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A Comparative Study of Phytoconstituents, Antioxidant Activity and Hptlc Finger Printing of Methanolic Extracts of E. *Globules* and *E.Hybrid*

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Abstract
In the present work we have estimated the phytochemical constituents and HPTLC finger printing of methanolic extract of two species of Eucalyptus, E. globulus and E. hybrid and also studied its antioxidant activity. The primary screening of phytoconstituents shows that these two species have wide difference of phytochemicals. The total polyphenolic content curve and flavonoid curve shows the difference of quantity. The HPTLC fingerprinting of these species confirms that these species have wide difference. Its antioxidant activity were assessed by DPPH Scavenging activity in terms of ascorbic acid IC_{50} = 36.23in which E. globulus (pure) represents more scavenging activity at IC_{50} = 48.42 in comparison of IC_{50} = 59.68.

Keywords: Myrtaceae, Eucalyptus globulus, Eucalyptus hybrid, DPPH, Scavenging activity, Methanolic extract

Introduction

India has a very long history of using different plants for the purpose of treating various diseases. Literature 1-20 reveals that since 3000 B.C to 5000 B.C. Peoples of India are using continue plants and its different parts as a source of medicine and for therapy. In the same order Eucalyptus has also been considered as a plant of medicinal importance and is used worldwide due to its activity against fungus and other harmful microorganism [1]. The extracts obtained by Eucalyptus plant showed significant antioxidant and antimicrobial activities [2] against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Staphylococcus aureus* and Candida albican. Eucalyptus species (Family *Myrtaceae*) is aromatic plant originated in Australia. It now grows in almost all tropical and subtropical areas and is also cultivated in many other climates including India. Family Myrtaceae have 133 genera and 3800 species [4]. Much research has been conducted on the antioxidant properties of different species of Eucalyptus in which E.globulus has been widely studied and it was found that by using three different antioxidant assays: 2,2 diphenylpicrylhydrazyl (DPPH), 2, 2 azino-bis [ethylbenzthiazoline-6-sulfonic acid] (ABTS) and β-carotene bleaching the methanolic extract showed higher antioxidant activity against both DPPH and ABTS radicals than petroleum ether extract [5] performed several tests on different parts of the Eucalyptus tree such as leaves, bark, seeds etc [6]. Polyphenolic phytochemical in Eucalyptus acts as a powerful antioxidant, and can be used as a supplement to a daily intake of bio active compounds. Phytochemicals like phenolic acids, polyphenols, flavonoids, flavonols terpenoids vitamin c, vitamin E, Carotenes, phenolic acid, phytate, and phytoestrogens scavenge the free radicals activity by inhibiting oxidative mechanisms which lead to emergence of various diseases because they possess electron rich molecules [7]. One important reason of using Plants and herbs as a traditional medicine from ancient time for treatment of diseases because of its easy availability, less expensive, safe and efficient working and have rare side effects [8]. Due to these special properties it introduced in phytotherapy and aromatherapy mostly related to the fragrances in which inhalation is introduced for the purpose to prevent from diseases and infections [9].

In the present paper two different species of Eucalyptus plant were taken and their different phytoconstituents, Antioxidant activity DPPH radical scavenging activity and HPTLC finger printing was studied.

Materials and Methods

The chemicals used for the present study are: DPPH (2, 2 Diphenyl-1-Picrylhydrazyl), Methanol (HPLC grade), acetic acid, ethyl acetate, Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, Qualigens, Cathecol, Quericitineetc. All the Chemicals used were of Analytical grade.

Sampling

Two species of Eucalyptus were collected from the plantation area of TFRI, Jabalpur, India. The plant leaves were harvested in the last week of march month, thoroughly washed with tap water, shade dried, crushed in homogenizer to fine powder weighed and filtered with sieve no. 28 and subjected to solvent extraction.

1 g of fine powdered sample of plant leaves were extracted with 10 ml HPLC grade methanol through maceration process at 30^0C for 30 min. Then the extract was filtered through filter paper (Whatman No. 1) to remove free unextractable substances. The filtrate was evaporated at room temp. Till 2/3 part remained than 10 ml of extract was further diluted with DMSO. The filtrate of plant extracts were preserved at 4-5^0C for further process, and the different phytoconstituents were analysed by following tandard methods, described in pharmacopia.

Determination of total Alkaloid: For this Wagner's test was done

10 mg of extract was taken and few drops of Wagner's reagent was added and observed the colour

Determination of total Carbohydrate:

Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. Observed the colour.

Determination of total Glycosides:

0.5 gm of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added, and observed the colour.

Determination of total polyphenolic content

The total polyphenolic content were estimated colorimetrically by folin-cetieu method. 100 µl of methanolic extract, 2.0 ml water, 2.0 ml of sodium carbonate and 500µl of folin reagent were mixed in a test tube and incubated for 30 min at 60 ºC, then absorbance was taken at 650 nm. by shimadzu 1608 spectrophotometer. Reference standard was taken cathechol.

Determination of total flavonoid content

A flavonoid content was estimated spectrophotometrically by aluminium chloride method 500 µl of test sample, 2 ml of water, 50 µl of sodium acetate and 50 µl of aluminium chloride were mixed in a test tube and incubated at room temperature for 30 min. and the absorbance was recorded at at 650 nm. Quercetin was used as reference standard.

Antioxidant activity
(DPPH radical scavenging activity)

The antioxidant activity of plant leaves extract was determined by in-vitro method (DPPH free radical scavenging assay). The free radical scavenging capacity of the methanolic extracts of Eucalyptus plants was determined by using DPPH (2,2Diphenyl -1-Picrylhydrazyl). DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanolic extracts of plants were further diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml & 1000 µg/ml by serial dilution. 100 µl of various dilution of plant extracts, 3 ml of Methanol & 100 µl of DPPH solution were mixed in a test tube. The reaction mixture was shaken well. DPPH decolourisation was recorded (517 nm) on simadzu 1608 after 30 min. incubation in the dark. The percentage of DPPH scavenging by plant extracts was obtained in terms of ascorbic acid. Percentage of DPPH scavenging was calculated as

% Inhibition of DPPH= Abs of Control – Abs of Sample/ Abs of Control × 100

HPTLC Finger printing of plant sample: Camag Switzerland HPTLC system equipped with an automatic (TLC sample applicator) Linomat 5 fitted with 100 µl syringe (Hamilton, Switzerland) TLC scanner device 5, TLC visulizer, win CATS planar chromatography manager software verson 4.5 and twin trough glass tank (5×5cm) was used for the HPTLC finger printing.

Results

The results are tabulated in the Table -1to-6

Table 1: Screening Of Phytoconstituents in Eucalyptus Hybrid.

Experiment	Observation	Inference
1.10 gram of sample + few drops of reagent.	Reddish brown p.pt formed.	Shows presence of alkaloids
2. 5 gm. of extract + Fehling solution(5ml) and boiled	Change of yellow or red p.pt was observed	Carbohydrate present (Molish test)
3. 0.5 of extract+1ml. of water + NaoH	Yellow colour was observed	Presence of glycosides

Table 2: Screening of Phytoconstituents in Eucalyptus globulus

Experiment	Observation	Inference
1.Same as in table -1	Same as in table-1	Same as in table-1
2.Same as in table-1	Same as in table-1	Same as in table-1
3.Same as in table-1	No change	Glycosides were absent

Table 3: Comparative table of E. globulus and E. hybrid

S. No.	Test Perform	Observation in plants	
		Plant1(E. Hybrid)	Plant2 (E. globulus)
1	Alkaloids	+	+
2	Cabohydrates	++	++
3	Glycosides	+	Not seen

+- Represent mild quantity of phytochemicals.
++- Represent massive quantity of phytochemicals.

Estimation of total polyphenolic content

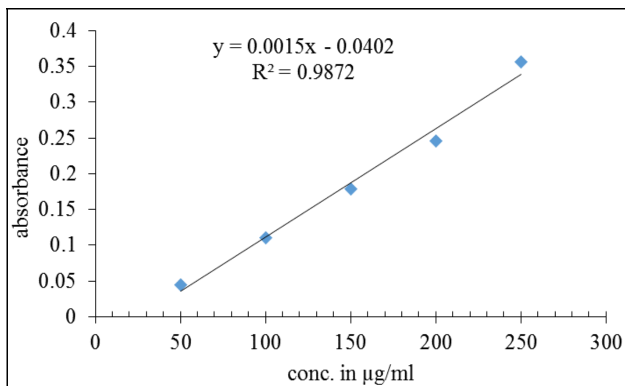

Fig 1: Standard curve of Cathecol for ploypheno l.

Table 4: Representing of polyphenolic content

S. No.	Sample Name	Quantity in µg/ml±SD
1.	Plant1(E.hybrid)	135.0±0.002
2.	Plant2 (E.globulus)	167.0±0.005

Estimation of total flavonoid content

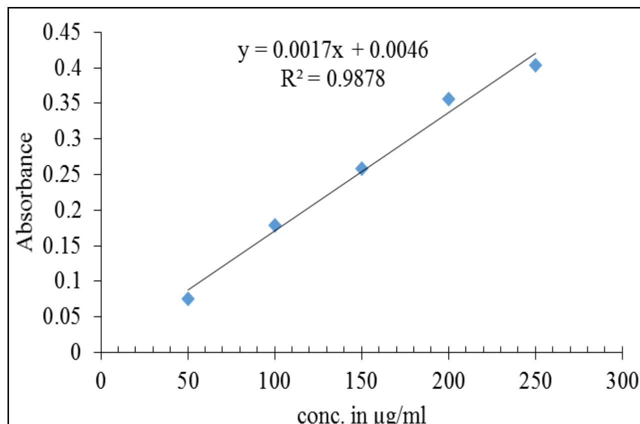

Fig 2: Standard curve of Cathecol for Flavonoid

Table 5: Estimation of Flavonoid in plant extract

S. No.	Sample Name	Quantity in µg/ml±SD
1	Plant1(E.hybrid)	234.0±0.004
2	Plant2 (E.globulus)	185.0±0.007

DPPH radical scavenging activity

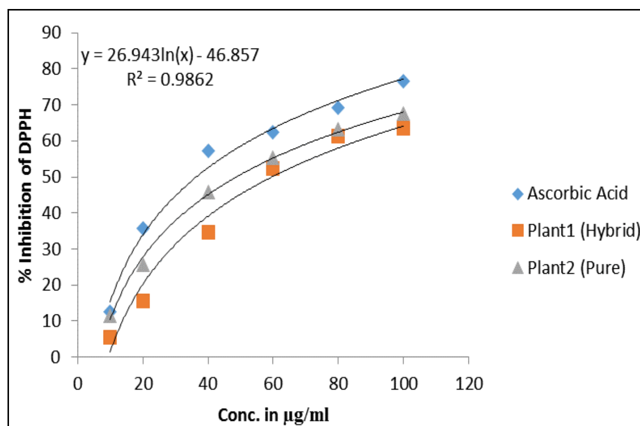

Fig 3: Representing DPPH Scavenging Activity.

Table 6: Representing IC50 Value of plant Extracts

S. No.	Plant Name	IC_{50} Value
1	Ascorbic Acid	36.23
2	Plant 1 (E.Hybrid)	59.68
3	Plant 2 (E.globulus) or pure	48.42

HPTLC Finger printing of plant sample

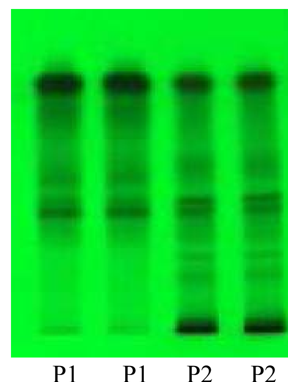

Fig4: HPTLC fingerprinting of plant leaf

Discussion

Table 1, 2, 3 reveals that Alkaloids, Carbohydrates, were present in both the species of Eucalyptus while glycoside was totally absent in Eucalyptus globulus. Table -4, reveals that plant E. hybrid contains less amount of polyphenol i.e135.0±0.007µg / ml. while plant E.globulus has high polyphenolic contents i.e167.0±0.005µg / ml. and perhaps this is the reason that E. globulus is used in various formulation of medicines.

The flavonoid content was less in E. globulus. e 185.0± 0.007µg /ml. while it is high in E. hybrid that is 234.0±0.004µg /ml., Further plant extracts were assayed for there antioxidant activity with the help of DPPH and it was found that E.globulus have the highest antioxidant activity, While E. hybrid has low antioxidant activity.

HPTLC fingerprinting was done and scan at 254 nm and found several spots on TLC plate which reveals that methanolic extract have several active component in E. globulus. The results concluded that E. globulus is found to be effective against some pathogenic microorganisms involved in wounds, burns and skin infections. The extracts of the plants have potent antioxidant low concentration. The scented volatile oil in *E. globulus* may be incorporated in pharmaceuticals e.g. *Eucalyptus* syrup, anticough solutions and suppositories for its strong antibacterial action. It could also find much use as expectorants and decongestants.

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