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Preliminary Phytochemical Screening of *Cymbopogon martinii* (Roxb.) Wats. Leaves from Talode Taluka of Nandurbar District

[1]Sharad B. Kakurde and [2*]Nissar A. Reshi

[1,2]Department of Biological Sciences, School of Science,
Sandip University, Nashik - 422213 (India)
*[2]Corresponding author- Nissar A. Reshi, Associate Professor, Department of Biological Sciences, School of Science, Sandip University, Nashik - 422213 (India)

Abstract

In the current study preliminary phytochemical analysis of various extracts of *Cymbopogon martinii* leaves were carried out. The plant material was collected from the Taloda taluka of Nandurbar district, shade dried and coarse powdered followed by successive extraction using different organic solvents like petroleum ether, acetone, methanol and water. The preliminary phytochemical analysis was carried out by using various reagents. Results revealed the presence of alkaloids, flavonoids, phytosterols, tannins, anthocyanins and other phytochemicals. The results validate the traditional medicinal use of the plants by revealing the presence of the various types of secondary metabolites. Further bioactivity and purification of metabolites needs to be carried for the identification of potential therapeutic agents.

Key words : *Cymbopogon martinii* (Roxb.) Wats, Extraction, Qualitative phytochemical test, Bioactive metabolites, Physical parameters.

India has long been known for its abundant reservoir of medicinal plants, which have been utilized across various regions of the country since ancient eras. The genus Cymbopogon comprises aromatic grasses belongs to the family Poaceae. Monocots form the Poaceae family, some grasses from the said family contain essential oils which are useful in the pharmaceutical sectors. It has more than 180 species, subspecies, varieties and subtypes worldwide[17]. Medicinal plants, also referred to as medicinal herbs, have been used for centuries for their healing properties. The ancient medicine system of India is one of the oldest systems of medicine in the world. There are various medicinal resources found in plants that can be used to produce medication. Moreover, these plants remain crucial for

[1]Research Scholar, [2]Associate Professor

human wellness.

Nandurbar district lies in the in North western part (Khandesh Region) of Maharashtra state in India. Taloda taluka is situated in the district of Nandurbar.

The *Cymbopogon martinii* grass is locally known as Roisa. Cymbopogon martini is a perennial herb, erect, leaves are simple, alternate, aromatic, 9-15 x 0.5-1.0 cm, linear or lanceolate, sessile spikelet's, elliptic-oblong[10-16]. *Cymbopogon martinii* (Roxb.) Wats. is a grass commonly known as Palmarosa. The different common names of this plant are Roshisa (Sanskrit), Rosha gavat (Marathi), Rusa ghas (Hindi), and Rosha grass (English).

II. Taxonomic position of *Cymbopogon martini* (Roxb.) Wats :

Kingdom	-	Plantae
Division	-	Magnoliphyta
Class	-	Liliopsida
Order	-	Poales
Family	-	Poaceae
Genus	-	*Cymbopogon*
Species	-	*martinii*
Scientific name	-	*Cymbopogon martinii*

A variety of medicinal plant species are used to make natural remedies, which are useful for treating a wide range of ailments. Locals refer to this plant as Roisa. Leaves are used to treat fever. To prepare the medicine, boil three leaves in a cup of water, let it cool and drink one cup daily[2]. *C. martinii* is locally known as Rohiso in Navapur area and is rare[7]. Cymbopogon martinii can been found in Nandurbar district and is also referred to as Roshghash. For joint pain, the entire plant as well as its parts are therapeutic[19]. *C. martinii* is found in Dhule district, and is also referred as Roicha. Leaves are used therapeutically to treat colds and coughs[12]. Valuable fragrant oil-producing plant Cymbopogon martinii is found within the talukas in Taloda, Dhadgaon and Akkalkuwa. In Dhadgaon, this plant holds sacred importance for the Ashwatthama fair, when they return from the fair, devotees carry rosha grass with them.

Phytochemicals are natural bioactive compounds found in plants. Natural bioactive compounds are found in various plant parts, such as fruits, flowers, stems, leaves, and roots. Plants contain a mixture of various phytochemicals, such as alkaloids, tannins, and flavonoids. Medicinal plants have always played a major role in the primary health care system. Essential oils from this aromatic species are widely used in cosmetics, fragrances, soaps, detergents, and perfumery[4]. Previous studies on Cymbopogon essential oil and extract have shown the plant's potential to be investigated for therapeutic purposes due to its diverse range of bioactivities[1]. The purpose of this study is to determine the phytoconstituents of Cymbopogon martinii in various solvents using qualitative phytochemical screening.

Collection of plant material :

In the Taloda taluka, the *Cymbopogon martinii* (Roxb.) Wats can be found on the hill slopes in a few villages and the surrounding area. Dr. S.K. Tayade, Head of the Botany Department of PSGVPM Science College at Lonkheda, verified the authenticity of the plant. The plant voucher specimen was kept at the college for future reference.

Macroscopic evaluation :

In the macroscopic evaluation of *Cymbopogon martinii* leaves, different parameters such as colour, shape, size, and smell were studied. The leaves are simple, long, linear, thin, and tapering to a very fine point. Macroscopic evaluation is shown in table-1.

Cymbopogon martinii

Leaves of *Cymbopogon m.*

Preparation of extracts :

The plant was collected from Taloda taluka. Fresh leaves were washed under running tap water and dried under shade in the lab. The dried material was coarse powder, which was sequentially extracted in a Soxhlet apparatus using various organic solvents like petroleum ether, acetone, methanol, and water. The extract was stored in a refrigerator until further use[14,18]. The extractive yield of Cymbopogon martinii leaf is shown in table- 2.

Phytochemical screening :

Preliminary phytochemical investigations were carried out as per the standard methods prescribed by Harborne. Preliminary phytochemical analysis of the extracts was carried out to detect the presence of various phytoconstituents[5,6,8,15]. Preliminary phytochemical analysis is shown in table-3.

Test for alkaloids :

i) Dragendroff's test: 1 ml of the extract was treated with a few drops of Dragendorff's reagent. The formation of the orange-reddish precipitate indicates the presence of alkaloids.

ii) Mayer's test: 1 ml of extract was treated with 2-3 drops of Mayer's reagent. The formation of a whitish cream precipitate

indicates the presence of alkaloids.

iii) Wagner's test: Add 1 ml of diluted HCL, 1 ml of extract, and a few drops of Wagner's reagent. The formation of the reddish-brown precipitate indicates the presence of alkaloids.

iv) Hanger's test: To the 1 ml of extract, add a few drops of Hanger's reagent. The formation of the yellow precipitate indicates the presence of alkaloids.

Test for flavonoids :

i) Alkaline acetate test: To 1 ml of extract, 2 ml of a 2% sodium hydroxide (NaOH) solution and a few drops of HCL were added. The yellow precipitate becoming colourless shows the presence of flavonoids.

ii) Ammonia test: To 1 ml of extract was treated with 1 ml of diluted ammonia solution and a few drops of concentrated H_2SO_4 were added. The yellow precipitate's appearance indicates the presence of flavonoids.

iii) Lead acetate test: To a small quantity of extract, a few drops of 10% lead acetate solution were added. The formation of a yellow precipitate showed the presence of flavonoids.

iv) Sulfuric acid test: 1 ml of H_2SO_4 should be added to 1 ml of extract. Flavonoids can be identified by the formation of an orange precipitate.

Test for tannins :

i) Braymer's test: A 10% ferric chloride solution was added to 1 ml of extract along with 3 ml of distilled water. The formation of a green colour indicates the presence of tannins.

ii) Gelatin test: 1 ml of the extract was dissolved in 5 ml of distilled water using a 10% sodium chloride and 1% gelatin solution. The formation of a white colour indicates the presence of tannins.

Test for phytosterols :

i) Salkowski test: To the 1 ml extract, add a few drops of conc. H_2SO_4 (shake well and allow to stand). The formation of a red colour in the lower layer indicates the presence of phytosterols.

ii) Test for sulphur powder test: A pinch of sulphur powder was added to few ml of extract. Sulphur appears to sink to the bottom, which indicates the presence of phytosterols.

Test for anthocyanins :

i) HCL test: 2 ml of 2N HCL was added to the 1 ml extract, along with a few ml of ammonia. The formation of a pink-red solution that later turns blue-violet indicates the presence of anthocyanins.

Test for Carbohydrates :

i) Barfoed's test: To the 1 ml of extract, 1 ml of Barfoed's reagent was added and boiled in a water bath for a few minutes. The formation of a reddish-brown precipitate indicates the presence of carbohydrates.

ii) Molish test: To the 1 ml of

extract, a few drops of Molish reagent, and 1 ml of concentrated H_2SO_4 were added.The formation of a violet ring indicates the presence of carbohydrates.

iii) Seliwanoff's test: To 1 ml of extract, 3 ml of Seliwanoff's reagent was added and boiled in a water bath for a few minutes. The formation of a pink to cherry-red precipitate shows the presence of carbohydrate.

Test for Protein :

i) Million's test: 1 ml of extract with a few drops of Millon's reagent. The formation of a white precipitate indicates the presence of protein.

ii) Xanthoprotein test: 1 ml of extract with a few drops of concentrated nitric acid. The formation of a yellow precipitate indicates the presence of protein.

Test for Amino acids :

Ninhydrin test : 1 ml of extract was boiled in a water bath for a few minutes along with 2-3 drops of ninhydrin solution. The formation of the purple precipitate indicates the presence of amino acids.

Test for reducing sugar :

i) Benedicts test: 0.5 ml of extract, add 0.5 ml of Benedict's reagent and boiled in a water bath for a few minutes. The formation of a green precipitate indicates the presence of reducing sugar.

ii) Fehling test : 1 ml of extract and equal quantities of Fehling solutions A and B were added, then heated in a water bath for a few minutes. The presence of reducing sugars is indicated by the formation of a brick-red precipitate.

Test for phenolic compounds :

Ferric acid test : 1 ml of extract with a few drops a solution containing 5% ferric chloride was added. The formation of a dark green colour indicates the presence of phenolic compounds.

Test for fixed oil :

Spot test : Between two filter papers, a small amount of extract is pressed. Oil traces on paper as they appear.

Physical parameters :

In the current study, Cymbopogon martini leaves powder was examined. Moisture content, total ash, acid insoluble ash, water soluble ash and sulphated ash were studied. For precision, all experiment was conducted three times, and the results were expressed as mean ± SD [13].

1) Moisture contents : Checking the moisture content of the air-dried crude drug is crucial. After accurately weighing two grams of the air-dried crude drug, it was transferred to a china dish. In a hot air oven, the loaded dish is heated to 105°C and weighed periodically until a uniform weight is obtained. To ensure accuracy, this experiment was performed three times and the percentage moisture content of the sample was determined[3,11].

2) Ash Content: Ash content is the inorganic content that remains after burning a crude drug .

i) *Total ash value :*

In a tarred silica dish, 2 grams of the air-dried crude drug were precisely weighed. It was then burned in an incinerator at a temperature of no more than 450 ^{0}C, cooled in a desiccator, and the weight was recorded. Until the weight remained constant, the procedure was repeated and it is used to calculate the percentage of ash[3].

ii) *Acid insoluble ash :*

Following the previously described procedure, the total ash was recovered. It was then heated with 25 ml of hydrochloric acid for 5 minutes, filtered, and the insoluble material was collected on ashless filter paper. After that, it was burned, washed with hot water, let cool in a desiccator, and then weighed. The percentage of acid-insoluble ash was calculated using drugs that had been air-dried[3,11].

iii) *Water soluble ash :*

The ash was obtained using the aforementioned procedure, and after five minutes of boiling in 25 ml of water, it was filtered, and the insoluble material was collected on ash-less filter paper. After that, hot water is used to wash it, and it will burn for fifteen minutes at a temperature not exceeding above 450°C. Finally, his weight was measured. By deducting the weight of insoluble materials from the weight of the ash, the water-soluble ash is given as the weight difference. The percentage of water-soluble ash is calculated with regard to drugs that were air-dried[9].

iv) *Sulphate ash :*

The silica crucible was heated for ten minutes, let to cool in a desiccator, and finally weighed. Weighing out 2 g of the drug that had been air-dried, gently lit it until it was cool and charred. The residue was moistened with 1 ml of sulfuric acid. It was gradually heated until the white fumes stopped emerging, and then it was ignited at 800 °C + 25 °C until the

Table-1. Macroscopic evaluation *Cymbopogon martinii* leaf

Sr. No	Characters	Observation
1	Color	Green
2	Shape	Leaf blades linear
3	Odor	Aromatic
4	Size	Varying in size

Table-2. Yield of *Cymbopogon martinii* leaf extract

Sr. No.	Extractives	Color	Yield (%)
1	Petroleum ether	Dark green	7.44 %
2	Acetone	Light yellow	6.22 %
3	Methanol	Dark brown	10.84 %
4	Water	Light brown	22.16 %

Table-3. Quantitative phytochemical analysis of *Cymbopogon martinii* leaf extract

Sr. No	Phytochemicals	Chemical Test	Petroleum ether extract	Acetone extract	Methanol extract	Aqueous extract
1	Alkaloids	Dragendroff test	-	-	-	+
		Mayer's test	-	-	-	+
		Wagner's test	+	-	+	+
		Hanger's test	+	-	+	+
2	Flavonoids	Alkaline acetate test	+	-	-	-
		Ammonia test	-	-	+	-
		Lead acetate test	-	-	+	-
		Sulfuric acid test	-	+	-	-
3	Tannins	Braymer's test	-	+	-	-
		Gelatin test	+	-	-	-
4	Phytosterols	Salkowski test	-	-	+	-
		Sulphur powder test	+	+	-	-
5	Anthocyanins	HCL test	-	+	-	-
6	Carbohydrates	Barfoed's test	-	-	-	-
		Molish test	-	-	+	-
		Seliwanoffs test	-	+	-	-
7	Protein	Million's test	-	-	-	-
		Xanthoprotein test	-	-	-	+
8	Amino acids	Ninhydrin test	-	-	+	-
9	Reducing Sugar	Benedicts test	-	-	+	+
		Fehling test	-		-	- -
10	Phenolic compound	Ferric acid test	-	+	-	-
11	Fixed oil	Spot test	+	-	-	-

Note, (+) sign indicate presence and (-) sign indicated absence of phytoconstituent.

Table-4. Physical analysis of *Cymbopogon martinii* leaf

Sr.No.	Parameters	Values (% w/w)
1	Moisture content	8.66 ± 0.4
2	Total ash value	9.16 ± 0.3
3	Acid insoluble ash	5.12 ± 0.4
4	Water soluble ash	6.16 ± 0.5
5	Sulphate ash	12.5 ± 0.5

black particles completely vanished. In an area shielded from air currents, ignition was carried out. The crucible was cooled down, followed by the addition and ignition of a few drops of sulfuric acid. Subsequently, it was left to cool once again before being weighed[9]. Various physical parameters are mentioned in table-4.

Cymbopogon martinii is an aromatic and medicinal plant of great ethnopharmaceutical importance. The presence of the different metabolites in the plant material confirms the traditional therapeutic usage of the plant. The phytochemical investigation was conducted quantitatively. The results of the preliminary phytochemical screening table-3 showed that petroleum ether, acetone, methanol, and water extracts of Cymbopogon martinii contain phytochemicals, namely alkaloids, proteins, flavonoids, phenols, tannins, carbohydrates, reducing sugar, phenolic compounds, amino acids, and phytosterols. The findings indicated that major phytochemicals were more prominently present in petroleum ether and methanol solvents than in acetone and aqueous extracts. Phytochemical analysis showed the highest content of alkaloids and flavonoids in petroleum ether and methanol extracts, followed by acetone and aqueous extracts with the lowest content of tannins and proteins. The results showed that the sample's moisture content was 8.66 ± 0.4, the total ash value was 9.16 ± 0.3, the water-soluble ash value was 6.16 ± 0.5, the acid-insoluble ash value was 5.12 ± 0.4, and the sulphate ash value was 12.5 ± 0.5.

The study concluded that there are potential bioactive metabolites. Also, various physical aspects have been examined in the study. Various extracts have shown the presence of several phytochemicals, such as alkaloids, flavonoids, carbohydrates, tannins, and others. The findings indicate that petroleum ether and methanol extracts have a higher concentration of major phytochemicals compared to acetone and aqueous extracts. The present study determined physical parameters and was carried out. The moisture contents, total ash value, acid-insoluble ash, water-soluble ash, and sulphate ash were 8.66%, 9.16%, 5.12%, 6.16%, and 12.5%, respectively.

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Conflict of Interest :

The authors declare that there is no conflict of interest.

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