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Phytochemical Screening, FTIR, GCMS and Antioxidant Activity of Aqueous Extract of *Pogostemon speciosus* Benth.: An Endemic Medicinal Plant

Sathiya Sheela D*[1], P. Viswanathan[2], K. Kalimuthu[3], E. Shanthi Priya[4] and A. Vanitha[5]

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A B S T R A C T

Many phytochemicals protect plants from various diseases; however, recent studies prove that many of these chemicals can also protect humans against infectious diseases. Aqueous extract of *Pogostemon speciosus* was used in the study to analyze the phytochemicals, FTIR, GCMS, and antioxidants. As per standard methods, phytochemical analysis was performed on aqueous extract. In order to measure the FTIR of the aqueous extract, Jasco FT/IR-6300 was used. Perkin-Elmer Gas Chromatography-Mass Spectroscopy was used to investigate the chemical composition of *P. speciosus* aqueous extract. *In vitro* antioxidant activity studied through 1, 1-diphenyl-2-picrylhydrazil (DPPH), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$), ferric reducing ability of plasma (FRAP) assay, hydrogen peroxide radical scavenging activity, nitric oxide scavenging activities. Preliminary phytochemical screening of aqueous extract of *P. speciosus* showed positive result for alkaloids, flavonoids, tannins, steroids, triterpinoids, and saponins. In FTIR analysis functional groups like alcohols, aliphatic primary amines, amine salt, carbon dioxide, alkene, halo compound etc. were identified. The GCMS analysis of *P. speciosus* plant aqueous extract exhibited the presence of 49 compounds with 12 known bioactive uses. In antioxidant activity DPPH, $ABTS^+$, FRAP, hydrogen peroxide and nitric oxide scavenging activities are dose dependent manner. When the concentration increases the percentage of inhibition also increased. The results revealed that the plant has rich in bioactive compounds, which serve as a novel therapeutic application for drug discovery.

***Key words*:** Phytochemical screening, FTIR, GCMS, Antioxidant activity, *Pogostemon speciosus*, Aqueous extract

Medicinal plants have been used for therapeutic purposes by mankind for thousands of years. The use of these drugs in traditional medicine has contributed to the isolation of an impressive number of modern drugs that are based on natural sources [1]. Additionally, some medicinal plants are still hidden, which have needed to be scientifically evaluated. These herbal medicines were becoming significant attention in global health debates. Traditional medicine has recognized preventive, healing, rehabilitative, and primitive role. Every plant is a possible source of drugs because it consists of phytochemical compounds and biological screening is necessary to know more about the activities of those phytochemical compounds. Furthermore, plant-based drugs are easily available, less expensive, efficient, safe as well as having less or no side effects as compared to allopathic medicines [2-3]. According to WHO reports, 80% of the population of developing and developed countries rely on plant-based medicines for their safety and cheap health care because they are highly safe and eco- friendly [4].

The primary metabolites contribute in vital metabolic pathways. The secondary metabolites are accomplished non vital functions in plant. In chemical resistance against pathogens and predators' secondary metabolites are involved and act as photoprotectant and allelopathic agents, assist in pollination, dispersal and in prevention of diseases in the form of medicine in humans [5-6]. Medicinal plant contains organic compounds such as tannins, alkaloids, terpenoids, steroids, flavonoids and carbohydrates. These are widely used in various fields including scientific research, the medical field, agriculture, veterinary medicine, etc. [7]. Based on the peak value ratio, the FTIR spectrum identifies the functional groups in the plant extract. For the chemical analysis of non-polar constituents and volatile essential oils, fatty acids and lipids, gas chromatography and mass spectrometry have been increasingly useful for the investigation of most medicinal plants [8].

Antioxidants are defined as substances that smooth at low concentration considerably delay or prevent oxidation of effortless oxidizable substrates. Antioxidants in food play a crucial role as a health-protecting factor. However, the numbers

* **Sathiya Sheela D**
✉ manavaisheela1357@gmail.com

[1-5] P. G. and Research Department of Botany, Government Arts College (Autonomous), Coimbatore - 641 018, Tamil Nadu, India

of those protective antioxidant principles present under the conventional physiological conditions are sufficient only to deal with the physiological rate of free-radical generation. It is obvious that any additional burden of free radicals either from environment or produced within the body can alter the pro-oxidant and antioxidant balance resulting in oxidative stress. In humans, the over production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) may result in tissue injury and has been implicated in disease progression and oxidative damage of nucleic acids, proteins and lipids [9]. Scientific evidence suggests that antioxidants reduce the chance of chronic diseases including cancer and cardiovascular disease. Plant sourced food antioxidants like Carotenes, Vitamin E, Phytate, Vitamin C, Phenolic acids, and Phytoestrogens are known as having the possible activity to avoid disease risk. When there is lack of antioxidants to extinguish the excess reactive free radicals were end up in development of cardiovascular disease, cancer, neurodegenerative, alzheimer's and inflammatory diseases within the body [10].

Pogostemon speciosus is native to the Western Ghats region, belonging to the Lamiaceae family. It is a shrub, reaching heights of 3 ft and having brown branches with pilose-hispid sides, white flowers tinged with pink and nearly black leaves when dry. The very long stamens give a bottle –brush appearance to the racemes [11]. This species leaf, stem and root used for anti-inflammatory, local anesthetic, antifungal properties [12-13] and cytotoxicity activity [14-16]. Also, it reported anti-asthmatics, muscle relaxants, neuro degenerative disorder and antibacterial activity [17]. Further, it has antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic, reductase inhibitors [18-19]. Moreover, it has antinociceptive, food additive, and antibacterial properties [20], treatment of urinary, intestinal, and ophthalmic infections, as well as ulcerative colitis [21], rheumatoid arthritis [22], and antispasmodic properties [23]. To our knowledge and literature survey there is no report on FTIR, GCMS and antioxidant studies in *Pogostemon speciosus*. The aim of this work was to investigate the preliminary phytochemical, FTIR, GCMS and antioxidant studies from *Pogostemon speciosus*.

MATERIALS AND METHODS

Preparation of plant extract

A 100 g of plant powder of *P. speciosus* leaf was soaked in 500 ml distilled water and boiled it 60°C for 20 to 25 minutes. For further studies, the extract was filtered through Watmann No. 1 filter paper.

Preliminary phytochemical analysis

The aqueous extract was subjected to preliminary phytochemical qualitative screening by various chemical test such as alkaloids: Dragendorff's test [24], Mayer's test [25], Wagner's test [26], Hager's test [27], flavonoids: 10% HCl, 5% NaOH test [28], tannins: Ferric chloride test [29], triterpenoids [30], saponins [31], glycosides [32], gum and mucilage [33], fixed oil [31] and anthraquinones [34] to determine the presence of secondary metabolites.

FTIR analysis

The FTIR analysis of the aqueous extract was conducted using the potassium bromide (KBr) pellets (FTIR grade) using the Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with a JASCO IRT-7000 Intron Infrared Microscope using transmittance mode with a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan).

GCMS analysis

GCMS analysis was carried out to identify the phytochemical constituents of the *P. speciosus* leaf sample. GCMS model, Perkin Elmer, Clarus 680 and Helium (1 mL/min) was used as a carrier gas. 1 µL of each extract was injected into the instrument and the injector temperature was set at 260 °C during the chromatographic run. The mass detector conditions were transfer line temperature 240 °C, ion source temperature 240 °C and ionization mode electron impact at 70 eV, a scan time 0.2 second and scan interval of 0.1 second. The oven temperature was fixed in starting at 60 °C for 2 min then the temperature was changed increased into 300 °C at the rate of 10 °C min^{-1} and this temperature was held for 6 min (Anupama *et al*., 2014). The GCMS analysis was performed in Sophisticated Instrument Facility (SIF), VIT University, Vellore and TurboMass ver 5.4.2 software was used. The interpretation of mass spectrum GCMS was conducted using database of National Institute Standard and Technology (NIST), library 2008.

Antioxidant methods

Diphenylpicryl Hydrazyl (DPPH) radical scavenging method [35]

The principle of this assay is based on the measurement of the scavenging ability of antioxidant towards the stable radical DPPH. Microtitre plates containing 96 wells were used for the assay. To 200 µl of DPPH solution, 10µl of each of the sample or the standard solution was added separately in well of the microtitre plate. The final concentration of the lest and standard solution used are 1000 to1.953µg/ml. The plates were incubated at 37 °C for 20 minutes and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding test and standard blank and the remaining DPPH was calculated. IC_{50} (Inhibition Concentration) is the concentration of the sample required to scavenging 50% of DPPH free radicals.

$$\text{Percent of inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

ABTS radical scavenging method [3].

The pre-formed radical monocation ABTS is generated by oxidation of ABTS with potassium per sulfate (a blue chromogen) and is reduced in the presence of such hydrogen donating antioxidant. To 0.2 ml of various concentrations of the extract or standards, 1 ml of distilled DMSO and 0.16 ml of ABTS solution were added to make a final volume of 1.36 ml. absorbance was measured spectrophotometrically, after 20 min at 734 nm using ELISA reader. Blank is maintained without ABTS. IC_{50} represents the concentration of sample required to inhibit 50% of ABTS radical monocation.

FRAP assay [37]

As a measure of antioxidant power, the ferric reducing ability of plasma FRAP assay was used to determine the total antioxidant potential of the sample. It is measuring the change in absorbance at 593nm owing to the formation of a blue colored Fe II-tripyridyl triazine compound from colorless oxidized Fe III form by the action of electron donating antioxidants. The stock solution of 2,4,6-tripyridyl-s-triazine (TPTZ) was prepared at 10 mM in a buffer consisting of 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 0.3 M acetate (pH 3.6).2.5 ml of TPTZ solution, 2.5 ml of ferric chloride solution, and 25 ml of acetate buffer compose the FRAP reagent. Freshly prepared, it was gently warmed to 37 °C. Then, 900 µl FRAP reagent was mixed with 90 µl water and 30 µl test sample/methanol/distilled

water/standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 minutes and the absorbance was recorded at 593 nm. An intense blue coloured complex were formed when ferrictripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. In both aqueous and ethanol solutions, absorbance at 593 nm was plotted versus ferroussulphate concentration in the range of 0.1 mM. In turn, $FeSO_4$ concentrations were plotted against concentrations of L-ascorbic acid, a standard antioxidant.

Scavenging of hydrogen peroxide radicals

H_2O_2 is mainly produced by enzyme reactions. In plant and animal cells, super oxide dismutase is able to produce H_2O_2 by dismutation of O_2^-, thus contributing to the lowering of oxidative reactions. The natural combination of dismutase and catalase contributes to remove H_2O_2 and thus has a true cellular antioxidant activity. H_2O_2 and thus has a true cellular antioxidant activity. H_2O_2 is able to diffuse easily through cellular membranes. The ability of plant extracts to scavenging H_2O_2 is followed by decay in H_2O_2 concentration.

$$O_2^- + H_2O_2 \text{ ---------- } OH^+ + O_2^-$$

A solution of H_2O_2 (20 mM) was prepared in PBS, (pH 7.4) various concentrations of 1 ml of sample or standard in methanol were added to 2 ml of H_2O_2 solution in PBS. In a blank solution that contained extracts dissolved in PBS without H_2O_2, the absorbance at 230 nm was measured after 10 minutes.

Nitric oxide radical inhibition activity [38-39]

Nitric oxide is naturally formed in activated macrophages and endothelial cells and is considered as an active agent in several pathological based on inflammation, organ reperfusion and also may play an important role in atherosclerosis. Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrate ions, which can be estimated by the use of Griess Illosvoy reaction. In the present investigation, Griess Illosvoy reagent is modified by using NEDD (0.1% w/v) instead of 1-naphthylamine (5%). Scavengers of NO complete with oxygen leading to reduced production of NO. The reaction mixture (6 ml) containing SNP (10 mm, 4 ml) and 1 ml of DMSO were incubated at 25 ºC for 90 minutes. After incubation 0.5 ml of the extraction mixture containing nitrate was removed and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of NEDD was added, mixture and allowed to stand for 30 minutes in different light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution. IC_{50} value obtained is the concentration of the sample required to inhibition 50% NO radical.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

(Table 1) summarized the obtained results of the preliminary phytochemical analysis in *P. speciosus* Leaf Aqueous (PSLA) extract. The presence of alkaloids, flavonoids, tannins, steroids, triterpinoids, and saponins were observed. However, glycosides, gum and mucilages, fixed oils and anthraquinones are absent.

Table 1 Preliminary phytochemical analysis of PSLA extract

Compounds	Tests	PSLA
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Flavonoids	10% HCl and 5% NaOH test	+
	Alkaline test	+
Tannins	5% $FeCl_3$ test	+
Steroids	Libermann-Burchards test	+
Triterpenoids	Libermann-Burchards test	+
	Salkowskis test	-
Saponins	Foam test	+
Glycosides	Killer and Kilian test	-
Gum and Mucilages	Whistler and BeMiller test	-
Fixed oils	Spot test	-
Anthraquinones	NH_4OH test	-

FTIR analysis

FTIR characterization studies are used to identify the functional molecules of the phytochemicals present in the plant extract. Figure1 shows seventeen different absorption peaks at wave numbers which are corresponds to functional groups of the PSLA extract. The medium and sharp band was observed at 3865.35 cm^{-1} corresponds to O-H stretching andalcohols. The weak band at 2931.80 cm^{-1} strong and broad band indicates the presence of N-H stretching amine salt. A strong band was observed at 2306.86 cm^{-1}corresponds O=C=O stretching carbon dioxide. The bands 1612.49 (strong), 1411.89 (strong) and 1355.60 (strong) cm^{-1} are assigned to C=C stretching α, β-unsaturated ketone and S=O stretching sulfate and S=O stretching sulfonamide respectively. The medium bands shown at absorption peak 1026.13 and 840.96 cm^{-1} are designated to C-N stretched amines and C=C bending alkene respectively. The strong bands 702.09 and 655.80$cm^{-1}$are corresponds to the alkene and halo compound respectively. Hence this result concluded that the PSLA extract has active functional groups like alcohols, aliphatic primary amines, amine salt, carbondioxide, alkene, halocompound etc. These functional groups are associated with the bioactive phytochemicals in the PSLA extract.

Table 2 FTIR analysis of PSLA extract

S. No.	Frequency cm^{-1}	Bond	Functional groups name
1	3865.35 (medium, sharp)	O-H stretching	Alcohol
2	3749.62 (medium, sharp)	O-H stretching	Alcohol
3	3371.57 (strong, broad)	O-H stretching	Alcohol
4	3332.99 (medium)	N-H stretching	aliphatic primary amine
5	2931.80 (strong, broad)	N-H stretching	amine salt
6	2384.02 (strong)	O=C=O stretching	carbon dioxide
7	2306.86 (strong)	O=C=O stretching	carbon dioxide
8	1612.49 (strong)	C=C stretching	α,β-unsaturated ketone
9	1411.89 (strong)	S=O stretching	Sulfate
10	1355.60 (strong)	S=O stretching	sulfonamide
11	1234.44 (strong)	C-O stretching	alkyl aryl ether

12	1064.71 (strong)	C-O stretching	primary alcohol
13	1026.13 (medium)	C-N stretching	Amine
14	840.96 (medium)	C=C bending	Alkene
15	763.81 (strong)	C-Cl stretching	halo compound
16	702.09 (strong)	C=C bending	Alkene
17	655.80 (strong)	C-Br stretching	halo compound

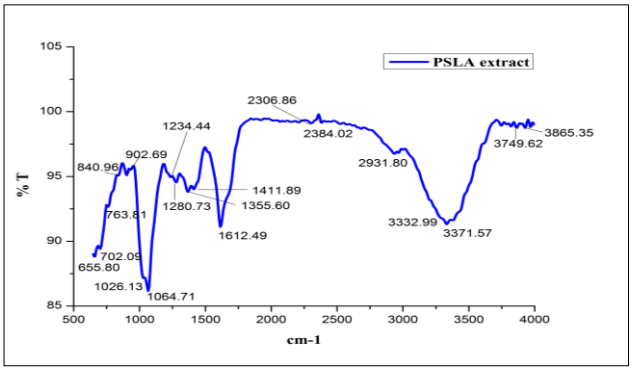

Fig 1 FTIR analysis of PSLA extract

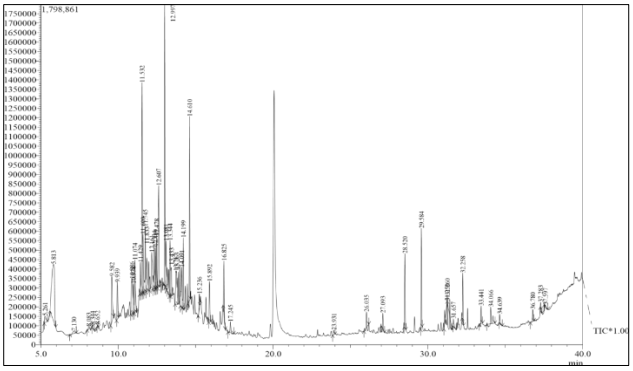

Fig 2 GCMS analysis of PSLA extract

GCMS analysis

GCMS analysis of PSLA extract showed the presence of 49 components at the different retention time and by interpretation of their mass spectra with 12 known bioactive principles (Fig 2, Table 3). Among these there are twelve major peaks which indicating the presence of major phytochemical constituents. From the forty-nine compounds identified, the most prevailing compounds were Methanamine N-[3-methyl-2-butenylid (10.74%), Dodecane (08.43%), Dodecane 4,6-dimethyl-(04.80%), Tetradecane (08.57%), Heptadecane (02.12%), Hexadecanoic acid, methyl ester (02.76%), Hexadecanoic Acid, Ethyl Es (03.54%) and Heptadecanoic Acid, 15-Meth (01.38%) (Table 3). The identification of the phytochemical compounds was confirmed based on the retention time, peak area and molecular formula. These 12 bioactive uses compounds namely Undecane, Naphthalene, Dodecane, Tetradecane, Hexadecane, 2,6,10,14-tetrame, Heptadecane, n-Pentadecanol, 2-Pentadecanone, 6,10,14-trimethyl, Hexadecanoic acid, methyl ester, Phytol, Heptadecanoic acid, ethyl ES and 9,12-Octadecadien-1-ol, (Z,Z)- were present in PSLA extract. The main properties of bioactive compounds are antimicrobial, carcinogenic, cytotoxic, antioxidant, anthelmentic, tumour, bronchitis, asthma, tuberculosis, dyspepsia, constipation, anemia, throat diseases, elephantiasis, antineurodegenerative, anti-psychotic, anti-convulsant, antidiabetic, anti-inflammatory, antidiarrheal, allelopathic activity, oligosaccharide provider, increase zinc bioavailability, skin care lotionand perfumery industry.

Table 3 Phytochemical constitutes with bioactivities of PSLA extract

R. time	Compound name	Molecular formula	Molecular weight	CAS No.	Bio active uses
9.939	Undecane	$C_{11}H_{24}$	156.31	1120-21-4	Antimicrobial, carcinogenic [40]
11.429	Naphthalene	$C_{10}H_8$	128.17	91-20-3	Anticancer, anti-microbial, anti-inflammatory, antiviral, antitubercular, antihypertensive, antidiabetic, antineurodegenerative, anti-psychotic, anti-convulsant and anti-depressant activities [41]
11.532	Dodecane	$C_{12}H_{26}$	170.34	112-40-3	Antibacterial activity [42]
12.997	Tetradecane	$C_{14}H_{30}$	198.39	629-59-4	Antimicrobial [43], Cytotoxicity [44] Antipyretic, Anthelmentic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabatic, Anti-inflammatory, Antidiarrhoeal [45]
14.199	Hexadecane, 2,6,10,14-tetrame	$C_{20}H_{42}$	282	638-36-8	Biomarkers in petroleum studies [46]
16.825	Heptadecane	$C_{17}H_{36}$	240.46	629-78-7	Antimicrobial activity [47]
26.035	n-Pentadecanol	$C_{15}H_{32}O$	228	629-76-5	Antioxidant, antimicrobial [46]
27.093	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268	502-69-2	Allelopathic activity, antibacterial [46]
28.520	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	112-39-0	Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antioxidant, Acidulant [40]
29.584	Hexadecanoic acid, ethyl es	$C_{18}H_{36}O_2$	284	628-97-7	Antioxidant, flavor, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor [48]
31.260	Phytol	$C_{20}H_{40}O$	296.5	150-86-7	Antinociceptive, Antioxidant, anticancer, antiinflammatory, antimicrobial, diuretic, chemopreventive properties [20]
31.657	9,12-Octadecadien -1-ol, (Z,Z)-	$C_{18}H_{34}O$	266.5	506-43-4	Oligosaccharide provider, increase zinc bioavailability [40]

Antioxidant activity

DPPH radical scavenging activity

The results of DPPH scavenging activity of PSLA extract were depicted in (Table 4). PSLA extract exhibited best antioxidant activity compared to standard ascorbic acid and rutin at different concentrations (15.62, 31.25, 62.5, 125, 250, 500 and 100 µg/ml). In a dose dependant manner, percentage of the antioxidant activity of PSLA extract was increased as increasing the concentration (Fig 3). The PSLA extract at a concentration of 1000 µg/ml showed a percentage inhibition 82.51% and for 500 µg/ml it was 72.23%. The ascorbic acid and rutin at a concentration of 1000 µg/ml exhibited a percentage inhibition was found to be 88.40% and 86.33% for 500 µg/ml it was noted as 77.48±1.11and 75.44±0.99 (Table 4). The 50% of inhibition concentration (IC_{50}) value of PSLA extract, ascorbic acid and rutin was found to be 107.31 µg/ml, 115.68 and118.47 µg/ml, respectively (Table 9; Fig 8). Degeneration study shows the good linear relation in plant extract towards concentration and inhibition action.

Table 4 Antioxidant activity of DPPH free radical by PSLA extract

Sample concentration (µg/ml)	Percentage of DPPH free radical inhibition		
	PSLA	Ascorbic acid	Rutin
1000	82.51±1.10^{b}	88.40±1.09^{a}	86.33±0.92^{a}
500	72.23±0.98^{b}	77.48±1.11^{a}	75.44±0.99^{a}
250	63.26±0.93^{b}	67.64±1.25^{a}	65.64±1.27^{a}
125	52.37±1.08^{b}	54.47±0.80^{a}	55.57±1.36^{a}
62.5	41.56±1.20^{b}	44.39±0.80^{a}	43.52±1.14^{a}
31.25	31.47±1.05^{b}	33.73±1.18^{a}	33.56±0.97^{a}
15.62	21.05±0.68^{b}	23.63±1.00^{a}	22.70±1.43^{b}

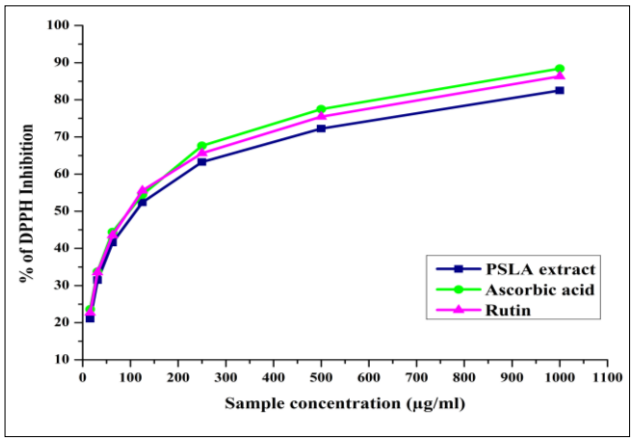

Fig 3 DPPH radical scavenging activity of PSLA extract

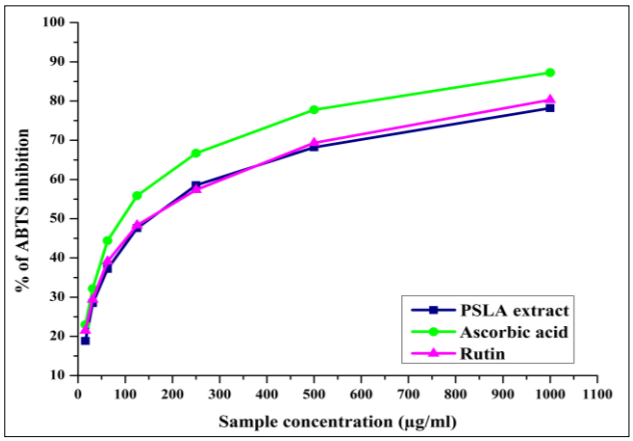

Fig 4 Antioxidant activity of ABTS free radical by PSLA extract

ABTS^{+} radical scavenging assay

To evaluate the antioxidant activity of the substrates PSLA extract, the ABTS^{+} coloring method is one of the assays. The results of ABTS^{+} scavenging activity of PSLA extract are presented in fig. 4 and the result demonstrated that the extract have a good ability to scavenge ABTS^{+} radicals with an IC_{50} value of PSLA extract 173.55 µg/ml, against the control ascorbic acid and rutin (104.75 and 105.98 µg/ml) (Table 9). The percentage of inhibition was achieved at 1000 µg/ml concentration with 77.30, 88.38 µg/ml and 86.35 percentages in PSLA extract and ascorbic acid, rutin control respectively (Table 5, Fig 4 & 8).

Table 5 Antioxidant activity of ABTS free radical by PSLA extract

Sample concentration (µg/ml)	Percentage of ABTS free radical inhibition		
	PSLA	Ascorbic acid	Rutin
1000	77.30±1.89^{b}	88.38±0.84^{a}	86.35±0.92^{a}
500	66.69±1.48^{b}	74.55±0.87^{a}	75.50±0.85^{a}
250	55.83±1.30^{b}	62.51±0.92^{a}	65.25±0.92^{a}
125	46.72±0.71^{b}	53.65±1.05^{a}	54.03±1.45^{a}
62.5	35.45±1.15^{b}	42.60±1.25^{a}	43.69±1.02^{a}
31.25	25.92±1.65^{c}	30.64±1.14^{b}	35.13±1.58^{a}
15.62	16.74±1.65^{b}	22.61±1.05^{a}	23.33±1.59^{a}

FRAP free radical scavenging activity

(Fig 5) represented the results of reducing power of solvent extract PSLA. In PSLA extract showed improved ferric reducing power activity with increasing concentrations (Fig 5). At the concentration of 1000 µg/ml, the highest absorbance is 77.76 in PSLA extract and standard ascorbic acid, rutin was 85.30%, 87.27%, whereas the second highest inhibition percentage was observed at 500 µg/ml concentrations in PSLA and standard, it is 66.46, 77.70 and 75.64% respectively (Table 6). The IC_{50} values of plant extract, standard ascorbic acid and rutin absorbance was 187.15, 105.37 and 107.23 µg/ml (Table 6, Table 9, Fig 5 & 8).

Table 6 Antioxidant activity of FRAP free radical by PSLA extract

Sample concentration (µg/ml)	Percentage of FRAP free radical inhibition		
	PSLA	Ascorbic acid	Rutin
1000	77.76±1.81^{b}	85.30±1.02^{a}	87.27±1.18^{a}
500	66.46±1.46^{b}	77.70±1.07^{a}	75.64±1.26^{a}
250	56.61±1.32^{b}	65.78±1.35^{a}	64.81±1.43^{a}
125	45.82±1.14^{b}	53.93±1.30^{a}	53.90±1.25^{a}
62.5	36.89±1.46^{b}	42.51±1.22^{a}	43.01±1.74^{a}
31.25	25.66±1.56^{b}	32.74±1.21^{a}	33.81±1.22^{a}
15.62	16.60±1.53^{b}	23.79±1.67^{a}	22.57±1.05^{a}

Hydrogen peroxide scavenging activity

The hydrogen peroxide radical scavenging activity of PSLA plant extract and its graphical representation is shown in (Fig 6). As per results, the maximum percentage of inhibition was recorded in PSLA extract with 78.19% at the concentration of 1000 µg/ml. This is followed by 68.22 at 500 µg/ml concentration, respectively (Table 7). The IC_{50}values in PSLA, ascorbic acid and rutin were 158.37, 104.58 and 139.58 µg/ml, correspondingly (Table 9, Fig 6 & 9).

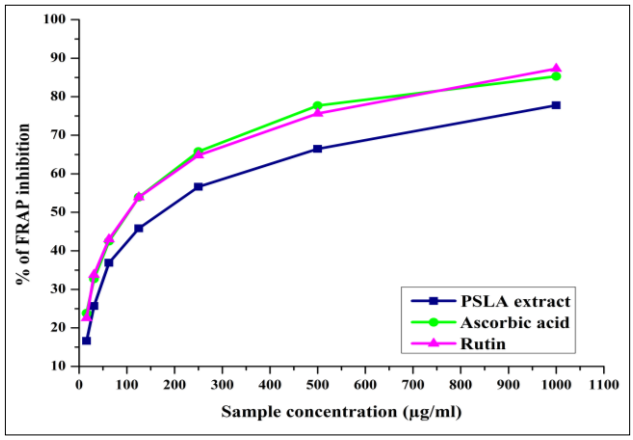

Fig 5 Antioxidant activity of FRAP free radical by PSLA extract

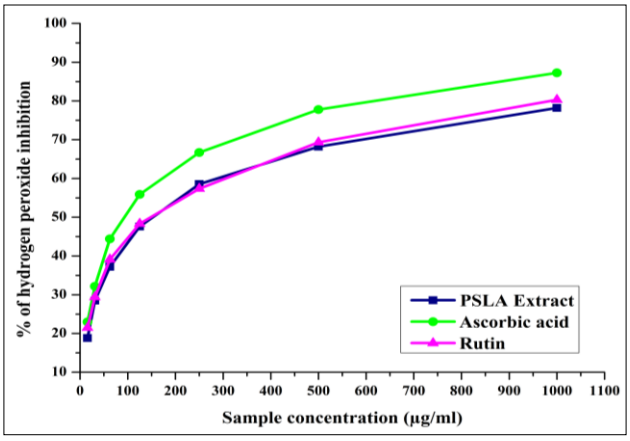

Fig 6 Antioxidant activity of hydrogen peroxide by PSLA extract

Table 7 Antioxidant activity of hydrogen peroxide by PSLA extract

Sample concentration (µg/ml)	Percentage of hydrogen peroxide free radical inhibition		
	PSLA	Ascorbic acid	Rutin
1000	78.19±1.06^{c}	87.26±0.81^{a}	80.30±1.08^{b}
500	68.22±1.00^{b}	77.76±1.58^{a}	69.32±0.87^{b}
250	58.54±0.78^{b}	66.70±1.41^{a}	57.36±1.00^{b}
125	47.58±1.14^{b}	55.87±1.50^{a}	48.31±0.97^{b}
62.5	37.20±0.83^{b}	44.41±0.77^{a}	39.11±0.59^{b}
31.25	28.50±1.34^{b}	32.17±0.58^{a}	29.41±0.90^{b}
15.62	18.82±1.04^{b}	22.99±1.50^{a}	21.48±1.16^{a}

Nitric oxide scavenging activity

The results observed for nitric oxide scavenging activity and the graphical representation is presented in (Fig 7). In the present study, PSLA extract at the concentration of 15.62, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml were studied in the nitric oxide scavenging activity. All the concentrations of extract have excellent inhibition percentage. Among this concentration, the highest inhibition percentage 78.25±0.73 was recorded at 1000 µg/ml concentration. The second maximum inhibition was reported 67.54±0.96 at 500 µg/ml concentration (Fig 7). The IC_{50} value was calculated using linear regression analysis was found to be 147.25±2.48, 135.85±1.25, and 136.12±1.96 µg/ml in PSLA extract, and standard ascorbic acid, rutin, respectively (Table 9, Fig 8). In PSLA extract, nitrogen reduction capacity was noticed as extract concentrations-dose dependent manner.

Table 8 Antioxidant activity of nitric oxide scavenging activity by PSLA extract

Sample concentration (µg/ml)	Percentage of nitric oxide free radical inhibition		
	PSLA	Ascorbic acid	Rutin
1000	78.25±0.73^{b}	82.42±1.27^{a}	81.31±0.99^{a}
500	67.54±0.96^{b}	68.47±0.67^{a}	68.31±1.00^{a}
250	59.23±0.77^{b}	58.36±1.23^{a}	58.52±1.23^{a}
125	48.35±1.17^{b}	49.27±0.96^{a}	49.28±0.77^{a}
62.5	37.36±1.01^{b}	37.21±0.89^{a}	37.39±1.01^{a}
31.25	28.39±1.14^{b}	28.39±0.65^{a}	28.36±0.82^{a}
15.62	18.59±1.02^{c}	19.50±0.88^{b}	20.63±0.73^{a}

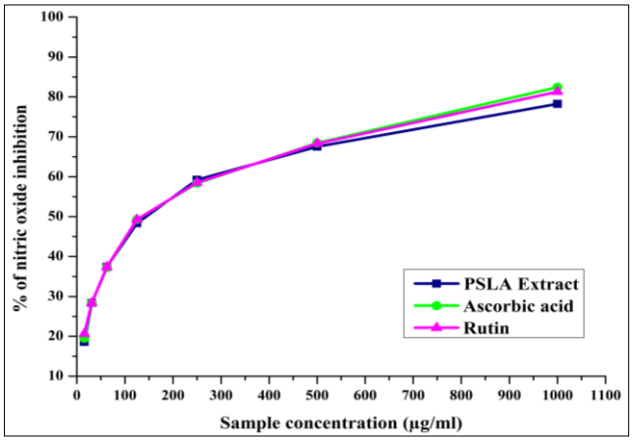

Fig 7 Antioxidant activity of nitric oxide scavenging activity by PSLA extract

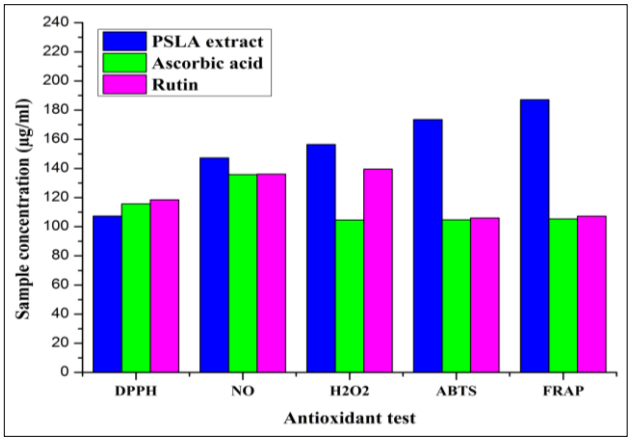

Fig 8 IC_{50} value of antioxidant activity of PSLA extract

Table 9 IC_{50} value of antioxidant activity of PSLA extract

Antioxidant test	IC_{50} value in µg/ml				
	DPPH	ABTS	FRAP	H_2O_2	NO
PSLA extract	107.31±1.33^{a}	173.55±1.39^{d}	187.15±1.38^{e}	158.37±1.33^{c}	147.25±2.48^{b}
Ascorbic acid	115.68±0.98^{b}	104.75±1.41^{a}	105.37±1.29^{a}	104.58±0.95^{a}	135.85±1.25^{c}
Rutin	118.47±1.41^{b}	105.98±1.75^{a}	107.23±2.48^{a}	139.58±1.12^{d}	136.12±1.96^{c}

Preliminary phytochemical results revealed the presence of alkaloids, flavonoids, tannins, steroids, triterpinoids, and saponins. However, glycosides, gum and mucilages, fixed oils and anthraquinones are absent. The presence of total phenolic and flavonoid contents was reported on some different Lamiaceae genera *Nepeta italic* and *Teucrium sandrasicum* [49]. The variety of phytochemicals present in the extracts of the plant may be responsible for its pharmacological properties [50]. Saponin-rich plant has analgesic, anti-inflammatory, and anti-edematous properties. Tannins and anthocyanins exhibit antioxidant, antimicrobial, and antiviral properties. The presence of total phenols, flavonoids, and alkaloids possess antiapoptotic, anti-ageing, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic properties and protect against cardiovascular diseases [51].

An FTIR spectrum is generally used in plant biological studies [52]. The functional constituent's presence in the extract, identification of medicinal materials from the adulterate and evaluation of the qualities of medicinal materials identified through FTIR analysis [53]. The present study FTIR analysis showed totally 17 peaks. In this the strong, medium, weak and sharp band was observed at 3865.35 cm^{-1}, 2931.80 cm^{-1}, 2306.86 cm^{-1}, 1612.4 $9cm^{-1}$, 1411.89 cm^{-1}, and 1355.60 cm^{-1}, 1026.13 cm^{-1}, 840.96 cm^{-1}, 702.09 cm^{-1} and 655.80 cm^{-1} respectively. Hence this result concluded that the PSLA extract has active functional groups like alcohols, aliphatic primary amines, amine salt, carbondioxide, alkene, halocompound etc. According to Janakiraman *et al.* [54], alkyl halides and alkanes in plant samples were found to be more significant against microbes.

GCMS analysis of PSLA extract showed the presence of 49 components at the different retention time and by interpretation of their mass spectra. Among these there are twelve major peaks which indicating the presence of major phytochemical constituents. GC-MS study of plant extracts was conducted to determine various biological properties, including antimicrobial activity [55], chemopreventive activity, antihelmintic activity and antiproliferative activity [56]. Arunkumar and Muthuselvam [4] investigated the non-polar fatty acid compositions of *Aloe vera* L. leaf extracts in aqueous, ethanol, and acetone. Bark and leaves of the *Cinnamomum* genus contain essential oils with wide range of applications, including antimicrobials in pharmaceuticals and antioxidants, spices, and flavoring agents in food [57]. In this analysis exhibited the presence of 49 compounds with 12 known bioactive principles. These 12 bioactive compounds namely Undecane with an Antimicrobial and carcinogenic activity [40], Naphthalene has Anticancer, anti-microbial, anti-inflammatory, antiviral, antitubercular, antihypertensive, antidiabetic, antineurodegenerative, and anti-depressant activities [41]. Dodecane have been reported to antibacterial activity [42]. Tetradecane with Antimicrobial [43], Cytotoxicity [44], Antipyretic, Anthelmentic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabatic, Anti-inflammatory, Antidiarrhoeal [45]. Hexadecane, 2,6,10,14-Tetrame has been various biological activities such as Biomarkers in petroleum studies [46]. Heptadecane with Antimicrobial activity [47], n-Pentadecanol have reported Antioxidant, antimicrobial activities [46], 2-Pentadecanone, 6,10,14-trimethyl showed allelopathic activity, antibacterial [46]. Hexadecanoic acid, methyl ester with Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antioxidant, Acidulant [40], Hexadecanoic Acid, Ethyl ES which has medicinal importance as an Antioxidant, flavor, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor [48], Phytol were also have Antinociceptive, antioxidant, anticancer, antiinflammatory, antimicrobial properties [20], 9,12-Octadecadien-1-ol, (Z,Z)-e have Oligosaccharide provider, increase zinc bioavailibility [40] were present in PSLA extract.

The antioxidant activity of DPPH, ABTS, FRAP, hydrogen peroxide and nitric oxide scavenging activities are reported in PSLA extract. This result is in accordance with the previously published antioxidant activity for *B. dracunculifolia* [58] (and *S. cretica*and *S. tmolea* extracts [59]. In a dose dependant manner, percentage of the antioxidant activity of PSLA extract was increased as increasing the concentration. Some other reports demonstrated that phenol [60-62] flavonoids [63], saponins [64] and tannins [65] have been pounds have been found to possess potent antioxidants, antimicrobial and anti-inflammatory activity. Free radicals could cause oxidative damages that are implicated in many health disorders. In several previous papers, plants prove potential antiradical effects through a different mechanism of action [66]. Therefore, in the plant antioxidant proprieties evaluation studies, the uses of different assays are essential to appreciate and understand exactly their antioxidant mechanism of action [67]. Studying some *Lamium* species including *Lamium maculatum*, *L. garganicum*, *L. purureum*, *Lamium album* and *L. eriocephalu*. All of them have a strong antioxidant potentiality and can be considered as a good source of natural antioxidants [68]. Additionally, noted that the plants of the same genera should have many uses (medical, pharmaceutical, industrial, and commercial), thus providing them with traditional applications in folk medicine [69]. Comparing with the *Lamium* genera; there are many more studies in the literature concerning *Stachys* genera. Sarikurkcu *et al*. [70] revealed that both ethyl acetate and methanol extracts of seven *Stachys* species (*S. alpine*, *S. officinalis*, *S. palustris*, *S. recta subsp. recta*, *S. recta subp. subcrenata*, *S. salviifolia* and *S. sylvatica*), as well as both *S. byzantine* and *S. iberica* ethyl acetate, methanol and water extracts, exhibit a powerful free antiradical propriety determined by DPPH, FRAP, phosphomolybdenum, superoxide anion and β-carotene bleaching assays; when the IC_{50} values are comparable to our results.

CONCLUSION

The present study was to analyze phytochemical screening, FTIR, GC–MS and antioxidant activity of PSLA extract. The extract has various phytocompounds which have many pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial and anticancer activities, etc. Further investigations are needed for antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. The study exhibited the presence of various useful compounds in the extract. The availability of various bioactive principles clearly precedes the purpose of *Pogostemon speciosus* for various diseases. However, isolation and purification of compounds may proceed to find new active drug.

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