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Research Article

Phytochemical Profiling from Leaf Extract of Wild Genotype of *Cymbopogon martinii* (Roxb.) Collected from Devarayana Durga Hill Karnataka

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Abstract

The *Cymbopogon martinii* (Roxb.) plant is commonly known as palmarosa or rosa grass belonging to *Poaceae*. It is a perennial aromatic grass known for its rose-like aroma and rich in essential oil composition. The present investigation is made to study the ecological, morphological and anatomical studies in wild genotype of *Cymbopogon* collected from Devarayana Durga hill, Karnataka, India. The studies were made by screening the phytochemicals using methanol extract from the leaf by adopting standard qualitative tests. The screening of phytochemicals from the plant investigated serves as important steps to determine the biologically active chemical constituents present in the plant. The study showed that the methanol extract contains alkaloids, steroids, tannins, terpenoids, flavonoids, cardiac glycosides and phenols with the absence of saponins were absent. The quantitative determination of total phenols, total flavonoids and total terpenoids were made and found to be 0.011 mg. Tannic acid equivalent/gm, 0.508 mg quercetin/gm and 1.606 mg linalool/gm, respectively. There was higher terpenoid content, which followed by the flavonoid and phenol content. Further the thin layer chromatographic studies revealed the presence of terpenoid compounds.

Keywords: *Cymbopogon martinii*, Anatomical studies, Methanol leaf extract, Qualitative and quantitative phytochemical analysis, Thin layer chromatography

INTRODUCTION

Plants are the vital source of secondary metabolites with medicinal properties and play a critical role in human health because of phytochemical constituents. There has been an increasing interest in natural products due to environmental, health and safety concerns. Plant-derived substances have recently become of great interest due to their medicinal properties. They gain much interest as food additives due to their relatively low or negligible toxicity, high volatility, and biodegradability and alternative to synthetic drugs, especially against microbial pathogens because of the growth of antibiotic resistance (Afolabi, 2007).

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Plant materials are a rich source of biologically active metabolites. Terpenoids comprise a large group of distinct natural metabolites, many of which are discovered in plants. Terpenes found and isolated from plants have been utilized widely in foods, cosmetics, pharmaceuticals and various biotechnological applications. The ability to isolate and purify these valuable molecules from plants is key to elucidating their potential applications. The elementary classes of terpenoids present in plants are the volatile essential oils, triterpenoids, steroids and carotenoids. The presence of phenolic compounds (phenolic acids, polyphenols and flavonoids) in plant, herbs and spices is gaining increases attention because of their various functions, such as antioxidant activity and flavouring properties. Phenolic compounds are commonly found in both edible and nonedible plants and they have multiple biological effects. Identified tannins as another class of phenolic in plants. And phenolic compounds also involved in the cell defence system against free radicals (Ashwini et al., 2019).

Cymbopogon martini (*Poaceae*), commonly known as palmarosa, yields an essential oil rich in geraniol. Palmarosa oil is of commercial importance, extensively used in perfumes, soaps, cosmetics, toiletry and tobacco products. It is used mainly as a high-quality geraniol used in high-grade perfumes and cosmetics. It exhibits beneficial effects on several central nervous system pathologies, mainly neuralgia, epileptic and anorexia. There are a few reports on its products still, *C. martinii* has attracted many researchers attention due to its antimicrobial, anti-genotoxic and antioxidant activities. Countries such as India, Brazil and Madagascar have the practice to produce essential oil from this plant. Geraniol, the principal constituent of *C. martinii* essential oil, is an acyclic monoterpenoid and acts as a new therapeutic agent class against pancreatic cancer and colon cancer. Besides, it possesses several biological properties, including antimicrobial, antioxidant and anti-inflammatory activities. The essential oils of *C. martini* acted as potent antimicrobial agents with broad-spectrum activity with possible potential for the control of pathogens in plants against post-harvest spoilage of many crops and human pathogenic diseases (Das Talukdar., 2002).

Chemical studies of the palmarosa oil reveals that it contains monoterpenes, sesquiterpenes and alcohols like geraniol, geranyl acetate, (E, Z)-farnesol, nerol, limonene, terpinene, myrcene, caryophyllene, linalool and fatty acid 16-hydroxypentacos-14-(z)-enoic acid. The plant collected from the research farm of central institute of medicinal and aromatic plants resource centre, Hyderabad, oils of whole herb and different parts of palmarosa is identified b-Ocimene (1.2%-4.3%), linalool (0.8%-2.0%), geraniol (70.1%-85.3%), geranyl acetate (4.3%-14.8%) and (E, Z)-Farnesol (1.6%-3.4%) were the main components. Extensive studies carried in the essential oil of *Cymbopogon martinii* to study the bioactive compound and their activities.

As our previous studies, GC-MS analysis of the leaf extract (methanol) revealed the presence of tetratetracontane (19.09%), tetradecanal (18.97%), heptadecanal, (11.53%) cubenol (6.47%), beta-sitosterol (6.06%) and 2,4-di tert-butylphenol (5.40%) in higher percentages. GC-MS analysis of the essential oil revealed that presence of geraniol (26.90%), cubenol (24.71%), geraniol acetate (15.98%), camphene (8.60%), limonene (4.65%), 1R-alpha-pinene (2.76%), borneol (2.39%) and linalool (2%) as major components. In the present investigation, the study carried out to explore wild species of *Cymbopogon martinii*, phytochemical screening and thin layer chromatography of bioactive compounds present in it (Geetha et al., 2014).

MATERIALS AND METHODS

Collection and identification of the plant

Wild genotype of the *Cymbopogon* species was collected during the month of June (onset of flowering period) from Devarayana Durga hill station, Tumkur district, Karnataka. The species of *Cymbopogon* was identified as *Cymbopogon martinii* from the Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD) with an authentication number RRCB1-1052.

Ecological studies

The species of *Cymbopogon martinii* collected from the Devarayana Durga hills, Tumkur is a rocky hill surrounded by forest and the hilltops and is dotted with several temples with 42.27 sq.km. The habitat and the habit of the *Cymbopogon martinii* collected was studied and photographed (Hossain et al., 2011).

Morphological studies

Plant morphology: The morphological characters such as appearance of the plant, height of the plant, leaf size and colour, stem size and colour and details of the inflorescence were recorded.

Anatomical studies

Hand-cut sections were prepared using fresh, young leaves and incubated in Schiff's reagent for 30 min at room temperature. The sections were taken, washed thrice for 10 min each with freshly prepared solution of 0.5% (w/v) sodium meta-bisulphite in 0.1% HCl. The stained sections were examined under light microscope (Iwalewa et al., 2006).

Preparation of leaf for extraction

The leaf samples of *Cymbopogon martini* were sorted

out by removing the culm portion and were washed thoroughly with tap water followed by distilled water to remove dirt and debris. The leaves were cut into small pieces and shade dried for three weeks at room temperature (28 ± 3°C). The dried leaves were pulverized into a fine powder using an electric blender.

The powdered material was stored in airtight polythene bags protected from direct sunlight until further use.

Extraction of the crude sample

The powdered leaves were extracted with methanol overnight using stoppered bottle by occasional stirring at room temperature (28 ± 3°C). The sample was filtered using whatmann No. 1 filter paper and the process was repeated for three times. The filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The dry extract obtained was stored in a refrigerator and the concentrated extract was used for further analysis (Jin et al., 2007).

Phytochemical studies

Test for alkaloids

Wagner's test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). The formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for flavonoids

Sulphuric acid test: To the leaf extract, add conc. sulphuric acid was added. The appearance of orange colour indicates the presence of flavonoids.

Test for terpenoids

2 ml of chloroform was added with 3 ml of concentrated sulphuric acid to 2 ml of leaf extract. The formation of a reddish layer at the interface indicates the presence of terpenoids.

Test for steroids

2 ml of chloroform was added with 3 ml of concentrated sulphuric acid to 2 ml of leaf extract. The formation of a reddish layer at the interface indicates the presence of steroids.

Test for glycosides

Liebermann's test: 2 ml of acetic acid and 2 ml of chloroform were added to leaf extract. The mixture was cooled and concentrated sulphuric acid was added. The presence of green colour indicated the presence of aglycone, steroidal part of glycosides.

Test for tannins

Ferric chloride test: To the leaf extract, 0.1% of ferric chloride was added. Brownish-green or black/blue colour indicates the presence of tannins.

Test for phenols

Ferric chloride test: To the leaf extract, 0.3% of ferric chloride was added. Green or blue colour indicates the presence.

Test for saponins

Foam test: Add 5 ml of distilled water to leaf extract and mix vigorously; the appearance of froth indicates the presence of saponins.

Quantitative analysis

Sample preparation: 20 mg of the leaf extract was dissolved in 1 mL methanol followed by centrifugation at 1,000 × g for 10 min. The clear supernatant obtained was used for further analysis.

Total phenolic content

The total phenolic determination, gallic acid was used to make the standard calibration curve. Different concentrations of gallic acid (10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml) and test sample (1 mg/ml) were used. To this 1 mL methanol and 0.5 mL of folin-ciocalteu's phenol reagent (1:1) were added. After 5 min, 1.5 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made upto 10 mL using distilled water (Mallavarapu et al., 1998).

The reaction was kept in the dark for 2 h and the absorbance of the samples were measured at 750 nm. The phenolic content was calculated as Gallic Acid Equivalents GAE/g of the dried leaf material by comparing gallic acid standard curve.

Total flavonoid content

Total flavonoid determination, quercetin was used to make the standard calibration curve with different concentrations of quercetin (100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml, 1000 µg/ml) and the test sample (1 mg/ml). To the sample 1 mL methanol, 4 mL distilled water and 0.3 mL of 5% sodium nitrite was added to all the test tubes. 0.3 mL of 10% aluminium chloride and 1 M sodium hydroxide was added after 5 min of incubation and the volume was made upto 10 mL using distilled water. The solution was incubated for 30 min at room temperature. The absorbance of the reaction mixture was measured at 510 nm with a UV-Vis spectrophotometer. The concentration of total flavonoid content in the test samples were calculated from the calibration plot and expressed as mg Quercetin Equivalent (QE)/g of the dried leaf material (Promila et al., 2018).

Total terpenoid content

For total terpenoid determination, linalool was used to make the standard calibration curve. Different concentrations of linalool (10 mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, 100 mg/ml) and test sample (1 mg/ml, 5 mg/ml, 10 mg/ml). To the used mixture 200 µl, methanol and 1.5 mL of chloroform were added. 100 µL of conc. sulphuric acid was added

after 3 min of incubation. The reaction was kept in the dark for 5 min for the standard and 1-2 h for the test extract. The reddish-brown precipitation was dissolved in 95% methanol after decanting the supernatant carefully. The absorbance of the reaction mixtures was measured against blank at 538 nm wavelength with a UV-Vis spectrophotometer. The terpenoid content was calculated as linalool equivalent linalool/g of dry plant material compared with the linalool standard curve.

Thin layer chromatographic analysis

The methanol leaf extract of *C. martinii* prepared applied on pre-coated TLC plates using capillary tubes and the plate was developed using toluene:ethyl acetate (9.3:0.7 V/V), n-Hexane:ethyl acetate (7.2:2.9 V/V) and petroleum ether:ethyl acetate (4:1 V/V) mobile phase. The TLC plates developed were air-dried and observed under ultraviolet light at 254 nm.

They were later sprayed with vanillin sulphuric acid reagents and were placed in a hot air oven for one min at 100°C for the appearance of the colour as separate bands (Raina et al., 2003). The analytes movement of the analytes was expressed by calculating the Retention Factor (Rf) and the values were calculated.

Retention time (Rf)=Distance travelled by the solute/distance travelled by the solvent.

RESULTS AND DISCUSSION

Identification of the plant

The wild genotype of *Cymbopogon martinii* (Roxb). collected plant from Devarayana Durga hill Tumkur presently studies for phytochemical showed following results.

Ecological studies

Devarayana Durga hill is a southern thorn forest of grassland covering an area of 42.27 sq. Km. The latitude and longitude of hill are reported as 13.375 and 77.123, respectively. The area has an elevation of 1290 mts above sea level with red sandy soil. The hill receives low rainfall throughout the year, with an average temperature of 22.9°C. The entire hill was covered with many types of medicinal plants and abundance occurrence of wild genotype of *Cymbopogon martinii* (Rajeswara., 2019).

Morphological studies

The morphological characteristics of the wild *C. martinii* studied showed that the plant perennial in nature, further the morphological characteristics of the different plant parts such as leaf, stem and inflorescence etc., were evaluated and recorded.

Stem (culm): Stem erect, green, 200 cm high, smooth glabrous; lower base of nodes and internodes swollen, internodes 15 cm high.

Leaf: Light green, 50 cm long, 25 mm wide, cordate at base, tapering at the end, glabrous/smooth. Ligule membranous, 1.5 mm-4 mm long.

Inflorescence: Large and complex, pink panicle, branches slender, racemes paired with spatheole and spikelets, one sessile, other pedicelled. sessile spikelets 2 mm-3 mm long, spathe green, 2.5 cm; spatheole light green, boat-shaped, 1.5 cm long glabrous; peduncle filiform. Racemes 20 mm long, heterogamous; lower raceme base and lowermost pedicel swollen; sessile spikelet 3.5 mm long, glabrous; lower glume 1 mm wide, broadly winged, 2-nerved; awn 12 mm-18 mm long. Pedicellate spikelet 4 mm long, glabrous; lower glume lanceolate, 8-nerved stamens 2, anthers 1.5 mm-2 mm long.

Anatomical studies

The adaxial layer or upper epidermis consisted of single layer of large bubble-shaped elongated cells termed as bulliform cells. The bulliform cells were followed by the ground tissue having 3-4 layers of cells (Sumit et al., 2020).

The vascular bundles were oval-shaped conjoint, collateral, having closed arrangement in the abaxial layer. The vascular bundle consisted of two metaxylem oriented towards the upper epidermis. The phloem, containing companion cells and sieve tubes were located near the lower epidermis. The large vascular bundles were surrounded by two layers of cells that define the bundle sheaths, each bundle capped by a small group of sclerenchyma fibers both on the upper and lower ends. Single large vascular bundles present in the midrib were connected to the lower epidermis *via* a group of sclerenchyma cells with lignified walls and the sclerenchyma cells formed 3 to 5 layers. The abaxial epidermis formed a single layer of small cells and a smaller nine vascular bundles between interpersed the massive vascular bundles. The lower epidermal cells found below the vascular bundles posessed projections and formed papillae (Figures 1 and 2) (Szeto et al., 2019).

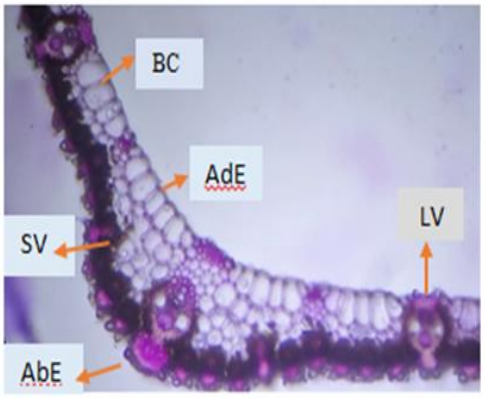


Figure 1. T.S of leaf showing arrangement of vascular bundles (10X and 40X).

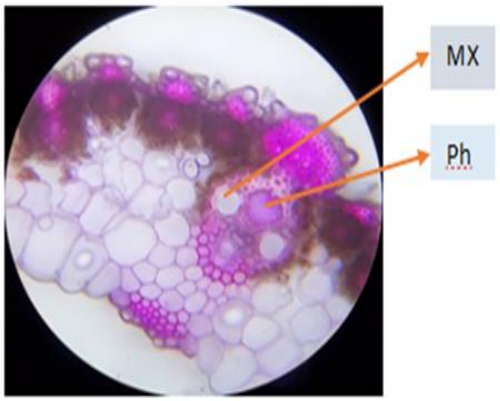


Figure 2. Single vascular bundle enlarged.

Phytochemical studies

The qualitative phytochemical analysis of methanol leaf extract of *C. martini* leaves revealed the presence of flavonoids, terpenoid, tannins, glycosides, steroids phenolic as secondary metabolites. The alkaloids and saponins were absent in the methanol extract (Table 1).

Quantitative phytochemical screening

The quantitative phytochemical analysis revealed the leaf extract studied showed the total phenol content of 0.011 mg/g, the flavonoid content of 0.508 mg/g and total terpenoid content of 1.606 mg/g (Table 2).

Cymbopogon species is mainly an essential oil-bearing plant that is rich in terpenoid. The quantitative analysis *C. martinii* leaf extract showed higher content of terpenoid when compared to flavonoid and phenol. The total phenol content of the methanol leaf extract was determined by extrapolation from the calibration curve ($Y=0.0078x+0.0096$; $R^2=0.994$) was found to be 0.011 mg/g of dry weight whereas the total flavonoid content of the extract ($Y=0.249x-0.0238$; $R^2=0.9821$) was found to be 0.508 mg/g dry weight and total terpenoid content of the extract ($Y=0.0126x+0.0056$; $R^2=0.9795$) were found to be 1.606 mg/g dry weight respectively (Figures 3-5).

Thin layer chromatography

The TLC profiling results were showed the presence of different colour bands when sprayed with vanillin sulphuric acid reagent after heating at 110°C for 5-10 min. The study revealed the presence of several phytochemical compounds in the leaf extract. The result showed the presence of terpene derivatives (purple and pink colour) and phenol compound (blue). It was observed that a combination of (hexane:ethyl acetate) is a better solvent system for the separation of compounds compared to the other solvents tried (Figure 6) (Raina et al., 2003).

Table 1. Qualitative phytochemical analysis of *C. martinii* leaf extract.

Sl. No.	Compounds	Methanol extract
1	Alkaloids	-
2	Flavonoids	+
3	Terpenoids	+
4	Tannins	+
5	Glycosides	+
6	Saponins	-
7	Phenols	+
8	Steroids	+

Table 2. Quantitative phytochemical analysis of *C. martini* leaf extract.

Test sample	Phenolic content (mg of gallic acid equivalent/g dry material)	Flavonoid content (mg of quercetin equivalent/g dry material)	Terpenoid content (mg of linalool equivalent/g dry material)
Methanol leaf extract	0.011 mg	0.508 mg	1.606 mg

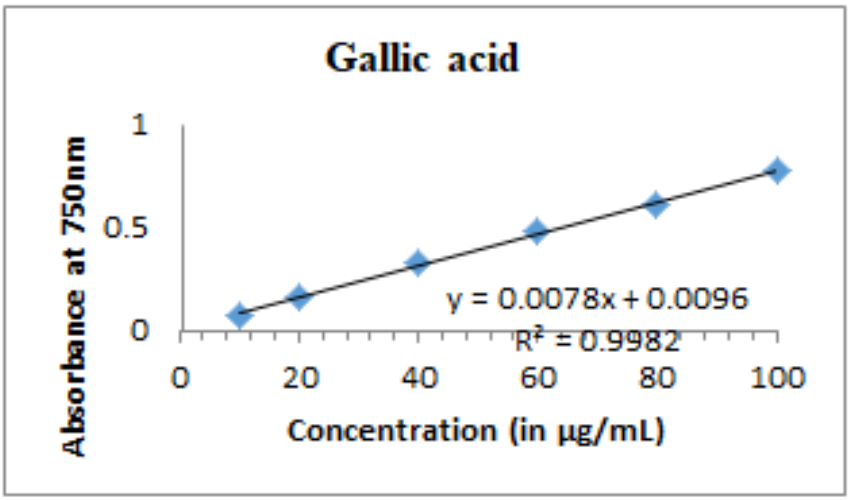


Figure 3. Gallic acid calibration curve.

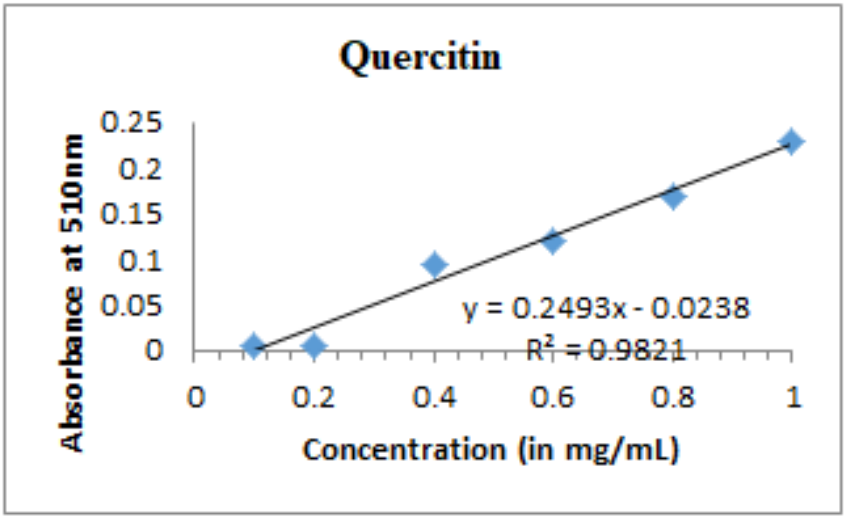


Figure 4. Quercetin calibration curve.

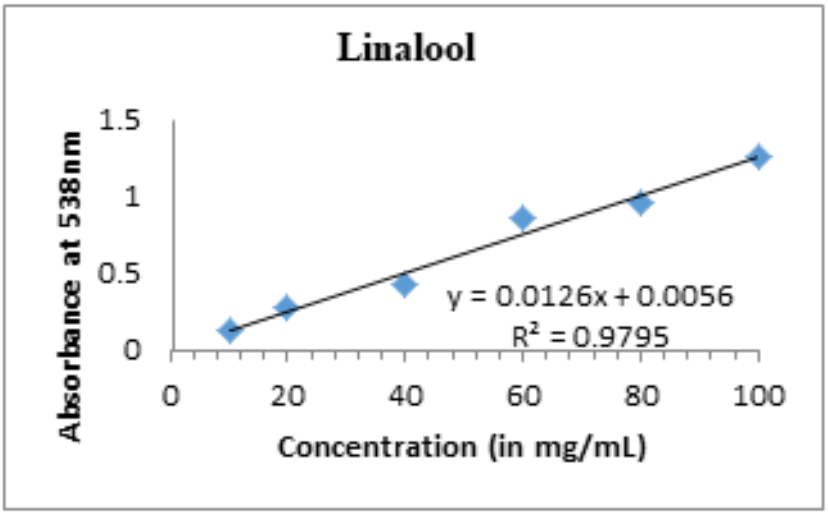
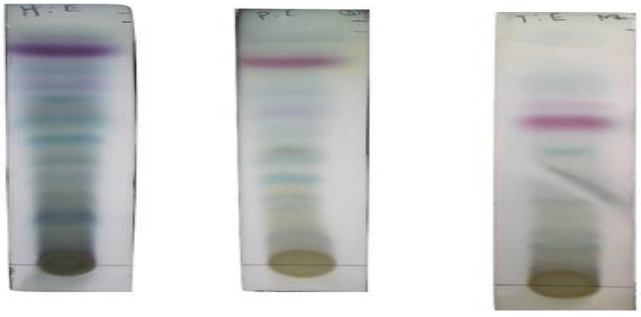


Figure 5. Linalool calibration curve.

Table 3. TLC screening of terpenoid compounds.

SL no.	Mobile phase	Spraying reagent	Colour of the spot/band	Rf value
1	n-Hexane:Ethyl acetate (7.2:2.9)	Vanillin sulphuric acid reagents	Purple	0.88
			Blue	0.79
			Pink	0.73
			Yellow	0.63
			Blue	0.51
			Blue	0,44
2	Petroleum ether:Ethyl acetate (4:1)	Vanillin sulphuric acid reagents	Purple	0.86
			Blue	0.45
			Blue	0.36
3	Toluene:Ethyl acetate(9.3:0.7)	Vanillin sulphuric acid reagents	Pink	0.71
			Purple	0.63
			Blue	0.52
			Blue	0.33
			Yellow	0.27



A) **B)** **C)**

Figure 6. TLC fingerprinting of Methanol leaf extract showing band variation.
A) n-Hexane:Ethyl acetate; B) Petroleum ether:Ethyl acetate; C) Toluene:Ethyl acetate

CONCLUSION

The phytochemical analysis result concluded that *Cymbopogon martini* produces many secondary metabolites of medicinal value. TLC profiling further confirmed the presence of terpene and phenol derivatives. Methanol was found as the best-suited solvent for extraction purposes. Thus, the plant can be used as a source to produce phytochemicals using advanced extraction techniques, screening, identification and isolation. The present study confirms many important phytochemicals in the unexplored plant *Cymbopogon martini* from Devarayana Durga hill region. The presence of some medicinally essential phytochemicals such as alkaloids, flavonoids and terpenoid and phenol strengthened further by thin-layer chromatography.The extracts were undertaken for TLC profiling to assess the nature of phytochemicals present in it. Since thin layer chromatography is the most widely used qualitative technique for the separation of constituents present in a mixture using stationary and mobile phase. Furthermore, this method helps in isolation, quantification and identification of separated components. Separations are based on differences in migration rates among the sample components. Several developing solvent systems were tried for all the extract and fractions. The solvent system, which gave the best resolution, was considered optimized, valid and useful. The satisfactory resolution was obtained in the mobile phase mentioned in Table 3, and photo documentation was shown in figure 6. However, presence of phyto-constituents in particular extracts and fractions was confirmed by spraying TLC plates with spraying reagents. Methanol is a polar solvent used to extract lipids polar compounds. Phenolic compound, flavonoids and the lower molecular weight of polyphenols, sugars and organic acid steroid, alkaloids and saponin and the methanol, which is more polar than chloroform, has been reported to extract terpenoids. Similarly, in this study, the TLC of methanol leaves extract showed terpenoid and phenol derivatives which is further characterized.

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