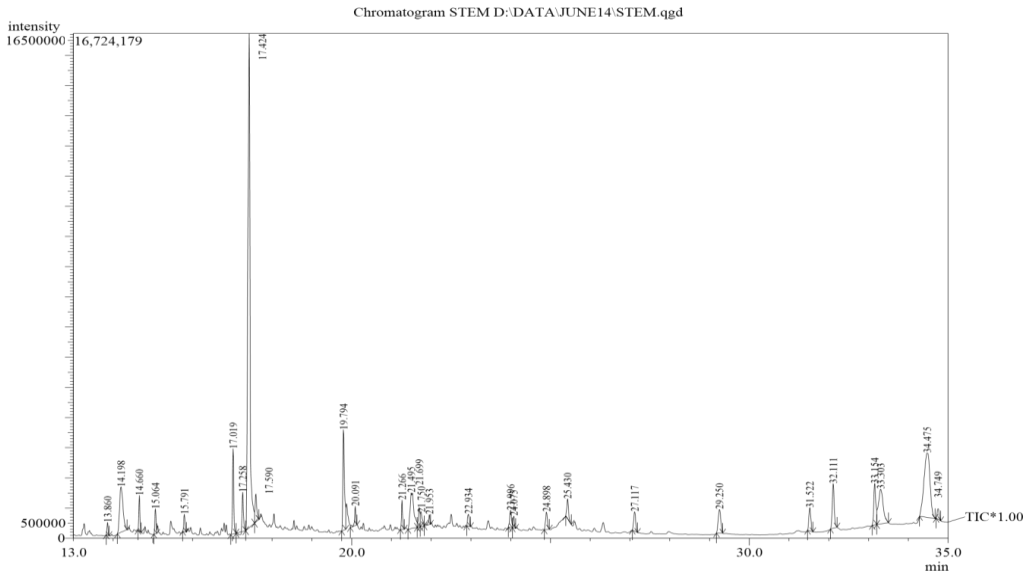

Figure 5: Gas chromatogram of phytochemical constitute of PSSE.

Table 8: Phytocomponents and bio active uses of PSSE.

S. N0	RT	Compound Name	Mole. Formula	Mole. Weight	Peak Area	
1	13.860	Caryophyllene	C15H24	204	0.47	Caryophyllene, a compound found in spice blends, citrus flavors, soaps, detergents, creams and lotions, and also in a variety of food and beverage products[26,27] is known for its anti-inflammatory, local anesthetic, and antifungal properties.[28,29] In experiments conducted on tumor cell lines, *β*-Caryophyllene has been reported to have a potent cytotoxic activity over a wide range of cell lines.[30,31]
2	14.198	2,4-octanedione	C8H14O2	142	5.27	
3	14.660	Hexadecane	:C16H34	226	1.25	
4	15.064	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahy	C15H26O	222	1.00	
5	15.791	cis-1-Chloro-9octadecene	C18H35Cl	286	0.72	
6	17.019	alpha.-Bisabolol	C15H26O	222	3.60	Bisabolol has been widely used as an ingredient in dermatological and cosmetic formulations such as aftershave creams, hand- and body-lotions, deodorants, lipsticks, sun-care and after-sun products, baby care products and sport creams.[33]
7	17.258	2-butynyl-5-hydroxy-3oxo-4	C10H12O4	196	1.58	
8	17.424	2H-Pyran-2-one, 3-acetyl-4-hydroxy-6-	C8H8O4	168	35.9 1	Antimicrobial activity, Throat disorder, Anti asthmatics, drugs for disorder of urinary system, wounds, ulcers, burns, scars, drugs for skeletal disorders, antiasthma, bronchodilators, anti-bacterial, tb and leprosy.[41]
9	17.590	Flopropione	:C9H10O4	182	1.55	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors.[42]
10	19.794	n-Hexadecanoic acid	:C16H32O2	256	5.43	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors.[34,35]
11	20.091	Hexadecanoic acid, ethyl ester	C18H36O2	284	0.81	

12	21.266	Phytol isomer	C20H40O	296	2.17	Antinociceptive and Antioxidant Activities.[36]
13	21.495	9-Octadecenoic acid, 1,2,3-propanetriyl	:C57H104O6	884	4.65	
14	21.699	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	1.06	
15	21.750	Ethyl Oleate	C20H38O2	310	0.87	
16	21.953	1-Nonadecene	C19H38	266	0.50	
17	22.934	Pregnenolone	C21H32O2	316	0.69	
18	23.996	1-Phenanthrenemethanol, 1,2,3,4,4a,9,1	C20H30O	286	1.06	
19	24.075	Dotriacontane	C32H66	450	0.53	Antimicrobial, antioxidant, Antispasmodic.[40]
20	24.898	1-phenanthrenecarboxylic a	C21H32O2	316	1.06	
21	25.430	Dotriacontane	C32H66	450	1.10	Antimicrobial, antioxidant, Antispasmodic.[40]
22	27.117	Tetrapentacontane	C54H110	758	1.28	
23	29.250	Dotriacontane	C32H66	450	2.02	Antimicrobial, antioxidant, Antispasmodic.[40]
24	31.522	Hexatriacontane	C36H74	506	1.52	
25	32.111	2,6,10,14,18,22-Tetracosahexaene, 2,6,1	C30H50	410	2.94	
26	33.154	Dotriacontane	C32H66	450	2.89	Antimicrobial, antioxidant, Antispasmodic.[40]
27	33.303	4,4a,6b,8a,11,11,12b,14a-octamet	C30H52O	428	5.50	
28	34.475	Friedelan-3-one $$ Friedelin $$ D:A-Fr	C30H50O	426	12.9 4	
29	34.749	Tetrapentacontane	C54H110	758	0.68	

The chromatogram of root shows 29 prominent peak in the retention time range 10.390 27.116 the peak at 17.496 retention time is having the peak area 44.05 shown in figure 6. This largest peak is due to the presence of 3-acetyl-4-hydroxy-6-methy. The second less prominent peak at 14.665 retention time has the peak area 7.25 is due to the presence of Heneicosane. The third less significant peak at 19.814 retention time with the peak area 5.21 is characteristics of l-(+). Ascorbic acid 2, 6-dihexadecanoat. The fourth less prominent peak at 20. 103 retention time with the peak area 4.29 is characteristics of 1(2h)-naphthalene, octahy. The other less prominent peak at other retention times are given in table 9.

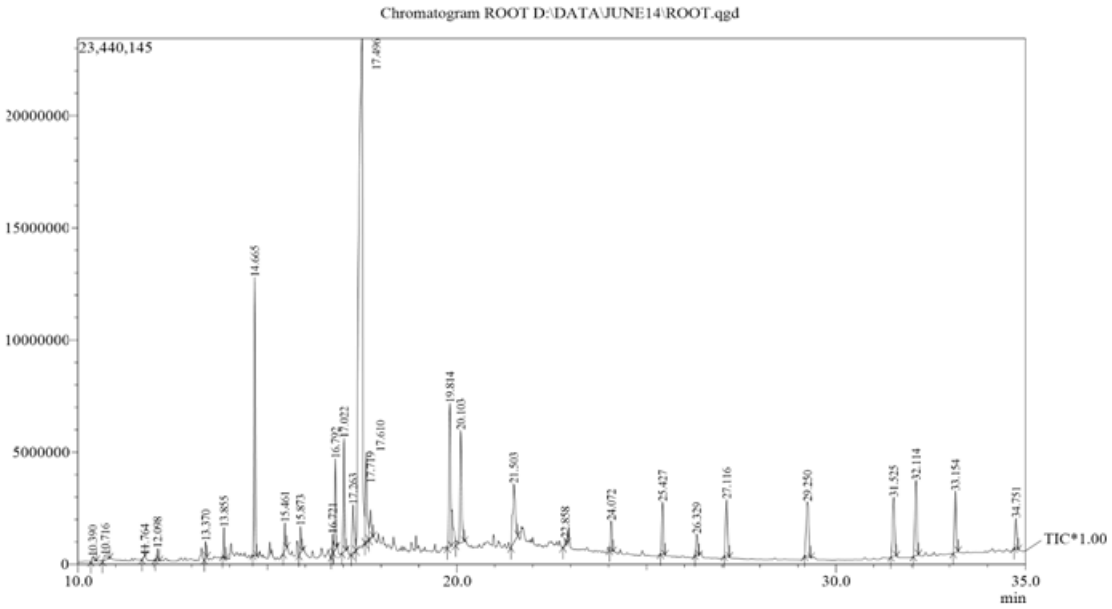

Figure 6: Gas chromatogram of phytochemical constitute of PSRE.

Table 3: Phytocomponents and bioactive uses of PSRE.

S. No	RT	Compound Name	Mole. Formula	Mole. Weight	Peak Area	Compound uses
1	10.390	1-Undecanol			0.21	
2	10.716	Pentanoic acid, 2methyl-, anhydride			0.32	
3	11.764	Ethyl 2-ethyl-2-methyl-3-oxobutyrate			0.02	
4	12.098	Cyclopentanecarboxyl ic acid, tridecyle			0.28	
5	13.370	Tetradecane	C14H30	198	0.40	
6	13.855	Caryophyllene	C15H24	204	0.76	Caryophyllene, a compound found in spice blends, citrus flavors, soaps, detergents, creams and lotions, and also in a variety of food and beverage products[26,27] is known for its anti-inflammatory, local anesthetic, and antifungal properties.[28,29] In experiments conducted on tumor cell lines, *β*-caryophyllene has been reported to have a potent cytotoxic activity over a wide range of cell lines.[30,31]
7	14.665	Heneicosane	C21H44	296	7.25	Anti-inflammatory.[24] Antifungal against fungal spores germination, Antioxidant, Antitumor, Antibacterial.[25]
8	15.461	Dodecanoic Acid	C12H24O2	200	0.85	
9	15.873	Octadecane	C18H38	254	0.73	
10	16.721	5-Heptadecene, 1-Bromo-	C17H36	240	0.52	
11	16.792	n-Pentadecanol	C15H32O	228	2.26	
12	17.022	Octadecane, 1-chloro-	C18H38	254	3.26	
13	17.263	Ethanone, 1-(2,4,6trihydroxyphenyl)-	C8H8O4	168	2.06	
14	17.496	:3-Acetyl-4-Hydroxy6-Methyl-2h-Pyran-2One	C8H8O4	168	44.0 5	Antimicrobial activity, Throat disorder, Anti asthmatics, drugs for disorder of urinary system, wounds, ulcers, burns, scars, drugs for skeletal disorders, antiasthma, bronchodilators, anti-bacterial, tb and leprosy.[41]

15	17.610	Flopropione	C9H10O4	182	3.21	
16	17.719	Tetradecanoic Acid	C14H28O2	228	1.16	
17	19.814	l-(+)-Ascorbic acid 2,6-dihexadecanoat	C38H68O8	658	5.21	
18	20.103	1(2h)-Naphthalenone, Octahy	C15H26O	222	4.29	
19	21.503	Octadec-9-enoic acid	C18H34O2	282	3.54	
20	22.858	1-Eicosanol			0.09	
21	24.072	Dotriacontane	:C32H66	450	0.92	Antimicrobial, antioxidant, Antispasmodic.[40]
22	25.427	Dotriacontane	C32H66	450	1.91	Antimicrobial, antioxidant, Antispasmodic.[40]
23	26.329	1,2-Benzenedicarboxylic acid, mono(2-	C16H22O4	278	0.83	
24	27.116	Tetrapentacontane	C54H110	758	2.63	
25	29.250	Dotriacontane	C32H66	450	3.25	Antimicrobial, antioxidant, Antispasmodic.[40]
26	31.525	Tetrapentacontane	C54H110	758	2.93	
27	32.114	2,6,10,14,18,22Tetracosahexaene, 2,6,1	C30H50	410	3.37	
28	33.154	Tetrapentacontane	C54H110	758	2.47	
29	34.751	Tetrapentacontane	C54H110	758	1.22	

4. DISCUSSION

Ethno botanical study of regional medicinal plants has always guided the search for new remedy. In spite of the development of modern drug discovery and advanced evaluation techniques, traditional knowledge system have given sign to the discovery of valuable drugs. Now a days the analysis of the organic compounds from plants and their biological activity has increased because of side effects of modern medicine. The importance of medicinal plants is due to the presence of biologically active compounds that produce during normal metabolic processes of the plant and it plays an important role in plant defense mechanism. Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species.

Spectroscopic technique is a powerful diagnostic mechanism for the quantitative and qualitative analysis of traditional and pharmaceutical metabolites. The FTIR technique is an outstanding method for the quantitative analysis because the compound spectrum is individual with the exception of optical isomers. It offers a rapid and nondestructive investigation to fingerprint herbal extract or powders.

The medicinal plants are exhibiting foundation of various secondary metabolites determined by GCMS spectra analysis.[20] The present research has been found helpful in the identification of many constituents present in the various extracts of *Pogostemon speciosus* The results of the qualitative analysis of the PSLE, PSSE and PSRE study plant, positive for the presence of the alkaloids, flavonoids, tannins, steroids, tri terpenoides, glycosides, gum& mucilage's, and fixed oils, negative for the absence of saponins, anthroquinones. Three different types of plant sample ethanol, water extract shows maximum constituents followed by, ethyl acetate and Petroleum ether.

The present results suggest that the phytochemicals properties for curing various ailments and having potential anti-inflammatory, antimicrobial and antioxidant and leads to the isolation of new and novel compounds. Alkaloids have a bitter taste while many to toxic to other organisms[21] Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory

agent.[22] The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable.[23,24] They also inhibit microbes which are resistant to antibiotics[25], which prevent oxidative cell damage and have strong anti-cancer activity[26], and also have anti-inflammatory actions.[27,28] Tannins are complex moieties produced by majority of plants as protective substances and they possess astringent, anti-inflammatory, antidiarrheal, antioxidant and antimicrobial activities.[29] Glycoside compounds are containing a carbohydrate and non-carbohydrates residue (moiety) in the same molecule. They are important in medicine because of their action on heart and are used in cardiac insufficiency.[30]

FTIR analysis of ethanolic leaf, stem, root extracts of *Pogostemon speciosus* showed possibility of identifying effective functional groups in the chemical constituents and possible whereby identify the different compounds, and to can distinguished between aromatic and non-aromatic compounds and alkenes, alkanes, esters, ethers and carboxylic acid, amines etc.

GCMS chromatogram of PSLE have seven known bioactive compounds like Caryophyllene, 1-Naphthalenal, Alpha-Bisabolol, N-Hexadecanoic acid, phytol, 1-t-Butyl-4(adamantyl-1) benzene and Hexatriacontane with anti-inflammatory, antifungal, antianesthetic, cytotoxicity activities and also used in dermatological and cosmetic formulation.[26,40] The result of GC-MS analysis of PSSE showed seven bio active compounds. Among the seven, the Caryophyllene compound is known for its anti-inflammatory and antifungal properties, and also used in soaps, detergents, lotions, food and beverage products.[26,27] It also have a potent cytotoxic activity.[28,31] Alpha. - Bisabolol has been widely used as an ingredient in dermatological and cosmetic formulations such as aftershave creams, hand- and body-lotions, deodorants, lipsticks, sun-care and after-sun products, baby care products and sport creams.[33] 2H-Pyran have antimicrobial, antiasthmatics activity, also used for urinary disorder, skeletal disorders, and leprosy.[41] Flopropione is used as antioxidant, hypocholesterolemic and nematicide.[42] Phytol and dotriacontane compounds have antioxidant, antiplasmodic anti-microbial activities.[36] N Hexadecanic acid with antioxidant, hypocholesterolemic, nematicide, pesticide activities.[34][35] Bisabolol has been widely used as an ingredient in dermatological and cosmetic formulations.[33]

The chromatogram of PSRE revealed the presence of 29 compounds with four bio active compounds. The bio active compounds are caryophyllene with antiinflammatory, antifungal and cytotoxic activity.[26,31] Also used in detergent, cream, beverage products, henicosane

with antiinflammatory, antifungal, antibacterial and antitumour activity.[24,25] 3-Acetyl-4-Hydroxy6-Methyl-2h-Pyran-2One compound having antiasthmatics, antimicrobial activities and drugs for tuberculosis and leprosy, dotriacontane with antimicrobial, antioxidant and antispasmodial activity.[41] The bio active compound caryophyllene is present in PSLE, PSSE and PSRE extracts, whereas alpha bisabolal, N-Hexadecanoic acid and phytol were present in PSLE and PSSE extracts. The compound dotriacontane present in PSSE and PSRE extracts. The presence of many known bioactive compounds confirm the use of *P speciosus* for various ailments by traditional practitioners.

5. CONCLUSION

The presence of phytochemicals in leaf, stem and root demonstrated expansive range of various organic exercises and mechanical applications like cancer prevention agent, mitigating, antiviral, antibacterial, antifungal, anticancer. The GCMS profile can be utilized as pharmacognostical apparatus for the distinguishing proof of novel medications from *Pogostemon speciosus*. The results showed that *Pogostemon speciosus* plant can be used to find the bioactive normal items which may serve in the improvement of new pharmaceuticals. Further, the compounds can be utilized for the revelation of novel medications to treat various diseases.

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