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CHARACTERIZATION OF PHENOLIC COMPOUNDS IN *EUCALYPTUS GLOBULUS* AND *CYMBOPOGAN CITRATUS* LEAF EXTRACTS

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ABSTRACT
The use of natural plant resources for applying ecofriendly finish for textiles has opened up new avenues in this area of research. The present study was undertaken to identify the phytochemicals present in *Eucalyptus globulus* and *Cymbopogan citratus* fresh leaf extracts. Extraction of phytochemicals was carried out by different solvents *viz*., ethyl alcohol, methanol, chloroform and distilled water. The phytochemical screening was carried out for the presence of various phenolic compounds, total phenolic content (TPC) and total flavonoid content (TFC). Results for qualitative tests revealed the presence of alkaloids, flavonoids, tannins, saponins and terpenoids in both the plant sources. TPC of *E.globulus* was high in ethyl alcohol (302.67mg/g) extract compared to methanol and distilled water extracts, whereas in case of *C.citratus,* high TPC was found to be in methanol extract (145.53mg/g). TFC was found highest in methanol extract (251.32 mg/g) of *E.globulus* and ethanol extract (81.93 mg/g) of *C.citratus*. Results were found to be highly significant which implies that there is a greater influence of different solvents used for extraction of phenolic compounds.

INTRODUCTION

Crude plant extracts (e.g. infusion, tincture, decoction or others) are traditionally used by populations all over the world for medicinal purposes. Although their effectiveness and mechanism of action have not been scientifically tested in the majority of the cases, they often mediate beneficial responses due to their bioactive chemical components (Barnes *et al*., 2007). The organic compounds usually related with physiological actions on the human body include alkaloids, phenolics, flavonoids, tannins, terpenoids and steroids (Yadav and Agarwala, 2011). These bioactive compounds commonly found in plants have been shown to have possible health benefits with anticarcinogenic, antihypertensive, antimut agenic, *i.e.* antimicrobial and antioxidant activities (Sweetie *et al*., 2014).

Eucalyptus globulus is a member of Myrtaceae family, it is well known as medicinal plants because of their biological and pharmacological properties. The essential oils from *E.globulus* are having great demand in the market, because of their anesthetic, antiseptic, astringent, disinfectant properties. They are also used as a folk remedy for abscess, arthritis, asthma, boils, burns, cancer, diabetes, dysentery, encephalitis, enteritis, fever, leprosy, malaria, rhinitis, sores, spasms, worms and wounds (Elliot and Jones, 1986). *E.globulus* leaf extract is used to treat influenza, chest problem, skin rashes and its vapour inhaled in cases of inflammation of respiratory tract at household level.

Cymbopogan citratus belongs to Poaceae family, it is a tufted perennial erect herb with a short rhizome. *C. citratus* oil is used in treatment of cholera. Leaves are chewed for soregums, also used for elephantiasis. The juice is applied during headache. This aromatic plant has also been reported for its antimicrobial activity against *Aspergillus* species, *Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pneumonia* (Matasyoh *et al*., 2011; Aboaba *et al*., 2006). *C.citratus* is widely used for treating high fever, stomach ache, gut problems and headache. Due to the pleasant aroma of its infusion, this plant is widely used in tropical and subtropical countries. It can also act as an antidepressant and as a mood enhancer. Other researches also reported hypoglycemic, hypolipidemic, anxiolytic, sedative properties (Blanco *et al*., 2009). In a study, isoorientin, isoscoparin, swertiajaponin, isoorientin 2"-O-rhamnoside, orientin, chlorogenic acid and caffeic acid were isolated from *C.citratus* extracts (Cheel *et al*., 2005).

Traditional technologies, especially the science of healing using natural herbs is origin to India, called the ayurveda. Ayur based clothing is now gaining popularity, there is a lot of awareness created among consumers with respect to the hazards caused by long term use of synthetics and chemical finishes. Roots, stems, leaves, flowers, fruits and seeds of plant sources contain bioactive components that are made use in treating diseases. The leaves yield dye that is used to color clothes and the medicinal properties of the plants could be

due to the phytochemicals present in them (Jyoti and Giridhar, 2016).

Textile materials have found different end uses in medicinal and healthcare applications. Depending on the specific end use, different products have to meet the demands for the specific end use performances. Irrespective of their applications, internal (surgical threads and various implants) or external (gauzes, bandages, surgical masks, gowns and apparel, nappies and so on), medical textiles have to be comprised of basic bioactive properties, especially antimicrobial. Phytochemical surveys are being seen as the first step towards the discovery and structural elucidation of useful natural organic constituents for textile or medicinal applications (Hostettmann *et al*., 2000). The mode of action of plants producing dyeing effects on selected textile materials can be better investigated if the active ingredients are identified and characterized (Wanyama *et al*., 2011).

Clothing being the second skin needs to be hygienic and is one of the major factors responsible for disease transmission. There is a need for intense research for sanitational clothing for a healthy living. The awareness for health and hygiene among consumers is increasing the demand for functional products. Medicinal and aromatic plants possess numerous properties *viz*., antimicrobial, aroma and wound healing; attributed to the presence of various complex chemical substances with different composition. Hence the present study was undertaken for evaluation of bioactive components present in *E.globulus* and *C.citratus* leaf extracts. The extracts were further quantified for total phenolic content (TPC) and total flavonoid content (TFC).

MATERIALS AND METHODS

Plant sources

Fresh leaf samples of *E.globulus* and *C.citratus* were selected for the study which were collected from the forest region of western ghats (Dharwad district) of Karnataka state in India.

Chemicals

Ammonia, chloroform, ethyl alcohol, methanol, hydrochloric acid, sulphuric acid, ferric chloride, sodium hydroxide, sodium chloride, sodium carbonate, lead acetate, gelatin were purchased from Rankem chemicals, Bangalore which were of AR grade. Sodium nitrite and aluminium chloride were purchased from Thomas-Baker, Mumbai. Folin-Ciocalteu and Dragendorff's reagent was purchased from Merck, Germany. Gallic acid and rutin standards were procured from Sigma-aldrich, Germany.

Preparation of extracts

The leaf samples were cleaned using distilled water and dried for a while to remove moisture from its surface. 2gm of fresh leaf was weighed, chopped into fine pieces and ground in mortar and pestle. Solvents used for extraction were ethyl alcohol, methanol, chloroform and distilled water. 2gm each of finely ground fresh leaf sample was mixed in 25mL of each of the solvent and incubated under agitation at 200forward and backward strokes in incubator cum shaker (Inkarp, Germany) for 24 hours at 25°C. The extracts were centrifuged (Remi Laboratory Equipments, Mumbai, India) at 10000 rpm at 4°C for 15 minutes and supernatant was separated. Residue was re-extracted with another 25mL of the respective solvent and the process repeated. The supernatants obtained were pooled and measured, stored under refrigeration at 8°C until further analysis within a week.

Phytochemical screening

Qualitative phytochemical screening of plant extracts was performed for the identification of various classes of phenolics like alkaloids, flavonoids, tannins, saponins and terpenoids using different chemical test methods.

Test for alkaloids

Dragendorff test

To 1mL of extract, few drops of Dragendorff's reagent were added. A prominent yellow precipitate indicates the positive test.

Wagner test

Few drops of Wagner's reagent were added by the side of test tube to 1ml of extract. A reddish-brown precipitate confirms the test as positive.

Test for flavonoids

Ammonia test

Few drops of 1% NH_3 solution was added to 1ml of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Sodium hydroxide test

Few drops of 20% NaOH solution was added to 1ml of extract. On addition of HCl, the yellow colour of the extract turns to a colourless solution that depicted the presence of flavonoids.

Test for tannins and phenolic compounds

Ferric chloride test

1ml of extract was separately stirred with 10ml of distilled water and then filtered. A few drops of 5% $FeCl_3$ was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of tannins.

Gelatin test

2ml of 1% solution of gelatin containing 10% NaCl is added to 1ml of the extract. White precipitate indicates the presence of phenolic compounds.

Lead acetate test

3ml of 10% lead acetate solution was added to 1ml of extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds.

Test for saponins

Foam test

About 1ml of the sample extract was boiled in 20ml of distilled water in a water bath and filtered; 10ml of the filtrate was mixed with the 5mL of distilled water and mixed vigorously for 15min to form a stable persistent froth. The presence of froth after 5min was taken as an indication of presence of saponins.

Test for terpenoids

Salkowski test

1ml of each extract was mixed with 0.5mL of chloroform and

1ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface indicates positive results for the presence of terpenoids.

Total Phenolic Content (TPC)

TPC in the extracts was determined using Folin-Ciocalteu assay method (Singleton and Rossi, 1965) using gallic acid as the reference standard. All the solvent extracts were diluted to appropriate volumes and were mixed with 100μL of Folin-Ciocalteu reagent, incubated at room temperature for 3 minutes. Then 2ml of 10% Na_2CO_3 solution was added to the mixture and the resulting solution was incubated for 60 minutes at room temperature under dark. The absorbance was measured at 765nm using the UV-Visible Spectrop hotometer (Cary 50, Varian, Middelburg, Netherlands). TPC was expressed as milligrams of gallic acid equivalent per gm (mg/g GAE) of fresh sample.

Total Flavonoid Content (TFC)

TFC was determined by a colorimetric method (Jyoti and Giridhar, 2015) with minor modification. Aliquots (1ml) of appropriately diluted extracts or standard solutions were pipette into 15ml polypropylene conical tubes containing 2ml double distilled H_2O and mixed with 0.15ml of 5% $NaNO_2$. After 5min, 0.15ml of 10% $AlCl_3.6H_2O$ solution was added and the mixture was allowed to stand for another 5min and then 1ml of 1M NaOH was added. The resulting solution was mixed well, kept for 15min and the absorbance was determined at 415nm using the UV-Visible Spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). TFC was expressed as milligrams of rutin equivalent per gm (mg/g RE) of fresh sample.

RESULTS AND DISCUSSION

Yield of extracts

Yield of extract as ml per (ml/50ml) of solvent is recorded in Figure I. It is observed that the yield of *E. globulus* using distilled water (41ml/50ml) was higher followed by methanol (38ml/50ml) and ethanol (37ml/50ml) solvents. Similarly *C. citratus* extract exhibited higher yield using distilled water (44ml/50ml) solvent compared to methanol (42ml/50ml) and ethanol (41ml/50ml).

Phytochemical screening

Preliminary phytochemical screening of *E.globulus and C.citratus* leaf extracts using ethanol, methanol, chloroform and distilled water is depicted in Table 1, wherein the results revealed that test for alkaloids for both the sources were positive for all the solvents except in chloroform extract for dragendorff's test in *E.globulus*.

The test for flavonoids revealed positive results in ammonia

Table 1: Phytochemical screening of *E.globulus* and *C.citratus* leaf extracts

S.No	Chemical tests	*E. globulus*				*C. citratus*			
		E*	M*	C*	D*	E*	M*	C*	D*
I.	Alkaloids								
1.	Dragendorff's test	+	+	-	+	+	+	+	+
2.	Wagner's test	+	+	+	+	+	+	+	+
II.	Flavonoids								
1.	Ammonia test	+	+	+	+	+	+	+	+
2.	Sodium hydroxide test	-	+	-	-	-	+	-	+
III.	Tannins and phenolic compounds								
1.	Ferric chloride test	+	+	+	+	+	+	-	+
2.	Gelatin test	+	+	+	+	-	+	+	-
3.	Lead acetate test	+	+	+	+	+	+	+	-
IV.	Saponins								
1.	Foam test	+	-	+	+	+	-	+	-
V.	Terpenoids								
1.	Salkowski test	+	+	+	+	-	+	-	-

*E = Ethanol, M = Methanol, C = Chloroform, D = Distilled water; (+) = positive, (-) = negative.

Table 2: TPC of the leaf extracts

Plant source	Extraction solvent Distilled water (mg/g GAE*)	Ethanol (mg/g GAE*)	Methanol (mg/g GAE*)
E. globulus	197.94	302.67	276.53
C. citratus	90.37	85.50	145.53

*GAE: Gallic acid equivalent of fresh sample; All values are mean of triplicate experiments

Table 3: TFC of the leaf extracts

Plant source	Extraction solvent Distilled water (mg/g RE*)	Ethanol(mg/g RE*)	Methanol(mg/g RE*)
E. globulus	145.11	241.90	256.32
C. citratus	60.62	81.93	51.34

*RE: Rutin equivalent of fresh sample; All values are mean of triplicate experiments

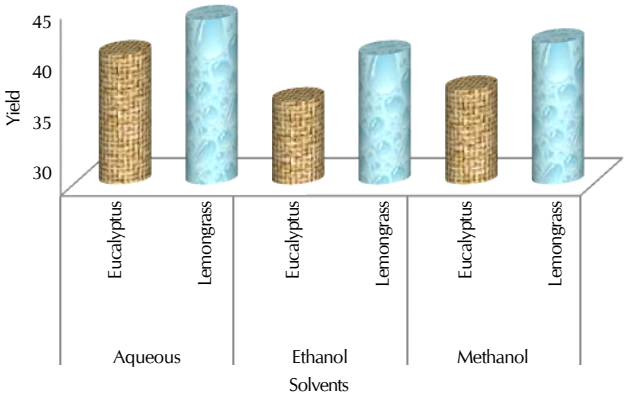

Figure I: Yield of different solvent extracts

test for both the sources in all the solvents, whereas with respect to sodium hydroxide test, the results were positive in methanol extract of *E.globulus* and for *C.citratus* it was positive in methanol and distilled water extract, but was negative for all other extracts.

The test for tannins was performed by three different tests *viz*., ferric chloride, lead acetate and gelatin tests. For ferric chloride test the results were positive in all the extracts for both the sources *i.e., E.globulus and C.citratus* except in chloroform extract of *C.citratus*. On the other hand, lead acetate test exhibited positive results in all the extracts for both the sources except in distilled water extract of *C.citratus* which showed negative results. Also for gelatin test the results were positive in all the extracts of *E.globulus* and in methanol and chloroform extracts of *C. citratus,* but negative in ethanol and distilled water extracts of *C.citratus*. In a similar study, phytochemical screening was conducted in the plant extract of *Tridax procumbens* (Tejaswini *et al*., 2011).

Test for saponins was done by foam test wherein except for methanol extract, the test was positive for all other extracts of *E.globulus,* whereas in *C.citratus* extracts the test was positive for ethanol and chloroform solvents and negative for methanol and distilled water extracts.

Test for terpenoids revealed positive results in all the extracts of *E.globulus*, whereas the test was positive only in methanol extract of *C.citratus* and negative in ethanol, chloroform and distilled water extracts.

Total Phenolic Content (TPC)

Table 2 reveals that TPC concentration of *E.globulus* leaf was found to be high in ethanol (302.67mg/g) extract followed by methanol (276.53mg/g) and distilled water (197.94mg/g) extracts. On the other hand, methanol extract (145.53mg/g) of *C.citratus* yielded higher amount of TPC compared to distilled water (90.37mg/g) and ethanol (85.50mg/g) extracts. Results of both the sources in different solvents were found to be highly significant. In a similar study, total phenolic content was evaluated in leaf sample of *Ocimum tenuiflorum* (Archana *et al*., 2011).

Total Flavonoid Content (TFC)

Table 3 reveals the TFC of *E.globulus and C.citratus* extracts. TFC in methanol extract (256.32mg/g) of *E.globulus* was found to be high followed by ethanol (241.90mg/g) and distilled water (145.11mg/g) extracts. Whereas, ethanol extract (81.93mg/g) of *C.citratus* yielded higher amount of TFC compared to distilled water (60.62mg/g) and methanol (51.34mg/g) extracts. Results of both the sources in different solvents were found to be highly significant.

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