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GCMS, phytochemicals and antioxidant activities of *in vitro* callus extracts of *Strobilanthes kunthiana* (Nees) T. Anderson *ex* Benth: An endemic plant of Acanthaceae

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Abstract. *Strobilanthes kunthiana* (Neelakurinji) is an endemic and underexploited plant belongs to the Family Acanthaceae. The aim of our study was to evaluate phytochemical analysis, GC-MS analysis and antioxidant activity of *in vitro* callus extract of *Strobilanthes kunthiana.* In this present study the phytochemical analysis of various extract of *S. kunthiana in vitro* callus were studied. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, terpenoids and phenols in the methanolic extract comparing to the other extracts. In this study GC-MS analysis revealed the presence of 10 bioactive phytochemical compounds were identified in the methanolic extract. The prevailing compounds were 9,12-octadecadienoic acid (Z,Z) (50.32%), hexadecanoic acid, methyl ester (20.69%), 9-octadecenoic acid (Z)-,methyl ester (10.45%), heptadecanoic acid, 16-methyl-, methyl ester (5.78%), 2,6-bis(1,1-dimethylethyl)-4-methyl phenol (4.70%). 3-methyl-2-ketobutyric acid tbdms (2.79%), 2,2,3,4-tetramethyl-5-hexen-3-ol (2.41%), N-(tert-butoxycarbonyl)-2-(4-methoxyphenyl) allylamine (1.06%), cyclotrisiloxane, hexamethyl (0.94%), benzenesulfonamide (0.87%). The antioxidant property was evaluated for methanol and ethanolic extract by DPPH method. The higher percentage of inhibition (79.23 ± 0.37) was observed in 250 µg/mL of ethanol extract followed by (90.35 ± 0.54) methanolic extract against the standard ascorbic acid (91.25 ± 0.33). The results show that the methanolic extract possesses more antioxidant activity than ethanol. The plant *S. kunthiana* may be exploited as a source of natural antioxidant and as herbal alternatives for various disorders.

Keywords: *In vitro* callus; Phytochemical; GC-MS analysis; DPPH; Antioxidant.

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Introduction

India has a rich culture of medicinal herbs which includes about 8,000 species of known medicinal plants and about more than 2,000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani,

and Siddha medicines but only very few have been studied chemically and pharmacologically for their potential medicinal values (Guptha et al., 2005). The phytochemical produced from medicinal herbs are curative constituents for several diseases. The plant natural compounds used as alternative sources of medicines continuous to play major roles in the general wellness of people all over the world. Plants have been provided mankind with sources of medicinal agents, natural products, once serving as the source of all drugs (Balandrin et al., 1993). It plays an important role in searching for novel drugs based on their new modes of pharmacological actions. This action may due to the presence of carbohydrates, carbohydrate derivatives, gums, mucilage's, pectin's, glycosides tannins, phenolic compounds, lipids, fixed and volatile oils, resins, alkaloids, terpenoids flavonoids, steroids etc.

Nowadays there was a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of phytochemicals. GCMS is the most commonly used techniques for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Hites, 1997).

A variety of these plant secondary metabolites have been reported to act as antioxidants and amongst them phenolic compounds form a major group. Antioxidants may have defined as free radicals scavengers which protect cells against damage.

Strobilanthes kunthiana (Nees) T. Anderson *ex* Benth locally known as Neelakurinji belongs to the family Acathaceae. It is a shrub that grow abundantly in the Shola forest of Western Ghats in South India (Paulsamy et al, 2007; Moylan et al, 2004) at 1300-2400 metres above Mean Sea Level (MSL) and endemic to Western Ghats. The plant belongs to the genus *Strobilanthes* which was first scientifically described by Christian Gottfried Daniel Nees von Esenbeck in India in the 19th century (Anonymous I and II). The plant grows 2m tall, bushy with reddish stout branches, hairless and glabrous. Leaves are base acute, margin crenate- serrate, dense hispid except veins above, floccose hispid below. The inflorescence spike branched or unbranched with many flowers. Bracts are elliptic to ovate, white villous, midrib not prominent. Bracteolate which are lanes shape, 10 mm long shorter than calyx. Calyx are lobed, divided almost half from the base, floccose- villous, linear-lanceolate. Corolla tubular ventricose portion, hispid inside and glabrous outside. Androecium are staminal filaments not grooved, pilose hispid. Gynoecium style 15mm ovary hairy at the apex. Fruit oblong, 4 seeded (Augustine et al., 2017).

S. kunthiana is well known to possess both ornamental and medicinal properties. The abundant source of unique active components shows anti-inflammatory, anti-osteoarthritic (Desu et al., 2011), analgestic properties (Desu et al., 2012) anticancer activity and antioxidant (Singh et al., 2014), antibioflim activity (Everlyne et al., 2016), enzyme inhibitor, central nervous depressant activity (Rajasekaran et al., 2000), antigiardial activity (Singh et al., 2012) antifungal, antibacterial, antiseptic, hypocholesterolemic 5-alpha reductase inhibitor, antimicrobial, cytotoxicity, protect skin against UV (Everlyne et al., 2015).

The aim of our study was to evaluate phytochemical analysis, GC-MS analysis and antioxidant activity of *in vitro* callus extract of *Strobilanthes kunthiana*.

Materials and methods

The application of biotechnological principles for the establishment of phytochemical, GC-MS analysis, antioxidant activity of *in vitro* callus of *Strobilanthes kunthiana* have been studied by following methods as given below.

Plant material and surface disinfestation

The plant material was collected in seed from Yercaud hills at altitude of ±1623m, in Eastern Ghats, Selam Dt., Tamil Nadu, India. Seeds were collected and used for various experiments for developing the protocol for explant preparation and regeneration. The collected seeds were washed with running tap water for 15 min and then washed with Tween 20 detergent solution (5% v/v) for 5 min. Surface sterilization of seeds was followed by rinsing with sterile distilled water 3 or 4 times to remove trace of detergent, rinsing in 70% ethanol for 30 s and finally treated with mercuric chloride (0%-12% W/V) ($HgCl_2$) for 3 min duration.

Culture medium and culture conditions

A culture medium containing MS salt supplemented with macro elements, micro elements, iron, vitamins, amino acid and 3% sucrose (Hi-Media, India) was used. The pH of the medium was adjusted to 5.8 by 1N NaOH or 1 N HCl after adding the growth regulators. The media were steam sterilized in an autoclave under 15 psi and 121 °C for 20 min. All of the cultures were incubated under 50 $\mu mol^{-2}S^{-1}$ light provided by cool while fluorescent lamp for a photo period of 16 h at 25 ± 2 °C.

Callus initiation

Node, internodes and leaf explants from *in vitro* grown plants from seeds were used as primary explants. The explants were cultured on MS medium supplemented with various concentrations of growth regulators (BAP, 2, 4 D and NAA). Twenty explants were used for each culture. The percent of explants responding for callus induction, nature of callus and number of days taken for callus induction were recorded after 40 days (Plate 1A-C).

PLATE-1

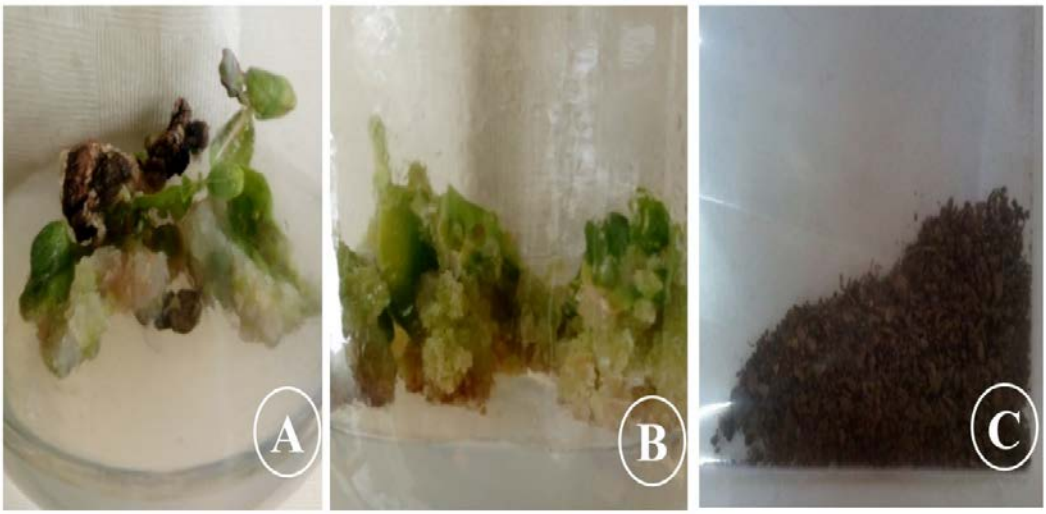


Plate 1– Callus initiation: A) Initial stage, B) Final stage, C) Callus powder.

Callus frequency

The percentage of callusing was recorded at the end of fifth week. Frequency of callus induction was calculated as shown below and was represented as percentage

$$\text{Frequency of response (\%)} = \frac{\text{Number of explants responded}}{\text{Total number of explants cultured}} \times 100$$

Preparation of extract

The leaf derived 30 days old *in vitro* callus collected from our laboratory was air dried and powdered and stored in room temperature. One gram of callus sample in 5ml of ethanol, methanol, chloroform, petroleum ether and water were added separately. It was then kept on a rotary shaker at 150-220 rpm for 24 h. The extracts were centrifuged at 2500rpm, the supernatant was collected. This were repeated for three times.

Phytochemical studies

The *in vitro* callus extracts were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material. Condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, and phenols (Gibbs, 1974); glycosides, terpenoids and saponins (Ayoola et al., 2008); tannins (Treare and Evans, 1985); flavonoids (Peach and Tracey, 1956).

Gas chromatography mass spectrometry analysis

The shade-dried 2 g powder of *in vitro* callus subjected to extraction in Soxhlet extractor with 70% methanol for 70 h (extract yield: 9%) and extract is collected. The collected extract is evaporated to dryness and stored at 4 °C until used.

The GC-MS analysis was 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 Turbomass 5.2 spectrometer with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30 m x 0.25 µm DF of capillary column. The instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 200 °C 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.

Antioxidant activity

The present investigations on *in vitro* callus extracts of *S. kunthiana* was carried out through antioxidant activity, were attempted.

DPPH Radical Scavenging activity

The antioxidant activity of the methanolic and ethanolic extract of *in vitro S. kunthiana* callus extract was measured on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical according to the method described by (Brand-Williams et al., 1995). 1.5 mL of 0.1 mM DPPH solution in methanol was mixed with 1mL of methanol and ethanol extract solution of varying concentrations (50, 100, 150, 200 and 250 µg/mL). Corresponding blank

sample were prepared and L-ascorbic acid (50-250 µg/mL) was used as reference standard. Mixer of different concentration 50-250 µg/mL methanolic and ethanolic and 1.5 mL DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 min in dark using UV-Vis spectrophotometer. The inhibition percentage was calculated using the following formula.

$$\text{Inhibition \%} = \text{Ac-As/Ac} \times 100$$

Where: Ac is the absorbance of the control
As is the absorbance of the sample

Result

In the present study preliminary phytochemical analysis of methanol, ethanol, petroleum ether, chloroform and water extracts of *Strobilanthes kunthiana* leaf callus are presented in the table 1. Qualitative phytochemical analysis of this *in vitro* leaf callus confirm the presence of various secondary metabolites. According to chemical test methanolic extract showed positive test with all the compounds like alkaloids, Flavonoids, Glycosides, Saponins, tannins, steroids, Terpenoids and Phenols. Both ethanolic and petroleum ether extracts showed the absence of glycosides and saponins. Followed by chloroform extract showed positive test with alkaloids, glycosides, saponins, tannins, steroids and phenols. Similarly, water extract showed positive to alkaloids, glycosides, tannins, steroids and phenols. It was concluded that the *in vitro* leaf callus of *S. kunthiana* extract contains important constituent for pharmacological activities.

Table 1. Qualitative phytochemical analyses of *S.kunthiana* methanol *in vitro* callus extract.

	Phytochemical test in methanol extract	**Inference**				
		Methanol	**Ethanol**	**Petroleum Ether**	**Chloroform**	**Water**
1	Alkaloids	+	+	+	+	+
2	Flavonoids	+	+	+	-	-
3	Glycosides	+	-	-	+	+
4	Saponins	+	-	-	+	-
5	Tannins	+	+	+	+	+
6	Steroids	+	+	+	+	+
7	Terpenoids	+	+	+	-	-
8	Phenols	+	+	+	-	-

The components present in the methanolic extract of callus of *S. kunthiana* were identified by GCMS (Figure 1). The ten compounds were detected belonging to various group like linoleic acid, palmitic acid methyl ester, unsaturated fatty methyl ester, stearic acid, lipophilic, allyl amino butane and phenolic compound. The active principle with their retention time (RT), Compound name, molecular formula, molecular weight, peak area (%), and activities related with medicinal uses are given in the (Table 2). The result

revealed that the presence of 9,12-octadecadienoic acid (Z,Z) (linoleicacid) (50.32%) shows highest percentage followed by hexadecanoic acid, methyl ester (palmitic acid methyl ester) (20.69%), 9-octadecenoic acid (Z)-,methyl ester (unsaturated fatty methyl ester) (10.45%), heptadecanoic acid, 16-methyl-, methyl ester (stearic acid) (5.78%), 2,6-bis(1,1-dimethylethyl)-4-methyl phenol (lipophilic) (4.70%), 3-methyl-2-ketobutyric acid tbdms (2.79%), 2,2,3,4-tetramethyl-5-hexen-3-ol (2.41%), N-(tert-butoxycarbonyl)-2-(4-methoxyphenyl) allylamine (allyl amino butane) (1.06%), cyclotrisiloxane, hexamethyl (phenolic compound) (0.94%), benzenesulfonamide (0.87%).

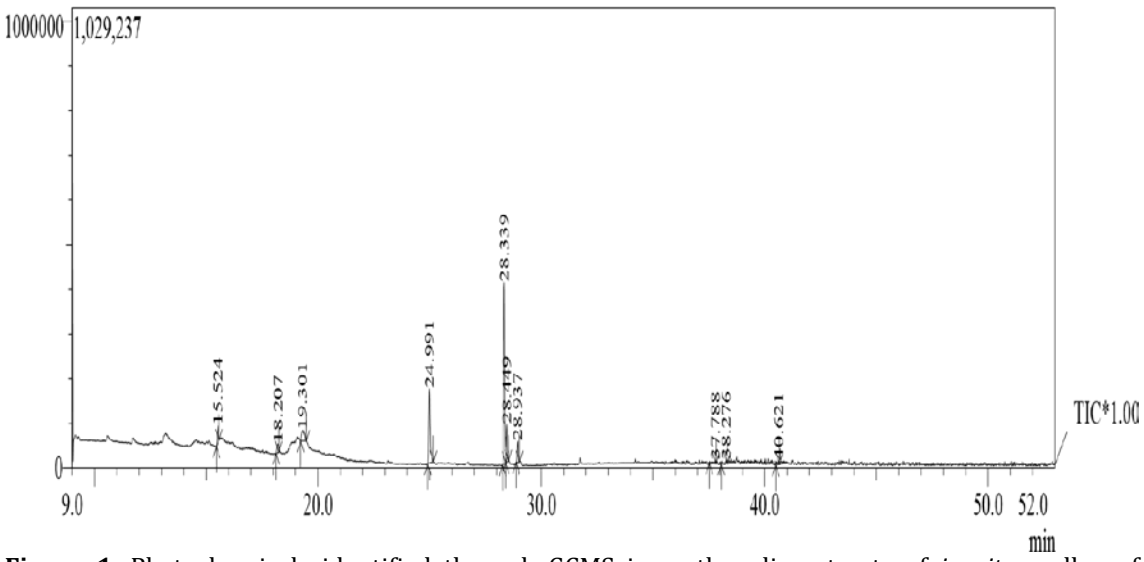


Figure 1. Phytochemicals identified through GCMS in methanolic extracts of *in vitro* callus of *S. kunthiana.*

Table 2. Compounds identified in the methanolic *in vitro* callus extract of *S. kunthiana*.

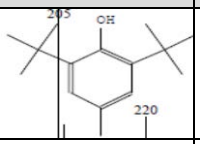
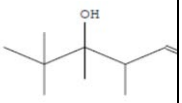
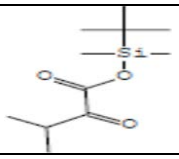
	RT	Compound	Formula	Molecular weight	Peak area	Structure	Medicinal uses
1	15.524	2,6-bis(1,1-dimethylethyl)-4-methyl phenol	$C_{15}H_{24}O$	220	4.70		Antioxidant (Ibtissem et al., 2010)
2	18.207	2,2,3,4-Tetramethyl-5-hexen-3-ol	$C_{10}H_{20}O$	156	2.41		No report
3	19.301	3-Methyl-2-ketobutyric acid tbdms	$C_{11}H_{22}O_3Si$	230	2.79		No report

Table 2. Continued.

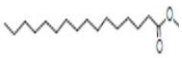
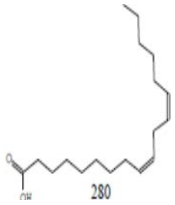
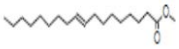
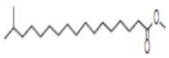
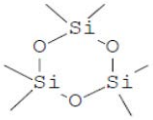
	RT	Compound	Formula	Molecular weight	Peak area	Structure	Medicinal uses
4	24.991	Hexadecanoic acid, methyl ester	C17H34O2	270	20.69		Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-alpha reductase inhibitor, antifibrinolytic, lubricant, antialopecic (Selvan and Velavan, 2015), anti-inflammatory (Hema et al., 2011), cancer preventive, hepatoprotective, antihistaminic, antieczemic, antiachne, antiarthiritic, anticoronary (Krishnamoorthy and Subramaniam 2014), antibacterial, antifungal (Chandrasekaran et al., 2011)
5	28.339	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	50.32		Anti-inflammatory, antiarthritic, antioxidant, anticancer (Mangunwidjaja et al., 2006). Hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, Insectifuge, antihistaminic, Antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic anticoronary insectifuge (Rajeswari et al., 2012). Antiarteriosclerotic, antianaphylactic, antiprostatic (Rajeswari and Srinivasan 2015).
6	28.449	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	10.45		Anti-inflammatory, antiandrogenic, Cancer preventive, dermatitigenic, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge (Rajeswari and Rani 2015), Antioxidant, anticancer (Asghar et al. 2011; Hema et al., 2011)
7	28.937	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	5.78		Used against skin cancer protein (Elaiyaraja, and Chandramohan, 2016). Antioxidant, antimicrobial, antiinflammatary (Vetha Merlin Kumari et al., 2016).
8	37.788	Benzenesulfonamide	$C_{23}H_{23}NO_2S_2$	409	0.87		Antimalarial (Andrews et al., 2013).
9	38.276	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222	0.94		Antimicrobial potential, antioxidants (Venkatesh et al., 2014).

Table 2. Continued.

	RT	Compound	Formula	Molecular weight	Peak area	Structure	Medicinal uses
10	40.621	N-(tert-butoxycarbonyl)-2-(4-methoxyphenyl) allylamine	$C_{15}H_{21}NO_3$	263	1.06		Phytocompound having liver susceptibility of reactions (Peter and Venky, 2012).

The antioxidant activity of *in vitro* grown leaf callus of methanolic and ethanolic extracts *S. kunthiana* were assessed by DPPH activity (Figure 2). The DPPH activity of different concentration of methanol and ethanol extract (50-250µg/ml) along with standard ascorbic acid is presented in the Table 3. With the increasing concentrations positive scavenging activity were noted. The percentage of scavenging activity is increasing with the increasing concentration in both the extracts. Among the five different concentrations, both extracts were tested, the higher percentage of inhibition 79.23±0.37 was observed in 250 µg/mL of ethanol extract followed by 90.35±0.54 methanolic extract against the standard ascorbic acid 91.25±0.33 followed by percentage of inhibition (76.31±0.34) 200 µg/mL of ethanol extract and 87.25±0.39 of methanol extract observed in 200 µg/mL against the standard ascorbic acid 89.5±0.42 200 µg/mL. The result concludes when compare the scavenging activity percentage of ethanol and methanol, the methanol extract shows higher activity.

Table 3. Antioxidant activity of *in vitro* callus extract of *S. kunthiana*: DPPH method.

	Sample	% of inhibition					Comparison of activity
		50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL	
1	Ethanolic Extract	54.98±0.33	60.13±0.32	74.3±0.34	76.31±0.34	79.23±0.37	Methanol > Ethanol
2	Methanolic Extract	78.16±0.40	81.03±0.53	83.28±0.41	87.25±0.39	90.35±0.54	
3	Ascorbic Acid	84.31±0.41	87.23±0.49	88.16±0.34	89.5±0.42	91.25±0.33	

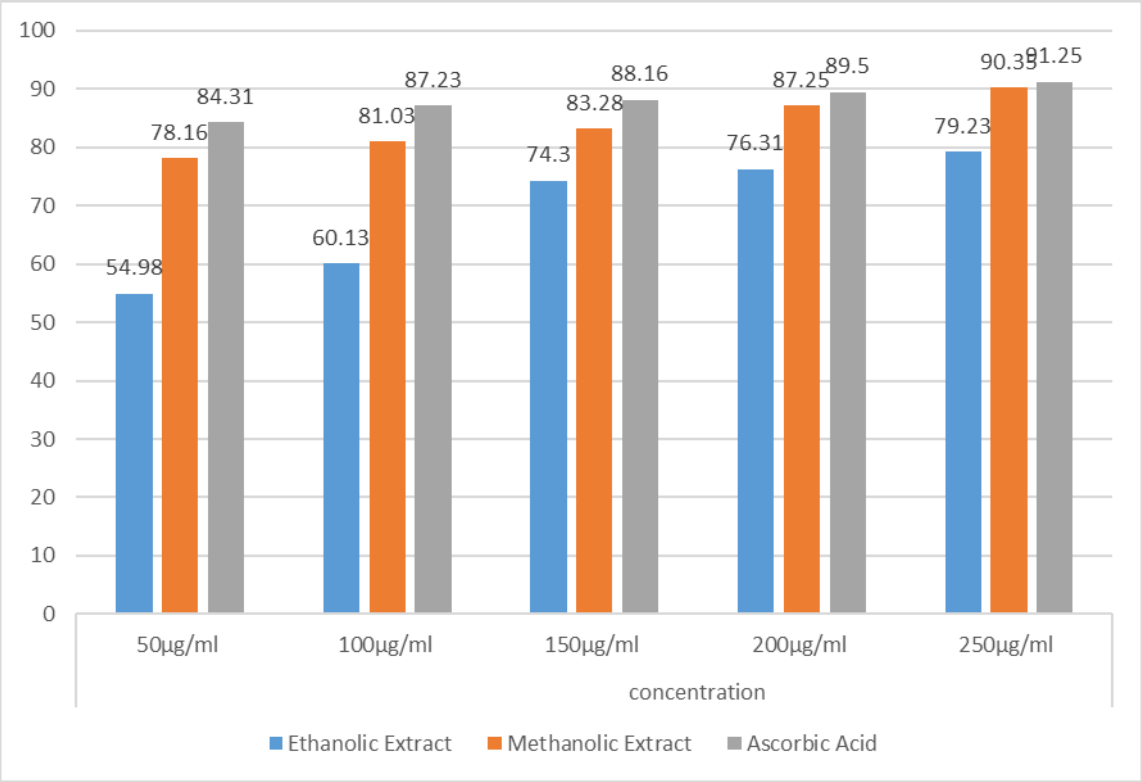


Figure 2. Antioxidant activity of *S.kunthiana in vitro* callus.

Discussion

In the present study, preliminary phytochemical screening of *in vitro* of methanol, ethanol, petroleum ether, chloroform and water extracts of *Strobilanthes kunthiana* leaf callus. The result concludes that methanolic extract only showed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, terpenoids and phenols. Similarly, in previous studies it was reported that methanolic flower extract of *Strobilanthes kunthiana* shows the presence of alkaloids, carbohydrates, phytosterols, tannins, proteins and flavonoids (Singh et al., 2014).

Secondary metabolites of plants serve as defense mechanism against predation by many microorganism, insects and herbivores. These secondary metabolites contribute many biological activities such as anti-inflammatory, antioxidants, antiosteoartritic, analgesic activities, antidiabetic, antimicrobial and heptaprotective (Desu et al., 2011; Singh, 2014).

The presence of alkaloids has pharmacological applications as anesthetics and CNS stimulants (Madziga et al., 2010). Likewise, flavonoids are important group of polyphenols have several proven medicinal properties, such as antioxidants or free radical scavengers (Kar, 2007) and also act as allergies, inflammation, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Barakat et al., 1993).

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and act as anticancer (Ruch et al., 1989; Motar et al., 1985).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). It possesses biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection, improvement of endothelial

function, as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007).

Steroids also have antibacterial properties and help in regulating the immune responses (Epand et al., 2007; Shah et al., 2009).

Terpenoids have been found to be useful in the prevention and therapy of several diseases such as cancer, possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties (Rabi and Bishayee, 2009; Wagner and Elmadfa, 2003).

The gas chromatogram shows that the relative concentrations of various compounds are getting eluted as a function of retention time. The peaks height indicate the relative concentrations of compounds present in the plant. Generally, the reliability of medicinal plant for its usage is evaluated by correlating the phytochemical compounds with their biological activities (Belkacem et al., 2013).

The present study is the first report on the GC-MS analysis in *Strobilanthes kunthiana* callus extract. Totally ten compounds were identified. Most identified compounds have been reported to possess interesting biological activities.

The prevailing major compounds were 9,12-octadecadienoic acid (Z,Z) (50.32%) which act as anti-inflammatory, antiarthritic, antioxidant, anticancer (Mangunwidjaja et al., 2006) hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic anticoronary insectifuge (Rajeswari et al., 2012) antiarteriosclerotic, antianaphylactic, antiprostatic (Rajeswari and Srinivasan, 2015). hexadecanoic acid, methyl ester (20.69%) similar percentage (21.25%) were reported in *Tesium humile* (Belakhdar et al., 2015) and it contains many biological activity such as antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic,5-alpha reductase inhibitor, antifibrinolytic, lubricant, antialopecic (Selvan and Velavan 2015), anti-inflammatory (Hema et al., 2011), cancer preventive, hepatoprotective, antihistaminic, antieczemic, antiachne, antiarthiritic, anticoronary (Krishnamoorthy and Subramaniam, 2014), antibacterial, antifungal (Chandrasekaran et al., 2011).

9-Octadecenoic acid (Z)-, methyl ester is reported to have anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge (Rajeswari and Rani, 2015), antioxidant, anticancer (Asghar et al., 2011; Hema et al., 2011).

Likewise, heptadecanoic acid, 16-methyl-, methyl ester is also used against skin cancer protein (Elaiyaraj and Chandramohan 2016), antioxidant, antimicrobial, anti-inflammatary (Vetha Merlin Kumari et al., 2016). 2,6-bis(1, 1-dimethylethyl)-4-methyl phenol have antioxidant (Ibtissem et al., 2010) followed by cyclotrisiloxane, hexamethyl reported to have antimicrobial and antioxidants activity (Venkatesh et al., 2014).

N-(tert-butoxycarbonyl)-2-(4-methoxyphenyl) allylamine have liver susceptibility of reactions (Peter and Venky, 2012) followed by benzenesulfonamide reported to have antimalarial (Andrews et al., 2013).

The result concludes that the major compound possess antioxidant, anti-inflammatory, anticancer and antimicrobial properties. In present study the stronger methanolic extraction capacity could have been produced number of active constituents responsible for many biological activities. So it is recommended as plant of pharmaceutical importance. However further studies will need to be undertaken its bioactivity and toxicity profile.

Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging (Yu, 1994; Halliwell and Gutteridge, 1999). Plants which contains phenols, flavonoids, vitamins, terpenoids are rich in free radical scavenging and antioxidant activity (Madsen and Bertelsen, 1995; Cai and Sun, 2003).

The plant which shows good source of natural antioxidant activity play a key role in prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegener-ative disorders, diabetic pregnancy, rheumatic disorder, DNA damage, ageing (Uddin et al., 2008; Jayasri et al., 2009).

Our current result showed that the highest inhibition percentage value of methanolic extract of *S. kunthiana* callus was found to be 90.35±0.54 than ethanol extract 79.23±0.37 against standard ascorbic acid 91.25±0.33. The ascorbic acid possesses the ability to scavenge the free radicals in human body. The result revealed that the methanolic extract of callus showed good amount of antioxidants to counteract the damaging effects of free radicals and may protect against mutagenesis.

In contrary other results the significant antioxidant activity were reported in ethyl acetate extract, n-butanol extract of *S. kunthiana* flower and n-hexane extract showed devoid of activity (Singh et al., 2014). Similarly, the crude extract of *Psidium guajava* showed maximum inhibition of 91% at 0.5 mg/mL which is comparable to 95% for vitamin C than *Carica papaya, Vernonia amygdalina*, and *Mangifera indica* (Ayoola et al., 2008) followed by DPPH scavenging activity of *Cassia tora* was evaluated at 20-80 µg/mL and the highest as found to be 71.18% (Sirappuselvi and Chitra, 2012). Likewise, the antioxidant activity of *Ipomoea cairica* was experimented with DPPH scavenging activity with 82.58% at 500 µg/mL (Arora et al., 2013).

The result of the present study suggests that *S. kunthiana* can be used as a source of antioxidants for pharmacological preparations which is which is very well evidenced by the present work.

Conclusion

The present study carried out on the *S. kunthiana* revealed the presence of medicinal active constituents by GCMS. So that those might be utilized for the development of traditional medicines. Based on the results of this study the *in vitro* callus contains alkaloids, flavonoids, glycosides, saponins, tannins, steroids, terpenoids and phenols. The extract also shows the antioxidant activity. From the above analyses, the possible mechanism of antioxidant activity of all the extracts include reductive ability, hydrogen-donating ability, and scavenging of superoxide, nitric oxide and free radicals, which may be due to the presence of phytoconstituents such as flavonoids and polyphenols present in the methanolic *in vitro* callus extract of *S. kunthiana.*

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Conflicts of interest

Authors declare that they have no conflict of interests.

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