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Phytochemical profiling of cat whisker's (*Orthosiphon stamineus*) tea leaves extract

Chitra Sivakumar and Karthikeyan Jeganathan

Abstract
Plants are renowned in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. Herbal medicines have gained magnitude in recent years because of their value and cost effectiveness and due to the serious complications of synthetic drugs. The objective of the present study is to scrutinize the phytochemicals present in the *Orthosiphon stamineus.* The bioactive profiling was done by preliminary phytochemical test for secondary metabolites, GCMS for volatile constituents and LCMS for nonvolatile constituents. HPLC analysis showed the presence of flavonoids. The GCMS and LCMS analysis shows the presence of many compounds with medicinal value. Thus the present study will make a way for the production of herbal medicines for various ailments by using this plant. Due to the various bioactive components the plant also can act as a potential source for the green synthesis of nanoparticles.

Keywords: *Orthosiphon stamineus*, GCMS, LCMS-MS, phytochemical screening

1. Introduction
The Plant still leftovers a major source for drug discovery in advance of synthetic molecules. The World Health Organization anticipated that about 80% of the world population relays on herbal medicine [1]. Substances resulting from the plants remain the basis for a large proportion of the commercial medications used today for the management of heart disease, high blood pressure, pain, asthma and infectious diseases [2]. Nowadays medicinal plants obtain more interest to researchers because of their safety and restorative property which is due to the complex mixtures. O*rthosiphon stamineus* is usually known as *misaim kucing. Cat's whiskers* and *kumis kucing*. *O. stamineus* is commonly grown in Southeast Asia and the tropical countries. Leaves of this plant are known as "Java tea" and are mostly used for the purpose of making herbal tea commonly in Southeast Asia and European countries [3]. Additional names for *O. stamineus* include *Orthosiphon aristatus*, *Orthosiphon spicatus*, *Orthosiphon blaetter*, *kumis kucing*, *Indischer Nierentee*, *Feuilles de Barbiflore*, and *de Java*. *Orthosiphon* species is categorized into two varieties: one with the white flowers (white variety) and the other with the light purple flowers (purple variety). Purple variety contains more bioactive compounds than the white one. Normally, the leaves and stem tips have medicinal values. Due to this property, this plant has extensively been conquered customarily to treat several human ailments and conditions such as diuretic, rheumatism, abdominal pain, kidney and bladder inflammation, edema, gout, and hypertension [4-6]. The leaves of *O. stamineus* exhibit brilliant pharmacological activities such as antioxidant, antibacterial, hepatoprotective, anti-inflammatory, cytotoxic, antihypertensive, and vasodilatation [7-9]. Previous report showed that this plant comprises high amount of flavones, polyphenols, bioactive proteins, glycosides, a volatile oil, and vast quantities of potassium [10]. The non-nutritive plant chemicals are termed as phytochemicals which have the properties to protect or prevent diseases. Plant yields these chemicals to protect themselves but the research have shown that they have the capacity to treat human diseases in an active way [11]. There are thousands of phytochemicals, each have their pharmacological properties of their own [12]. Keeping in view of the medicinal value of the plant the present study was implement to actuate the phytocompounds in the aqueous leaf extract of *O. stamineus* by qualitative screening of phytochemicals and to identify each specific compound with their concentrations by HPLC, Gas Chromatography – Mass Spectrum (GCMS) and LCMS-MS analysis.

2. Materials and Methods
2.1 Plant material Collection and Authentication
The Cat whisker's plant (*Orthosiphon stamineus*) plant was collected from an Organic farm. Salem District, Tamil Nadu, India and it has been identified and authenticated by Dr. S. Sahaya Satheesh, Associate Professor, Department of Botany, St. Joseph's college, Trichy, Tamilnadu, India.

2.2 Preparation of the crude plant extract
The dried leaves were blended using a blender and the powder was stored in a clean glassware container for further analysis. 50 grams of powdered leaves were mixed in 300 ml of distilled water in a clean beaker and was stored in a glass container. The contents were mixed by stirring for 15 minutes. It was kept undisturbed for overnight after 24 hours the mixture was then filtered using filter paper (what man no1). The filtrates were then used for further assay. The yield of the aqueous extract was 14% w/w.

2.3 Phytochemical analysis
The primary phytochemical screening test was carried out in aqueous extract of *O. stamineus* to find out the nature of chemical compounds as per the standard procedures [13]. The amount of phenol in the aqueous extract was determined by Folin-Ciocalteau reagent method with some modifications. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) [14]. Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. The results were determined from the standard curve and were expressed as quercetin equivalent (mg/g) of extracted compound [15]. The flavonoids present in the java tea leaves extract were identified through High performance Liquid chromatography. Finally the phyto constituents were identified through GCMS [16] and LCMS-MS [17].

UHPLC Conditions
UHPLC system (Shimadzu Corporation, Kyoto, Japan), Equipped with two Shimadzu UHPLC: Nexera UHPLC system Column: Shim-pack XR-ODS III (100 x 2 mm, 2.2 µm particle size) Column temp. 40 ºC. Mobile phase: (A) 0.1% formic acid in water and (B) Acetonitrile Both mobile phases were filtered through a cellulose nitrate filter, diameter 47 mm, pore size 0.45 µm (Sartorius, Goettingen, Germany). After the gradient separation, the column was re equilibrated for 5 min using the initial solvent composition. Flow rate: 1 mL/min, the samples were kept in amber vials at 4 °C in the autos ampler, and the injected volume was 5 µL. The separation was performed at 25.0 ± 0.1 °C.

GC Programme
Column BR-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm ID x 0.25µm df, Equipment Scion 436-GC Bruker, Carrier gas 1ml per min, Split 0:1,Detector TQ Quadrupole Mass Spectrometer, Software MS Work Station 8, Sample injected 2µl, Oven temperature Programme -110 °C hold for 3.50 min, Up to 200 °C at the rate of 10 °C/min-No hold, Up to 280 °C at the rate of 5 °C / min- 12 min hold, Injector temperature 280 °C, Total GC running time: 40.50 min

MS Programme
Library used NIST Version-11, Inlet line temperature 290 °C, Source temperature 250 ° C
Electron energy 70 Ev, Mass scan (m/z) 50-500 amu, Solvent Delay 0 - 3.5 min, Total MS running time: 40.50 min

MS/MS Conditions
LC-MS/MS System (Make: Shimadzu Corporation, Kyoto, Japan, Model LCMS 8040, Triple Quadrupole). Ionization: ESI (Positive / Negative). Ion spray voltage: +4.5 kV / −3.5 kV. MRM: 427 MRM transitions (2 MRMs / compound). Dwell time 5 msec. / Pause time 1 m sec. Ambient CDL Temperature: 250 ºC, Block Temperature: 400 ºC. Detector voltage: 1.3kv. Nebulizer Gas flow: 1.5 l/min drying gas: 10 L/min Detection.
The mobile phase was filtered through a 0.22 µ membrane and degassed using ultrasonicator.

3. Results and Discussion

Table 1: Preliminary Phytochemical screening of the aqueous extract of *O. stamineus* leaves

Test	Aqueous extract
Alkaloids	++++
Flavanoids	++++
Steroids	+
Cardiac glycosides	+
Terpenoids	+
Tannins	+++
Saponins	++
Reducing sugar	+
Anthraquinone	++
Phenol	++++
Carbohydrates	++

+ = detected, - = not detected

Table 2: Total Phenols and Flavonoids of the *O. stamineus* leaves extract

Plant	Part	Total Flavanoids Mg QE/100gm	Total phenolics Mg GAE/100gm
O. stamineus	Leaves	40.0	18.75

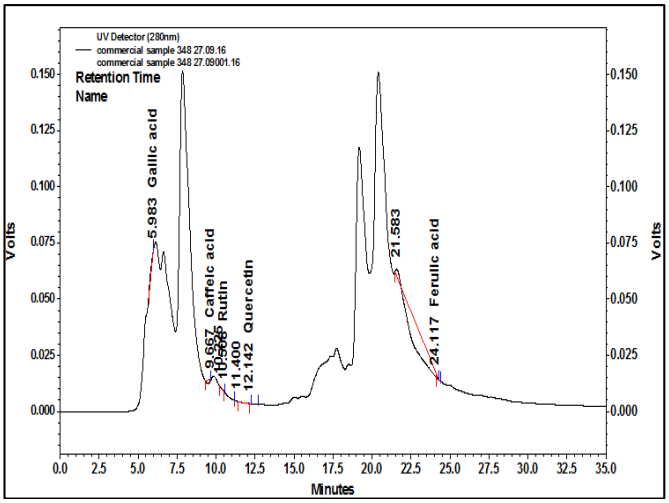


Fig 1: HPLC Chromatogram of flavonoids in *O. stamineus* leaves extract

Table 3: Components identified in HPLC of *O. stamineus* leaves extract

UV Detector (280nm)					
1	Retention Time	Area	Height	Concentration (mg/kg of sample)	Name*
1	5.983	35639	249	0.6	Gallic acid
2	9.667	24645	132	0.14	Caffeic acid
3	10.508	40230	2	0.9	Rutin
4	12.142	2957	54	0.2	Quercetin
5	24.117	9618	1940	0.6	Ferulic acid

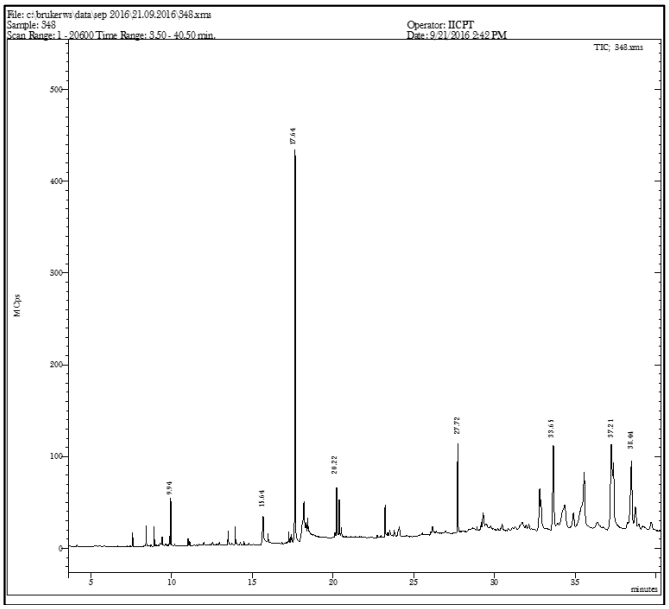


Fig 2: GCMS Chromatogram of *O. stamineus* Leaves extract

Table 4: Bioactive Components of *O. stamineus* identified in GCMS

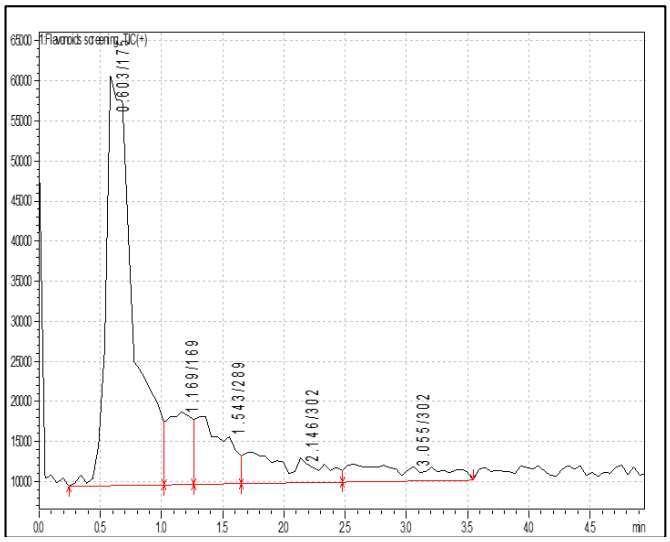
No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	4.13	1H-Pyrrole, 2,5-dihydro-	C_4H_7N	69	0.14
2.	7.26	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	$C_{11}H_{18}$	150	0.08
3.	7.58	γ-Elemene	$C_{15}H_{24}$	204	0.47
4.	8.25	α-ylangene	$C_{15}H_{24}$	204	0.03
6.	8.72	β-Guaiene	$C_{15}H_{24}$	204	0.07
7.	8.9	Caryophyllene	$C_{15}H_{24}$	204	0.6
8.	9.03	β-copaene	$C_{15}H_{24}$	204	0.03
9.	9.28	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	$C_{15}H_{24}O$	220	0.18
10.	9.41	Humulene	$C_{15}H_{24}$	204	0.35
11.	9.62	4,5-di-epi-aristolochene	$C_{15}H_{24}$	204	0.09
12.	9.87	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	$C_{15}H_{24}$	204	0.32
13.	9.94	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1α,3aβ,4α,7β)]-	$C_{15}H_{24}$	204	1.55
14.	11.01	β-Vatirenene	$C_{15}H_{22}$	202	0.25
15.	12	α-acorenol	$C_{15}H_{26}O$	222	0.1
16.	12.52	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	0.15
17.	13.5	Androstenedione	$C_{19}H_{26}O_2$	286	0.55
18.	13.92	3,7,11,15-Tetramethyl-2-hexadecen-1-o	$C_{20}H_{40}O$	296	0.6
19.	14.49	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4aα,7β,8aβ)-	$C_{14}H_{24}O$	208	0.14
20.	15.67	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.12
21.	15.96	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.3
22.	17.25	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.5
23.	17.4	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	0.41
24.	17.64	Phytol	$C_{20}H_{40}O$	296	17.35
25.	18.18	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	6.14
26.	18.42	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	1.83
27.	20.22	Z,E-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	2.26
28.	20.37	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	1.82
29.	23.21	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254	1.61
30.	24.07	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	0.85
31.	27.72	Squalene	$C_{30}H_{50}$	410	3.87
32.	29.3	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.98
33.	32.78	Vitamin E	$C_{29}H_{50}O_2$	430	5.94
34.	33.65	4H-1-Benzopyran-4-one, 5,6,7-trimethoxy-2-(4-methoxyphenyl)-	$C_{19}H_{18}O_6$	342	9.21
35.	34.33	Pregn-4-en-18-oic acid, 11-(acetyloxy)-7,9,20-trihydroxy-3-oxo-, γ-lactone, (7α,11α,20R)-	$C_{23}H_{30}O_7$	418	4.21
36.	35.54	Stigmasterol	$C_{29}H_{48}O$	412	11.67
37.	37.21	β-Sitosterol	$C_{29}H_{50}O$	414	10.33
38.	38.44	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-	$C_{20}H_{20}O_7$	372	8.67
39.	38.7	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	424	2.89
40.	39.67	α-Amyrin	$C_{30}H_{50}O$	426	0.62

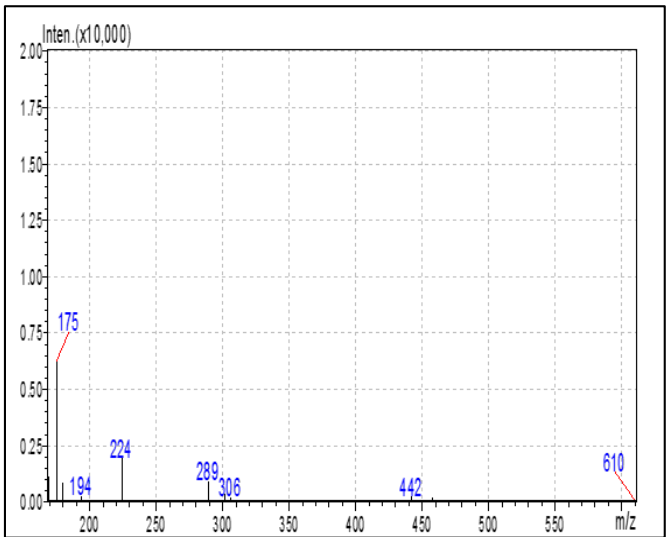
Table 5: Activity of the identified compounds of *O. stamineus*

Name of the compound	Compound Nature	**Activity
1H-Pyrrole, 2,5-dihydro-	Alkaloid	Antimicrobial, anti-inflammatory
Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	Aromatic compound	No activity reported
γ-Elemene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
α-ylangene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
α-Bulnesene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
β-Guaiene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
Caryophyllene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
β-copaene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	Sesquiterpene oxide	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
Humulene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
4,5-di-epi-aristolochene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1α,3aβ,4α,7β)]-	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
β-Vatirenene	Alkene compound	No activity reported
α-acorenol	Sesquiterpene alcohol	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	Flavonoid fraction	Antimicrobial, Anti-inflammatory
Androstenedione	Ketone compound	No activity reported
3,7,11,15-Tetramethyl-2-hexadecen-1-o	Terpene alcohol	Antimicrobial, Anti-inflammatory
2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4aα,7β,8aβ)-	Polyaromatic compound	No activity reported
n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 9.5-Alpha reductase inhibitor
Hexadecanoic acid, ethyl ester	Palmitic acid ethyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 9.5-Alpha reductase inhibitor
9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Anti androgenic, Hypocholesterolemic, 5-Alpha reductase, inhibitor, Antihistaminic. Anticoronary, Insectifuge, Antieczemic, Antiacne
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	Ester compound	No activity reported
Phytol	Diterpene compound	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid	Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne
Ethyl 9,12,15-octadecatrienoate	Linolenic acid ester	Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne
Z,E-2,13-Octadecadien-1-ol	Unsaturated alcoholic compound	No activity reported
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Unsaturated alcoholic compound	No activity reported
1,2-15,16-Diepoxyhexadecane	Alkane compound	No activity reported
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Alkane compound	No activity reported
Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
Ethyl iso-allocholate	Steroid	Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma, Hepatoprotective, Diuretic
Vitamin E	Vitamin E	Antiageing, Analgesic, Antidiabetic, Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic

		Antiulcerogenic, Vasodilator, Antibronchitic, Anticoronary, Antispasmodic.
4H-1-Benzopyran-4-one, 5,6,7-trimethoxy-2-(4-methoxyphenyl)-	Coumarin compound	Antimicrobial, Anti-inflammatory
Pregn-4-en-18-oic acid, 11-(acetyloxy)-7,9,20-trihydroxy-3-oxo-, γ-lactone, (7α,11α,20R)-	Steroid	Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma, Hepatoprotective, Diuretic
Stigmasterol	Steroid	Antioxidant Anti-inflammatory Sedative Antihepatotoxic Caner-preventive Antiviral Ovulant Hypocholesterolemic Estrogenic Artemicide
β-Sitosterol	Steroid	Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma, Hepatoprotective, Diuretic
4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-	Coumarin compound	Antimicrobial, Anti-inflammatory
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	Ketone compound	No activity reported
α-Amyrin	Triterpene	Antimicrobial, Anti-inflammatory, Anticancer

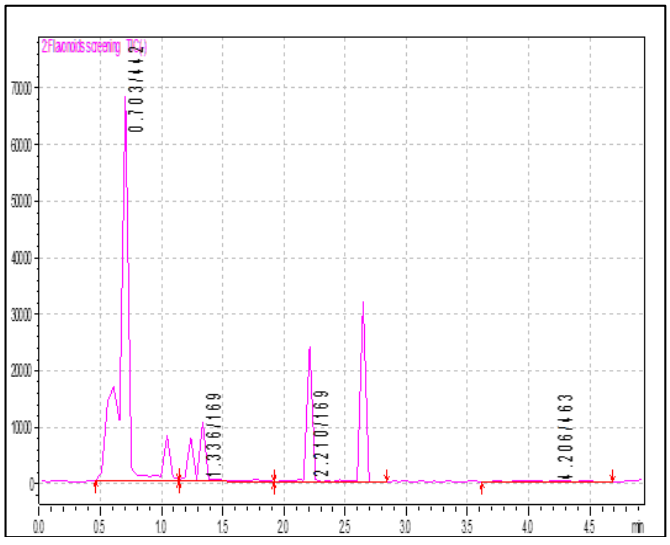
****Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases

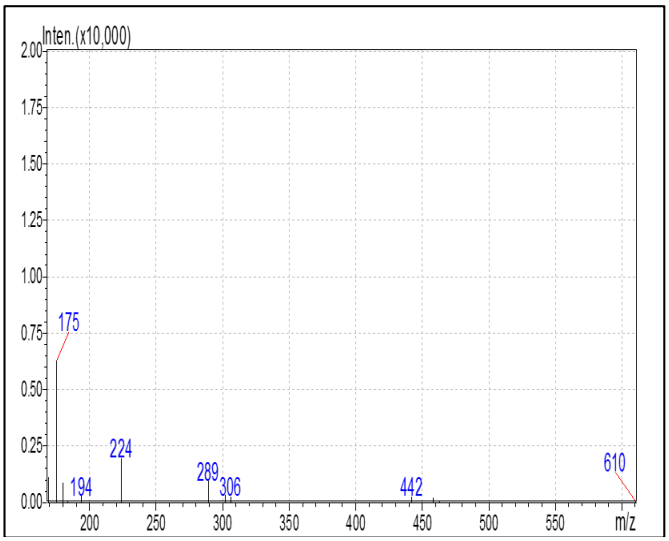




a) Positive Ionisation Chromatogram b) Negative ionisation chromatogram

c) Positive Ionisation spectrum





b) Negative Ionisation chromatogram

d) Negative Ionisation spectrum

Table 6: Compound screened by LCMS/MS of *O. stamineus* leaves Extract

S. No.	Compound	(Parent Ion) m/z	Rt	[M + H]		[M − H]	
				Positive Ionization		Negative Ionization	
				Absolute Intensity	Relative Intensity	Absolute Intensity	Relative Intensity
1.	Gallic acid	169	0.607	782	21.48	849	84.9
2.	Theanine	174.2	0.471	0	0	437	43.7
3.	Theobromine	180.164	0.505	3640	100	487	48.7
4.	Theophylline	180.164	0.505	774	21.26	330	33
5.	Caffeic acid	183.16	0.471	87	2.39	1	0.1
6.	Caffeine	194.19	0.514	287	7.88	40	4
7.	Ferulic acid	194.18	0.514	1073	29.48	111	11.1
8.	Theacrine	224.22	0.498	724	19.89	399	39.9
9.	Catechin	290.26	0.594	418	11.48	569	56.9
10.	Quercetin	302.236	0.612	70	1.92	1000	100
11.	EpiGallo Catachin	306.26	0.567	423	11.62	654	65.4
12.	catechin gallate	442	0.557	140	3.85	547	54.7
13.	Epicatachin gallate	458.37	0.536	136	3.74	553	55.3
14.	Quercetin hexoside	463	0.533	7	0.19	820	82
15.	Rutin	610.52	0.594	782	21.48	849	84.9

3. Discussion

In this study, the initial phytochemical test revealed the presence of Alkaloids, Carbohydrates, Glycosides, Steroids and Flavonoids. Through GCMS forty compounds and through LCMS analysis fifteen components were identified, in which nearly thirty compounds have biological activity. The GC-MS reports of *Orthosiphon stamineus* leaves revealed the presence of phytoconstituents that contribute the medicinal property of the plant., supported by the earlier reports [18].The results of preliminary phytochemical analysis (Table 1) and GCMS and LCMS analysis results were shown in Table 4, 5 & 6.The Chromatogram of GCMS and LCMS were shown in Figure 2 and 3.

GCMS chromatogram of the aqueous leaves extract of *Orthosiphon stamineus* (Figure 2) clearly showed forty one peaks indicating the presence of forty bioactive compounds. The documentation of the bioactive compounds was based on the peak area, retention time and molecular formula. The compound name with its molecular formula, Retention time, Peak area and % Peak area along with their pharmacological activity were represented (Table 5). The phytocompounds with pharmacological property identified are such as hexadecanoic acid, ethyl ester possess antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, histaminic, anti-eczemic, anti-acne, alpha reductase inhibitor, anti-androgenic, antiarthritic, anticoronary. Cosmetics/antipsychotic, medication/Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavour [19], Phytol found to be effective indifferent stages of arthritis, antimicrobial, antiinflammatory, antioxidant, diuretic, antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic, antimycobacterial activity against mycobacterium tuberculosis [20]. Octadecanoic acid, ethyl ester possess anti-inflammatory [21]. Stigmasterol possess anti-inflammatory, inhibit tumor promotion and anti-HIV reverse transcriptase [22, 23]. Vitamin E possess anti dermatitic. Anti-leukemic, Antitumor, Anticancer, Hepato protective and Antispasmodic [24]. N-Hexadecanoic acid possess antibacterial and antifungal activity [25]. These reports are in accordance with this study.

4. Conclusion

The present study has revealed the presence of various phytochemical constituents of aqueous leaves extract of *Orthosiphon stamineus*, which have potent pharmacological and biological property. This will support the researchers to carry out the research based on the active principles existing and to confirm the pharmacological activity with mechanism, this may support the use of the plant in folk medicine. Based on the pilot study we suggest that the *Orthosiphon stamineus* could be a potential source for treating various diseases and also have the potential to act as a reducing agent in the green synthesis of nanoparticles. The future success of the pharmaceutical industry depends on the identification of new compounds with novel activities or directed to more specific targets. The rapidly growing amount of secondary metabolite gene clusters identified and characterized provides new genetic tools for the generation of novel compounds by combinatorial biosynthesis.

5. Acknowledgement

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