

Research Article



Preliminary Phytochemical Screening of Members of Lamiaceae Family: *Leucas linifolia*, *Coleus aromaticus* and *Pogestemon patchouli*

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ABSTRACT

The members of Lamiaceae family include aromatic plants that are being used in traditional medicine for various disorders. The therapeutic application of these plants is attributed to the presence of secondary metabolites or phytochemicals such as alkaloids, saponins, flavonoids, glycosides and phenols. This study is a preliminary screening of phytochemicals of *Leucas linifolia*, *Coleus aromaticus*, *Pogestemon patchouli*, which belong to Lamiaceae family. Methanol, ethanol and chloroform extracts of each plant were subjected to both qualitative and quantitative phytochemical screening. The alkaloids, saponins, total phenols, tannins, flavonoids, steroids and proteins were quantified in the extracts by standard spectrophotometric methods. The results of the qualitative analysis of the study plants, revealed the presence of steroids and absence of terpenoids, amino acids in all the leaf extracts. In addition, methanol extracts were positive for alkaloids, saponins, tannins and phenols, flavonoids, carbohydrates and glycosides. Whereas, the ethanol extracts showed fewer constituents in different combination in all the three study plants. However, chloroform is a good solvent for extraction of steroids from these plants since only steroids were present in chloroform extract. The concentration of total phenols and tannins was higher in methanol extract. *Pogestemon patchouli* had the highest concentration of flavonoids more so in ethanol than either methanol or chloroform extracts. Although, protein concentrations was higher in the ethanol extracts of *Leucas linifolia* and *Coleus aromaticus*, methanol extract of *Pogestemon patchouli* had more protein than ethanol extract. However, chloroform which extracted steroids, is not a good choice for extraction of proteins from these plants. In conclusion, for further studies on the phytochemical and biological structure-function relationship, the use of appropriate solvent for extraction and purification of the specific phytochemical from plants is one of the crucial steps.

Keywords: chloroform extract, *Coleus aromaticus*, ethanol extract, *Leucas linifolia*, methanol extract, phytochemicals, *Pogestemon patchouli*.

INTRODUCTION

Plants are being used as remedies for diseases from time immemorial. There is a tremendous increase in the consumption of herbs as an alternate source of medicine to maintain health and improve the quality of life.¹ The chemical components in plants have diverse biological roles and are therefore of therapeutic value.² Phytochemicals, the compounds present in plants are valuable source of food and medicine. They are known to have various biological activities such as antimicrobial, antifungal, antioxidant, etc. The important bioactive components in plants are usually the secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compounds.³

One of the sources of pharmaceuticals for human ailments is plants, either as totally pure compounds or as synthetic analogs. Approximately 25% of drugs prescribed in the United States are plant derived natural products and 74% of the 119 most important drugs contain ingredients from plants used in traditional medicine.⁴ Hence, it can be stated that plants could be a source for the development of new molecules. Pharmaceutical research is now extensively focusing on natural compounds, for developing active molecules of plant origin.⁵

Herbs are being used for the promotion of health, prevention and treatment of diseases in India from ancient times.⁶ Phytochemicals are products of plant metabolism, mainly used by the plants for their defense. Hence attempts have been made to use them for therapeutic purposes.⁷ For example, saponins are shown to have beneficial effects on the blood cholesterol, against cancer, immune system and also have antiviral property.⁸ Cardiac glycosides play a role in the treatment of failing heart disorders and are known to show beneficial effects on cardiac arrhythmias.⁹ Although phytochemicals are said to be useful to the human body, they may have some toxic effects as well, as seen in the case of alkaloids.¹⁰ Alkaloids are reported to have cytotoxic activity which may be used in treatment of cancer.¹¹ Hence, it is desirable to know the phytochemical composition of the plant material before testing its efficacy for medicinal purpose.

The family Lamiaceae includes aromatic plants which are known to be used in conventional medicine. Various species from the genera "*Ocimum*" have been explored for their medicinal value and in connection with this the quantitative estimation of the phytochemicals have been studied to correlate the concentrations with their biological roles.¹² Reports of various extracts of *Ocimum* species have shown similar phytochemical composition.



Ethanol extract of *O. canum* leaves has shown the presence of carbohydrates, flavonoids, terpenoids and tannins.¹³ Chloroform extract of *O. sanctum* leaves contain phenols, tannins, flavonoids, glycosides, saponins, steroids, carbohydrates, amino acids and fats.⁶ Preliminary phytochemical screening of hydroalcoholic extracts, of yet another species of *Ocimum*, *O. basilicum*, indicated the presence of flavonoids and phenolic compounds.¹⁴ These studies indicate that there is a variation in the phytochemical composition in extracts with different solvents.

Therefore, the present work aims at evaluating the phytochemical composition, by qualitative and quantitative methods, of methanol, ethanol and chloroform extracts of three other members of the Lamiaceae family, namely, *Leucas linifolia* (*L. linifolia*), *Coleus aromaticus* (*C. aromaticus*) and *Pogestemon patchouli* (*P. patchouli*), which are known to be of medicinal use. The use of *Ocimum*, *L. linifolia*, *C. aromaticus* and *P. patchouli*, in traditional medicine is represented in table 1.

Table 1: Medicinal uses of the plants in the study

Plant species	Common name in English	Traditional uses
<i>Ocimum species</i>	Basil	Cough cold, chronic fever, sore throat, bronchial asthma, malaria, bronchitis, skin diseases, arthritis, diarrhea, dysentery. ¹⁵
<i>Leucas linifolia</i>	Dronpushpi	Leaf decoction used as sedative in nervous disorders, vermifuge, a poultice to sores and wounds, dermatosis. ¹⁶
<i>Coleus aromaticus</i>	Indian borage	Asthma, bronchitis, cold, cough, urinary infections, stimulates the function of liver, bruised leaves applied to burns, leaf juice applied to chapped lips. ¹⁷
<i>Pogestemon patchouli</i>	Patchouli	Poultice of leaves applied to boils, to relieve headache, antiasthmatic, skin infections, dandruff and eczema. ¹⁸

MATERIALS AND METHODS

Plant materials

Leaves of three plants from the family Lamiaceae - *Leucas linifolia*, *Coleus aromaticus*, *Pogestemon patchouli* were collected from the uncultivated areas in Mangalore, Karnataka, India. The plants were identified and authenticated by a taxonomist.

Preparation of crude extracts

Fresh leaves were collected, washed with distilled water, shade dried till it is crisp (approximately 15 days) and cut into small pieces. These dried samples were powdered and stored at 4°C until further use. Crude extracts (10% w/v) were made using 3 solvents i.e., methanol, ethanol and chloroform. The extracts were filtered through fine muslin cloth and the clear filtrate was evaporated to dryness to form the crude extract and stored at 4°C for further use.

Chemicals

Standard reagents and chemicals of analytical grade were used. Quercetin and bovine serum albumin were procured from HiMedia (Mumbai, India). Methanol, ethanol, chloroform, gallic acid, tannic acid, cholesterol, Folin Ciocalteu reagent were purchased from Merck.

Phytochemical screening

The chemical tests were carried out with the crude extracts of each plant i.e., methanol extract (ME), ethanol extract (EE) and chloroform extract (CE).

Qualitative analysis

Qualitative analysis was done to identify the presence of the following phytoconstituents; alkaloids, flavonoids,

tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids using standard procedures.^{19, 20}

Tests for Alkaloids

Each of the crude extracts was treated with dilute HCl and the tests were performed with the filtrate.

- Wagner's Test: Addition of Wagner's reagent to the filtrate results in formation of reddish brown precipitate indicating the presence of alkaloids.
- Hager's Test: Filtrates were treated with Hager's reagent. Formation of yellow coloured precipitate indicates positive test.

Tests for Flavonoids

- Alkaline Reagent Test: Extracts were treated with few drops of NaOH solution. Formation of an intense yellow colour which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoids.
- Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids.

Tests for Phenolic compounds and Tannins

- FeCl₃ Test: 0.5 g of the powdered sample is boiled in 20 ml distilled water and then filtered using a filter paper. 5% (w/v) FeCl₃ is added to the filtered samples and observed for the presence of brownish green or blue black color.
- Gelatin Test: To the extract 1% (w/v) gelatin solution containing 10% (w/v) sodium chloride is added and

observed for the formation of white precipitate. The method is based on the ability of tannins to precipitate proteins.

Tests for Steroids and Terpenoids

- Salkowski's Test: 2 ml of extracts were treated with 2 ml chloroform and filtered. Filtrates were treated with few drops of concentrated H_2SO_4 , shaken and allowed to stand. Appearance of golden yellow color interface indicates the presence of steroid ring.
- Liebermann Burchard's Test: Extracts were treated with chloroform and filtered. Filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated H_2SO_4 was added. The reagents, acetic anhydride and concentrated H_2SO_4 react with the hydroxyl group of phytosterols to produce a dark green color in the upper layer and red color in the lower layer indicating the presence of steroids and triterpenoids respectively.

Test for Saponins

- Foam Test: 0.5 g of the extract was shaken well with 2 ml of water. The test is based on the production of persistent foam, indicating positive test for saponins.

Tests for Carbohydrates

- Molisch's Test: Filtrates were treated with 2 drops of alcoholic α -naphthol solution and concentrated H_2SO_4 . Addition of concentrated H_2SO_4 hydrolyses glycosidic bond and dehydrates the carbohydrates producing furfural compounds. These compounds react with α -naphthol to produce violet colour indicating positive test.
- Benedict's test: Filtrates were treated with Benedict's reagent and heated gently in water bath for 5 minutes. The test is based on the principle that Cu^{++} in the alkaline medium is reduced by the reducing sugars. Orange red precipitate indicates the presence of carbohydrates.

Tests for Glycosides

- Keller Killiani Test: 0.5 g of the extract was treated with 2 ml of glacial acetic acid and a drop of 5% (w/v) $FeCl_3$ was added to it. Glacial acetic acid containing 1% (w/v) $FeCl_3$ gives a brown ring in the presence of 2-deoxysugar in the glycone portion of the phytochemical.
- Bromine water Test: Extracts were treated with bromine water. Yellow coloured precipitate indicates positive test for glycosides.

Tests for Proteins and Amino acids

- Biuret Test: Extracts were treated with 5% (w/v) NaOH and 1% (w/v) $CuSO_4$. Production of violet coloured complex indicates positive test for proteins.
- Ninhydrin Test: To the test solution 0.25% (w/v) Ninhydrin reagent was added and boiled for few

minutes. Ninhydrin reacts with aminoacids having free α -amino groups to produce a blue coloured complex called Ruhemann's purple.

Quantitative Analysis of chemical constituents

Total Phenolic Content²¹

The total phenolic content was estimated by Folin Ciocalteu method.²¹ The estimation is based on the principle that, in the alkaline medium, electrons from phenolic compounds are transferred to phosphomolybdic /phosphotungstic acid which is determined spectrophotometrically at 765 nm. Gallic acid was used as standard. The total phenol values were expressed as gallic acid equivalents in milligram per gram (GAE mg/g) of crude extract.

Total Flavonoid²²

The total flavonoid content was determined by aluminium chloride colorimetric technique.²² Acid stable complexes are formed by $AlCl_3$ with C-4 keto group and either C- 3 or C- 5 hydroxyl groups of flavones and flavonols. They also form acid labile complexes in the A or B ring of flavonoids with orthodihydroxyl groups which can be measured spectrophotometrically at 415 nm. Quercetin was used as the standard and the concentration of flavonoids was expressed as quercetin equivalents in milligram per gram (QE mg/g) of crude extract.

Alkaloid estimation³

The method Harborne (1973) is based on the complete precipitation of alkaloids upon addition of concentrated NH_4OH . 5 g of the dried leaf powder was added to 200 ml of 10% acetic acid in ethanol and allowed to stand for 4 hours. To the filtrate concentrated NH_4OH was added until precipitation was complete. The precipitate was allowed to settle and filtered. The residue was dried in the oven to a constant weight and expressed in percentage of leaf powder.

Tannin estimation³

In this method 500 mg of the crude extract was shaken with 50 ml distilled water for an hour and filtered. The filtrate was then subjected to reaction with 2 ml of (0.1 M) $FeCl_3$ in 0.1 N HCl and (0.008 M) potassium ferrocyanide after which the absorbance was read within 10 minutes at 120 nm. The concentration of tannins was expressed as tannic acid equivalents in milligram per gram (TAE mg/g) of crude extract.

Saponin detection³

According to this method, the 20 g of dried leaf powder was mixed with 20% aqueous ethanol and continuously heated over a water bath for 4 hours. 20 ml diethyl ether was added to the extracts and shaken. The ether layer was discarded and the purification process was repeated. 60 ml n-butanol was added and then treated with 10 ml sodium chloride (5% w/v) and heated. The residue remaining after evaporation was weighed and expressed as percentage of leaf powder.



Estimation of Proteins²³

The method is sensitive towards the detection of low concentration of proteins. The presence of tyrosine and tryptophan groups in the proteins produces a blue coloured complex with Folin Ciocalteu reagent, which was read spectrophotometrically at 660 nm. Varying concentrations of bovine serum albumin (10-200 µg/ml) was maintained for standard calibration curve. The concentration of proteins in the sample was expressed in milligrams/gram of the crude extract.

Estimation of Steroids²⁴

The Liebermann-Burchard reaction method was used to detect sterols and terpenoids that give dark pink to green colour, due to the hydroxyl group reacting with acetic anhydride and H₂SO₄. Varying concentrations of cholesterol (10-1000 µg/ml) was used for standard calibration curve, which was read spectrophotometrically at 640 nm. The concentration of steroids was expressed in milligrams/gram of the crude extract.

Analysis

The samples were analyzed in triplicates (n=3) and the results were expressed as mean ± standard deviation values.

Table 2: Phytochemical composition of the leaves of *L. linifolia*, *C. aromaticus* and *P. patchouli* in methanol, ethanol and chloroform extracts

Phyto-constituents	<i>Leucas linifolia</i> (<i>L. linifolia</i>)			<i>Coleus aromaticus</i> (<i>C. aromaticus</i>)			<i>Pogestemon patchouli</i> (<i>P. patchouli</i>)		
	EE	ME	CE	EE	ME	CE	EE	ME	CE
Alkaloids	+	+	-	-	+	-	-	+	-
Flavonoids	+	+	-	+	+	-	+	+	+
Tannin	+	+	-	+	+	-	+	+	-
Steroids	+	+	+	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-	-	-	-
Saponins	+	-	-	+	+	-	-	+	-
Carbohydrates	+	+	-	-	+	-	+	+	-
Glycosides	+	+	-	-	+	-	-	+	-
Proteins	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-

EE: ethanol extract; ME: methanol extract; CE: chloroform extract; '+': presence of phytochemical; '-': absence of phytochemical.

Steroids were present in all the extracts whereas terpenoids, proteins, amino acids were absent in all the extracts of the plants as shown in table 2. Methanol extracts showed the presence of most of the phytochemicals analysed i.e., alkaloids, flavonoids, tannins, steroids, carbohydrates and glycosides, with the exception of saponins. Saponins were present in *C. aromaticus* and *P. patchouli*, but absent in *L. linifolia*. However, ethanol extracts of the three study plants showed considerable differences in the phytochemical composition. Flavonoids, tannins and steroids were identified in the ethanol extracts of the three study plants. Alkaloids and glycosides were detected only in *L.*

RESULTS AND DISCUSSION

The commonly found phytochemicals in plants are alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids. Therefore the present study involves a preliminary screening of the phytochemicals in leaf extracts of three plants from the Lamiaceae family namely - *Leucas linifolia*, *Coleus aromaticus* and *Pogestemon patchouli*. Although in traditional medicine water is used as solvent for plant extraction, studies have shown that organic solvent extracts show greater biological activity than the aqueous extract.²⁵ Hence, three frequently used solvents, i.e., methanol, ethanol and chloroform were used to make the extracts of each plant to identify the best solvent for phytochemical extraction.²⁶

Preliminary screening for phytochemicals**Qualitative screening**

The phytochemical composition of nine extracts, i.e., extracts of the three study plants using three solvents (ME, EE and CE) is summarized in table 2.

linifolia; saponins were detected in *L. linifolia* and *C. aromaticus*; and carbohydrates were present in *L. linifolia* and *P. patchouli*. The use of three solvents for extraction has revealed that the phytochemical composition of the extract varies with the solvent used.

The results of this study indicate that the leaves of the three study plants contain alkaloids, flavonoids, tannins and phenols, steroids, saponins, carbohydrate and glycosides (table 2). These phytochemicals are known to be of therapeutic importance since they have biological roles. For example, flavonoids are shown to have antibacterial activity.²⁷ Phenols are shown to have

antioxidant activity.²⁸ Tannins are shown to have antiviral, antitumour, wound healing and antiparasitic effects.^{8, 29} Saponins being steroidal glycosides could be precursors for the synthesis of steroidal drugs like corticosteroids, sex hormones and contraceptives.⁸ Hence a quantitative evaluation of the flavonoids, total phenols, tannins, saponins and alkaloids was attempted to study the concentration of these phytochemicals.

Quantitative Analysis

The extractive yield of all the plants is shown in table 3. The extractive yield of *P. patchouli* was highest among all the three plants, with a maximum yield in ethanol extract (13.33% w/w) followed by methanol (12.6% w/w) and chloroform (7.16% w/w).

The results of the quantitative estimation of the chemical constituents - alkaloids and saponins by gravimetric analysis and total phenols, flavonoids, tannins, steroids and proteins by spectrophotometric method is summarized in table 4(a) and 4(b) respectively.

Alkaloids and saponins were estimated using the plant powder by gravimetric analysis and expressed in grams percentage (table 4a) of the leaf powder by weight. Percentage of alkaloids was higher in *L. linifolia* and saponin content was highest in *P. patchouli* than the other plants of this study.

Table 3: Extractive yield of the leaves of *L. linifolia*, *C. aromaticus* and *P. patchouli*

Plants	Solvents		
	Methanol	Ethanol	Chloroform
<i>Leucas linifolia</i> (% w/w)	6.43	5.00	2.23
<i>Coleus aromaticus</i> (%) w/w)	4.73	6.67	7.16
<i>Pogestemon patchouli</i> (% w/w)	12.60	13.33	7.16

The extractive yield of the crude extracts of the plants from three solvents - methanol, ethanol and chloroform; Extractive yield is expressed in % (w/w).

Table 4a: Quantitative analysis for alkaloids and saponins

Chemical constituents	Plants		
	<i>L. linifolia</i>	<i>C. aromaticus</i>	<i>P. patchouli</i>
Alkaloids (gms %)	1.6 ± 0.35	0.93 ± 0.23	1.53 ± 0.31
Saponins (gms %)	4.82 ± 0.08	6.23 ± 0.08	14.23 ± 0.21

Alkaloids and saponins estimated by gravimetric analysis and expressed in grams percentage of the leaf powder by weight. The dried leaf powder was used for the analysis as described in the methods. The values are expressed as mean ± standard deviation of three readings (n = 3).

Table 4b: Concentration of the phytochemicals (% W/W) in the leaf extracts of *L. linifolia*, *C. aromaticus* and *P. patchouli*

Plants	Phytochemical constituents	Concentration (% W/W)		
		Methanol	Ethanol	Chloroform
<i>Leucas linifolia</i>	Total phenol	23.79 ± 0.39	9.10 ± 0.56	0.50 ± 0.02
	Tannins	18.04 ± 0.32	2.83 ± 0.22	1.09 ± 0.00
	Flavonoids	11.66 ± 0.05	4.70 ± 0.13	ND
	Proteins	17.07 ± 0.06	20.40 ± 0.04	ND
	Steroids	13.56 ± 0.38	17.67 ± 0.76	40.57 ± 0.29
<i>Coleus aromaticus</i>	Total phenol	16.10 ± 0.45	15.13 ± 0.36	0.37 ± 0.01
	Tannins	12.06 ± 0.33	3.56 ± 0.17	1.47 ± 0.06
	Flavonoids	11.63 ± 0.05	2.53 ± 0.26	ND
	Proteins	9.09 ± 0.05	33.87 ± 0.12	ND
	Steroids	16.22 ± 0.38	15.33 ± 1.15	20.50 ± 0.87
<i>Pogestemon patchouli</i>	Total phenol	17.39 ± 0.27	7.13 ± 0.21	0.66 ± 0.01
	Tannins	13.60 ± 0.44	3.36 ± 0.02	1.17 ± 0.02
	Flavonoids	14.77 ± 0.08	18.60 ± 1.38	3.20 ± 0.02
	Proteins	18.22 ± 0.02	12.55 ± 0.05	ND
	Steroids	24.00 ± 0.50	49.49 ± 0.51	73.40 ± 0.29

ND: not detectable

Observations indicate that the concentration of total phenols and tannins are significantly higher in the methanol extract as compared to ethanol and chloroform extracts while the concentration of protein was higher in the ethanol extract as compared to the methanol extracts and there was no protein in the chloroform extract (table 4b). Chloroform extracts have minimum concentrations of all the phytochemicals analyzed quantitatively except for steroids, the highest being 73.40 ± 0.29 % in *P. patchouli*. Among all the extracts studied, methanol extract of *L.*

linifolia had the highest concentration of total phenols and tannins i.e., 23.79 ± 0.392 % and 18.04 ± 0.44 % respectively.

Although there was variation in the flavonoid concentrations of all the extracts, the highest (18.6 ± 1.38 %) was seen in the ethanol extract of *P. patchouli* compared to all the other extracts. *C. aromaticus* has been reported to possess antiepileptic, antimicrobial, antileishmanial and antitumor activity. This can be

attributed to the presence of specific phytochemical constituents. Flavones such as salvigenin, 6-methoxygenkwanin, quercetin, chrysoeriol, luteolin, apigenin, the flavanone eriodictol and the flavanol taxifolin triterpenic acids are reported in the leaves of *C. aromaticus*.³⁰ Three flavones, namely, salvigenin, cristimartin and chrysoeriol, were identified from the chloroform fraction of *C. aromaticus*.¹⁷ Contrary to these reports, in this study the qualitative and quantitative analysis of chloroform extracts of *C. aromaticus* were negative for flavonoids. This variation may be due to either absence of flavonoids in the chloroform extract or presence in such low concentrations that are undetectable in the method followed.³¹ However, it has been reported that the plant species grown in different geographical areas may contain different phytochemical constituents.⁸

Although the qualitative test, i.e., Biuret test, for presence of proteins, was negative, quantitative analysis by Lowry's method did show the presence of protein in methanol and ethanol extracts (table 4b). This could be due to the difference in the sensitivity of methods i.e., Biuret method (sensitivity-1 mg/ml) and Lowry method (sensitivity-5 to 10 µg/ml). Ethanol extracts of *C. aromaticus* had the highest yield of proteins (33.87 ± 0.12 %) when compared to the methanol extract. However, the chloroform extracts were negative for protein content which could be because proteins are hydrophilic and hence not soluble in chloroform.³²

CONCLUSION

This preliminary phytochemical profiling, both qualitative and quantitative has revealed that the phytochemical composition varies with the solvent used. Hence, based on the phytochemical of interest, it is necessary to use the appropriate solvent for extraction and isolation. This could be the crucial step in further studies on the phytochemical, biological structure-function relationship of the study plants which are already reported to be of therapeutic importance.

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