

Phytochemical and Physiochemical composition of leaves of *Chonemorpha fragrans* G. Don. and *Ellertonia rheedii* Wight.

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ABSTRACT

Chonemorpha fragrans belonging to Apocynaceae is also known as frangipani vine or climbing frangipani and is commonly used in the Indian Traditional medicine because of its wide variety of phytochemicals. *Ellertonia rheedii*, an indigenous plant of Apocynaceae is widely used in folklore medicine for the treatment of varicose veins. The present study aims at screening of extracts of aim of this work is to screen the extracts from leaves of *Chonemorpha fragrans* and *Ellertonia rheedii* for phytochemical and physiochemical composition. The preliminary phytochemical analysis of ethanol extracts of *Chonemorpha fragrans* showed the presence of phenols, flavonoids, alkaloids, terpenoids, sterols and carbohydrates while the extracts of *Ellertonia rheedii* showed phenols, flavonoids, alkaloids, saponins, terpenoids, glycosides and sterols. All the identified phytochemicals were subsequently quantified. The physiochemical composition analysis revealed the that total ash, water soluble, acid insoluble and sulphated ash in leaves were measured to be 13.36%, 6.01%, 2.34% and 15.13% respectively in *Chonemorpha* while they were found to be 13.11%, 5.44%, 2.03% and 14.21% respectively in *Ellertonia* species. The results were remarkable and both the plants can be further implicated in the treatment of various diseases.

Keywords: *Chonemorpha fragrans*, *Ellertonia rheedii*, phytochemical composition and physiochemical composition

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INTRODUCTION

Since ancient times, medicinal plants, aromatic plants, and their byproducts have been used to treat a variety of metabolic diseases. Studies on phytoconstituents from different plants have demonstrated that they scavenge reactive oxygen species and free radicals to control oxidative stress damage [1-2]. Previous research indicates a relationship between illness incidence and nutrition, with higher consumption of foods high in antioxidants lowering the risk of developing degenerative diseases [3-4]. Therefore, there is a need for the cutting-edge research to determine the phytochemical composition and assess the therapeutic potential of bioactive compounds from botanicals for future drug discovery and development. With their superior anti-oxidative qualities, many herbal preparations are used as infusions as home treatments to guard against a variety of illnesses [5-6]. *Chonemorpha fragrans* (*C. fragrans*) belonging to the family Apocynaceae is commonly known as Wood wine and is distributed in India, China and other parts of South East Asia. It is a lactiferous shrub (Climbing) with scented flowers [7]. Concerning the medicinal uses, roots are used in traditional medicine for the treatment of Jaundice, leprosy and scabies [8-10]. Further, the plant is also prescribed in the treatment of typhoid, urinary infections, common cough, intermittent fever, dyspepsia, cardiac debility etc. [11]. *Ellertonia rheedii* (*E. rheedii*) commonly known as Nara balli belongs to the family Apocynaceae. It is endemic to India and is widely distributed in Tamin Nadu, Karnataka and Keral districts. which means "climber used for the disease of vein". It is a plant native to the Western Ghats of Coorg, Shimoga, Chikamagalore, and Dakshina Kannada in Karnataka [12]. The plant is widely used in the treatment of varicose veins [13]. Therefore, we here in report the

qualitative and quantitative determination of phytochemical and physiochemical composition of *C. fragrans* and *E. rheedii* and we here in report the results of our investigative study.

MATERIAL AND METHODS

Plant material

The leaves of *C. fragrans* and *E. rheedii* were collected in Western Ghats near Sringeri, Karnataka (13° 25' 36.84" N 75° 15' 18.3456" E). A voucher specimen was deposited in AVK College Herbarium (AVK BOT 1023 & 1024).

Solvent extraction

The leaves of *C. fragrans* and *E. rheedii* were separately subjected to extraction using ethanol as a solvent. The extracts were filtered and stored at 4°C under nitrogen atmosphere for further experimentation.

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

The phytochemicals screening was done in accordance with the standard qualitative chemical methods [14]. Both the solvent extracts were screened for carbohydrates, alkaloids, Terpenoids, Anthraquinones, Tannins, monoterpene alcohols, and flavonoids

Test for Tannins

Plant extracts (0.5 g) diluted in Water (20ml) was boiled for 5 min, filtered and FeCl₃ (0.1 %) was added. Appearance of blue to black colour indicates the presence of tannins.

Test for Anthraquinones

This was done in accordance with Borntrager's procedure. Extracts (0.5g) was combined with 2 ml of benzene and shaken before being filtered. The filtrate was then mixed with 10 ml of 1% ammonium solution. After shaking the mixture for a minute, the colour shift was noticed. At the lower phase, the emergence of violet colour indicates the presence of anthraquinones.

Test for Glycosides

Extracts (1g) dissolved in FeCl₃ (0.5ml) followed by addition of acetic acid (4ml) and conc. sulphuric acid (2ml). Appearance of brown ring indicates the presence of glycosides.

Test for Saponins

Extract (1g) and water were shaken vigorously and then heated in a water bath. Appearance of froth indicates the presence of saponins.

Test for Flavonoids

To the plant extracts (0.5g), HCl (1ml) and Mg chip were added and observed for colour change. Appearance of red /red crimson/ orange/crimson magenta indicates presence of flavonoids.

Test for Steroids

To the plant extracts (0.5 g), chloroform (2ml) and conc. sulphuric acid (1ml) were added. Appearance of grass green colour indicates presence of steroids.

Test of Phenols

Plant extracts (0.5 g) and FeCl₃ (1ml) were mixed thoroughly where bluish black colour indicates presence of phenols

Test for Alkaloids

Hager's test was followed for detection of alkaloids, Briefly, plant extracts (0.5g) and HCl (4ml) were mixed thoroughly and filtered. To the filtrate, saturated picric acid was added in a drop wise manner where the appearance of yellow colour precipitate indicates presence of alkaloids.

Test of Terpenoids

Plant extracts (0.5g) and chloroform (2 ml) were mixed followed by addition of conc. sulphuric acid (3 ml) through walls of test tube so as to form a layer. A reddish-brown coloration at the of the interface indicates the presence of terpenoids.

Test for carbohydrates

Plant extracts (0.5g) were diluted with the solvent and Molisch reagent was added followed by conc. sulphuric acid. Appearance of a violet colour ring at the juncture of two layers indicates the presence of carbohydrates.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

ESTIMATION OF TOTAL PHENOLS

The total phenols present in both the extracts were determined by method described in the literature [15]. To an aliquot of plant extracts (100µl, 1µg/ml), 1M Na₂CO₃ and Folin-Ciocalteu reagent were added and mixed well. The mixture was incubated in dark for 15 min and the colour developed was measured at 760nm. Reagent and gallic acid were served as negative and positive control samples. The total phenols were determined as mg of gallic acid equivalent/g of sample using a calibration curve. All experiments were performed in quintuplicate.

ESTIMATION OF TOTAL FLAVONOIDS

The total flavonoids present in plant extracts were determined with the colorimetric assay using AlCl_3 as published in literature [16] and in quintuplicate. To the plant extracts (1ml; 1 $\mu\text{g}/\text{ml}$), AlCl_3 (10%, 0.2ml), Potassium acetate (1M, 0.2ml) and distilled water (5.6ml) were added. The reaction mixture and incubated for 30 min. at 37°C. The absorbance was recorded at 420 nm and the total flavonoids were determined using a calibration curve as mg of quercetin equivalent per gm of sample.

Estimation of Alkaloids

This was carried out as per the method of Harborne [17]. To the extracts (5 ml), acetic acid in ethanol (10%, 200 ml) was added and kept at room temperature for 4 h and then filtered. The filtrate was concentrated followed by addition of conc. NH_4OH drop wise to facilitate precipitation. The precipitate was washed with dilute NH_4OH and then refiltered and the residue was dried and determined the total alkaloids.

Estimation of Steroids

Volumetric flasks (10ml) were filled with plant extracts (1ml). Iron (III) chloride (0.5% w/v, 2 ml) and sulfuric acid (4N, 2 ml) were added first, then potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). After 30 minutes of intermittent shaking at $70 \pm 2^\circ\text{C}$ in a water bath, the mixture was diluted with distilled water to the proper level. At 780 nm, the absorbance was measured in comparison to the reagent blank.

Estimation of Terpenoids

Malik et al., 2017 method was followed for this assay [19]. Plant extracts (10 mg) were soaked in ethanol (9ml) for 24 h and then filtered. The filtrate was extracted with petroleum ether using a separating funnel. The ether extract was dried completely in pre-weighed glass vials. Then the ether was evaporated and the yield (%) of terpenoids was determined using

$$\frac{W_i - W_f}{W_i} \times 100$$

Where W_i = Weight of empty glass vial; W_f = Weight of glass vial with terpenoids

Estimation of Saponins

This was carried out as per Obdoni and Ochuko (2001) method [20] with slight modifications. A 100 ml of 20% aqueous ethanol was added to 20 g of the sample, and it was heated to roughly 55°C for two hours while being constantly stirred over a hot water bath. After filtering the mixture, 200 ml of 20% ethanol was used to further extract the residue. Over a water bath at roughly 90°C , the mixed extracts were reduced to 40 mL. 20 ml of diethyl ether was added to the concentration after it had been moved into a 250 ml separating funnel and vortexed. The ether layer was thrown away, but the aqueous layer was retrieved. After three iterations of the purification procedure, 60 ml of n-butanol was added. Aqueous sodium chloride (5%, 10ml) was used twice to wash the combined n-butanol extracts. The remaining solution evaporated, dried and the saponin content was determined.

Estimation of glycosides

This was carried out according to Nbaeyi-Nwaoha and Onwuka (2014) [21]. Plant extracts (10g) were mixed with Baljet's reagent (10ml), incubated at 37°C for 1h and then distilled water (20ml) was added. Subsequently, absorbance was recorded at 495 nm. Securidaside was used as a reference standard, and the amount of glycosides present was expressed as mg securidaside equivalent (SE)/g.

Physiochemical Analysis

Leaves of *C. fragrans* and *E. rheedii* were dried and powdered and used for the physiochemical analysis for determination of moisture content, total ash, water-soluble ash, acid insoluble ash, crude fibre content, alcohol soluble and water-soluble extractive values, nitrogen content and sulphated ash.

Determination of moisture content

The moisture content was estimated using the AOAC method (1990) [22]. After the extracts were placed in a hot air oven and dried at 105°C , the moisture content was determined as a weight loss indicator. After being placed aside in the oven and dried for 30 minutes at 100°C , the crucible was weighed (W_1). Two grams of the finely ground leaf sample were placed in crucibles, and their weight (W_2) was then calculated. After four hours of drying at 100°C , the samples and crucibles were cooled and weighed (W_3). Subsequently, moisture content was determined by the following

$$\frac{(\text{Initial weight of filled crucible}) - (\text{Final weight of filled crucible})}{(\text{Initial weight of empty crucible})} \times 100\%$$

Determination of ash content

The ash content was determined using the AOAC (1990) method [22]. Finely ground dry leaf material (1g) was stored in a muffle furnace and burned at 500 degrees Celsius. After cooling and weighing the resulting ash, the percentage of ash content was determined using

$\% \text{ Ash content} = \text{Weight of ash} / \text{Weight of original} \times 100$

Water soluble ash

After dissolving the entire amount of ash in 25 ml of water for 5 min, the filtered material was placed in a silica crucible, and the filter paper was then placed in a muffle furnace to be burned at 450 degrees Celsius until it was carbon-free and weighed. Using air-dried material as a reference, the amount of water-soluble ash was determined.

Acid insoluble ash

The total amount of ash was extracted, dissolved in 25 ml of HCl (2N), agitated, and then filtered through ashless filter paper. After that, the filter paper was placed in a silica crucible and burned at 650°C in a muffle furnace to remove carbon before being weighed. Air-dried material was used as a benchmark, and the proportion of acid insoluble ash was computed.

Determination of crude fiber content

This determination was made using the AOAC (1990) method [22]. The crucible was first dried at 105°C and then cooled before being weighed. Sulfuric acid (0.25N, 200ml) was used to dissolve the sample (1g) and filter agent (1g) of celite 545 diatomaceous earth. After filtering the hydrolyzed mixture, the residue was rinsed with water to get rid of extra acid before being filtered again. After that, 200 ml of NaOH (0.313 N) was added, and the mixture was left in a boiling water bath for 30 minutes. After filtering the hydrolyzed mixture, the residue was rinsed with water to get rid of extra alkali before being filtered again. It was then drained and washed with acetone. The residue was then dried in a crucible that was held in an oven at 105°C before being put in a muffle furnace that was run at 550°C. Then the crucible was kept in desiccators and fiber content was determined as

$(\text{Weight of residue without ash}) / (\text{weight Sample}) \times 100\% = \% \text{ Crude Fiber}$

Determination of alcohol soluble extractive value

After precisely weighing the 5g of air-dried leaf powder, it was macerated with 100 ml of ethyl alcohol and shaken for 6 h, with a final stand time of 18 h. It was then dried at 100°C after being filtered and evaporated to dryness in a crucible. The air-dried plant material was used as a reference standard to determine the ethanol soluble extractive value percentage (w/w).

Determination of water-soluble extractive value

The method was same as alcohol soluble extractive but chloroform and water (1:300) was used instead of ethanol.

Determination of nitrogen content

The nitrogen was determined using the AOAC (1990) method [22]. For roughly ten minutes, the Kjeldahl apparatus was steam-treated. Following that, five milliliters of boric acid/indicator were placed beneath the condenser in a conical flask. After adding the digest (5 ml) and washing water to the apparatus, 50 ml of NaOH (60%). Following steaming of the digest, $\text{NH}_4(\text{SO}_4)_2$ was gathered in a receiving flask. The receiving flask solution was treated with HCl (0.01M). Additionally, a blank was run concurrently and the nitrogen % was calculated as follows

$\% \text{ Nitrogen} = (V_1 - V_2) \times (\text{molarity of the acid used}) \times 0.01410 \times \text{weight of sample} \times 100\%$

Where,

V_1 = Volume of the acid used for titration of test solution

V_2 = Volume of acid used for titration of blank solution

Determination of sulphated ash

In a crucible, 2g of leaf powders were combined with sulfuric acid to create a paste. The crucible was placed in a desiccator to cool after being softly ignited until the white fumes stopped flowing. Using dried powdered plant material as a benchmark, the content was weighed and the proportion of sulphated ash was calculated.

RESULTS AND DISCUSSION

The preliminary analysis of phytochemicals of ethanol extracts from leaves of *C. fragrans* and *E. rheedii* was carried out and the results were depicted. As evident from table-1, *C. fragrans* extracts was found to contain phenols, flavonoids, alkaloids, terpenoids, sterols and carbohydrates while the extracts of *E. rheedii* showed phenols, flavonoids, alkaloids, saponins, terpenoids, glycosides and sterols.

Quantitative analysis of phytochemicals

The total phenols accounts for 28.74 and 23.47 mg of gallic acid/gm respectively for *C. fragrans* and *E. rheedii* extracts. While the total flavonoids were 14.45 and 11.74 mg of quercetin/gm respectively for

both the extracts (Table-2). The phytochemicals present in the ethanol extracts were carried out and the results depicted in table-3. The alkaloids, tepenoids, glycosides and saponins were found be 14.61, 11.26, 4.65 and 3.42 mg/100 g in the ethanol extract of *C. fragrans*. While, the ethanol extract of *E. rheedii* showed 13.28, 9.78, 4.13 and 3.02 mg/100 g respectively (Table-3). The Significant observation was that the ethanol extract of both the p[plants showed high amounts of phytochemicals.

Physiochemical Composition of extracts

The physiochemical properties were also determined and the results were depicted in Table-4. The total ash, water soluble, acid insoluble and sulphated ash in leaves were measured to be 13.36%, 6.01%, 2.34% and 15.13% respectively in *C. fragrans*, while they were found to be 13.11%, 5.44%, 2.03% and 14.21% respectively in *E. rheedii*.

Plants have drawn a lot of attention and play a vital role in the search for new effective medicinal agents because of presence bioactive components, such as antioxidants and hypoglycemic and hypolipidemic factors, India has a successful history of using various plant-based ingredients and powerful natural medicines to treat a variety of illnesses. Plants have always been excellent sources of pharmaceuticals, and many of the medications that are on the market today are either directly or indirectly derived from them [23]. Phenols and terpenes are well-known, significant chemicals found in plants that contribute to the antioxidant capacity of various botanicals [24-25]. Numerous studies have demonstrated that flavonoids are antioxidants and are valued nutraceuticals that can counteract the effects of free radical stress [25]. Cardiovascular problems have been linked to the hyperglycemic condition that is frequently observed in people with diabetes mellitus.

The most well-known aspect of flavonoids is their antioxidant properties. They are transformers that change the body's biochemical responses to allergens, infections, and chemicals that cause cancer. Numerous plants exhibit anti-inflammatory, anti-cancer, antibacterial, and anti-allergic properties, and they may have therapeutic applications [26]. Alkaloids are typically natural, organic substances that include nitrogen and have sedative and analgesic properties in addition to being physiologically active. They help lessen the symptoms of despair and stress. Because of their stimulatory properties, which cause excitation linked to cell and nerve problems, alkaloids are generally toxic when consumed in large quantities [27]. Because of their ability to function as an adjuvant and enhance the immune response, saponins are widely used in animal vaccinations. In intracellular histochemistry labelling, several of them are helpful because they allow antibodies to reach intracellular protein molecules [28]. Therefore, *C. fragrans* and *E. rheedii* plants serves as a repository of wide variety of bioactive compounds and could be explored as bio-friendly natural source of phytoconstituents, for the treatment of various diseases.

Table-1: Phytochemical composition of extracts of *C. fragrans* and *E. rheedii*

Parts used	Secondary metabolites	<i>C. fragrans</i> extract	<i>E. rheedii</i> extract
Leaves	Phenols	++	+
	Flavonoids	++	++
	Tannins	-	-
	Alkaloids	++	+
	Terpenoids	++	++
	Anthraquinones	-	-
	Glycosides	+	+
	Carbohydrates	+	-
	Saponins	-	++
	Steroids	+	+

Table-2: Quantitative determination of the total phenols and flavonoids content

Extract	Total phenol content (mg of gallic acid/g)	Total flavonoid content (mg of quercetin/g)
<i>C. fragrans</i> extract	28.74± 1.23	14.45± 1.02
<i>E. rheedii</i> extract	23.47± 1.09	11.74± 0.89

Data presented as Mean ± S.E (n=5)

Table-3: Quantitative determination of phytochemicals in *C. fragrans* and *E. rheedii*

Phytochemicals	<i>C. fragrans</i> extract	<i>E. rheedii</i> extract
	Amount (mg/100g)	Amount (mg/100g)
Alkaloids	14.61 ± 1.18	13.28 ± 1.12
Terpenoids	11.26 ± 1.02	9.78 ± 0.99
Glycosides	4.65 ± 0.56	4.13 ± 0.79
Saponins	3.42 ± 0.43	3.04 ± 0.54

Data presented as Mean ± S.E (n=5)

Table-4: Physiochemical properties of extracts of *C. fragrans* and *E. rheedii*

Properties	<i>C. fragrans</i> Percentage (%)	<i>E. rheedii</i> Percentage (%)
Total ash	13.36 ± 1.03	13.11 ± 1.05
Water soluble ash	6.01 ± 0.67	5.44 ± 0.73
Acid insoluble ash	2.34 ± 0.21	2.03 ± 0.28
Sulphated ash	15.13 ± 1.07	14.21 ± 1.13
Moisture	7.11 ± 0.63	6.68 ± 0.75
Water soluble extractive value	12.98 ± 1.12	12.01 ± 0.94
Alcohol soluble extractive value	25.79 ± 1.19	23.86 ± 1.13
Crude fibre content	13.32 ± 1.08	12.44 ± 1.14
Nitrogen	6.77 ± 0.91	6.09 ± 0.96

Data presented as Mean ± S.E (n=5)

CONCLUSION

The extracts from leaves of *C. fragrans* and *E. rheedii* showed substantial amounts of phenols, flavonoids, alkaloids, glycosides, saponins and sterols. The medicinal benefits of botanicals are due to the presence of wide variety of secondary metabolites called phytochemicals. It also expresses optimism for the creation of several new therapeutic agents or templates from these plants, which might eventually be used to create synthetically enhanced medicinal compounds. There is further scope of research in this area wherein, further are needed on various biochemical aspects of extracts where the data procured might help in the development of suitable formulations for the treatment of various diseases and disorders.

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COMPETING INTERESTS

The authors declare that there are no competing interests.

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