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Qualitative and Quantitative Phytochemical Analysis and Evaluation of Biological Activity of Leaf Extract of *Eucalyptus globulus*

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A B S T R A C T

Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Plant *Eucalyptus globulus* play a pivotal role in folk medicine. The present study was conducted with the main objectives of qualitative and quantitative phytochemical analysis and determination of antioxidant properties leaf extract of *E. globulus*. Leaves of *E. globulus* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol and acetone. The major phytochemicals found in leaf extract of *E. globules* were flavonoids, glycosides, proteins & amino acids, saponins, steroids, phenolic compounds/tannins, and terpenoids. Whereas, the phytochemicals alkaloids were found to be absent in leaf extract of *E. globulus*. The quantitative estimation of phytochemicals of leaf extract of *E. globules* delineated that flavonoid quantity of leaf extract of *E. globulus* was found to be highest (11.46 GAE) followed by total phenols (5.12 GAE), and tannins (1.26 GAE).The IC_{50} values exhibited by leaf extract of *E. globulus* was found to 210 µg/mL. In conclusion, this preliminary study confirms that the leaves of *E. globules* has wide variety of secondary metabolites. Biological activity such as antioxidant properties of leaf extract of *E. globules* depicted that *E. globulus* could be potential drug agent of folk medicines.

Introduction

Nature has been a source of medicinal agents for thousands of years. Recently, research is being done on medicinal plants worldwide. Plants have been used for the healing of diseases since ancient before the use of recent clinical drugs. Such medicinal plants are also recognized to have therapeutic properties or as precursors for the synthesis of useful drugs (Sofowora, 1982). More than 50% of these synthetic drugs are derivative of natural products. These natural products play a crucial role in drug development (Jeyachandran and Mahesh, 2007). With the increasing use of chemicals, antibiotics many pathogens have developed resistance against them; hence there is immense need to develop

new anti-agent with improved performance and wide applications.

Eucalyptus globulus is very huge and indestructible tree, which was first come across on the Tasmania Island in the year of 1792. It is one of the large genera of family Myrtaceae. There are approximately 900 species of eucalyptus and almost all of them are found in Australia. Eucalyptus is a very prominent tree in India because it was introduced in the year of 1843 as a fuel tree. *Eucalyptus globulus* is one of the convoluted species as it consists of peculiar four sub species these are *Eucalyptus bicostata*, *Eucalyptus pseudoglobulus*, *Eucalyptus globulus* and *Eucalyptus maidenii*. *Eucalyptus globulus* is well grown in the different part of India such as Nilgiris, Annamalai, Palani and Shimla hills, and there are numerous species of Eucalyptus which are cultivated in the variety of climatic conditions but the most suitable one is found in the warm temperature and subtropical province, because of their high economical value. There are more than hundred species which have been seen in India at contrasting period of time and some species are below planting (Kesharwani *et al.*, 2018)

Eucalyptus globulus has a long history of folk usage because of its rich medicinal values. The plant has been reported to possess potent antiseptic, astringent, deodorant, diaphoretic, expectorant, inhalant, insect repellant, rubefacient and suppurative properties (Fresquet Febrer, 1995; Javaid *et al.*, 2012). Various volatile phytochemicals like isoprenoids are found in the leaves of Eucalyptus which show a number of medicinal properties (Olorundare *et al.*, 1998). Eucalyptus extracts have been approved as food additives and are currently used in various cosmetics formulation. Saponins, tannins, steroids and flavonoids have been found in the leaf extract of Eucalyptus. Alkaloids and flavonoids possess antimicrobial activity (Sartorelli *et al.*, 2007). Traditionally, Eucalyptus leaves have been used to heal wounds and fungal infections. Eucalyptus leaves shown wide varieties of medicinal activities such as antioxidant, antiseptic and anti-inflammatory. Besides antimicrobial activity, the essential oil and its constituents also shown herbicidal (Setia *et al.*, 2007), anthelmintics (Bennet and Bryant, 1996), insecticidal (Rudin, 2005; Park and Shin, 2005), anti-tumor (Takasaki *et al.*, 1995) and anti-leech (Kirton, 2005) activities.

Free radicals are continuously being produced in our body as a result of various metabolisms. Some amounts of free radicals is very much necessary for body's defense system, signalling mechanism and in the induction of a mitogenic response. But the persistence of these free radicals even after their activity results in deleterious effects. These free radicals act on important biomolecules like nucleic acids (mutations), lipids (membrane lipid peroxidation), proteins (oxidation) and carbohydrates resulting in various diseases. Over 70 degenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss, depression, arthritis, cancer, ageing etc.) are caused due to free radicals (Fridovich, 1999; Pan *et al.*, 2011; Floyd *et al.*, 2011). Herbal medicines are the materials which are derived from one or more plant which possess some curative values to prevent human body from common diseases (Devi, 2011). Free radical scavenging property can be removed completely by the antioxidants and maintains the balance in the body (Mohani *et al.*, 2014).

Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Plant *Eucalyptus globulus* play a pivotal role in folk medicine. With this scenario, in the present study we mainly

aimed for qualitative and quantitative phytochemical analysis and evaluation of antioxidant properties of leaf extract of *E. globulus*.

Materials and Methods

Collection of plant material

The leaves of *E. globulus* were procured commercially from the local market of Bengaluru, Karnataka, India. The leaves of *E. globulus* were prayed with ethanol, and then shade dried at room temperature for 10 days. The dried leaves of *E. globulus* were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *E. globules* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of methanol and acetone with boiling temperature maintained at 67ºC and 56ºC for methanol and acetone respectively. The flask containing the extraction solvent was heated to reflux. The extraction was continued for 48 h. After extraction the solvent was removed. The non-soluble portion of the extracted solid remained in thimble and was discarded. Ultimately the extract was collected from the distillation flask and was filtered using filter paper. The filtrate was collected in the beaker was kept in water bath at 67ºC to remove the solvent so as to obtain a semi solid extract. The extract was preserved in airtight containers and stored at room temperature until further use (Jensen, 2007).

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of leaf extract of *E. globules* was carried out using standard procedures to detect constituents as described by Sofora (1993); Trease and Evans (1989) and Harborne (1973).

Test for Alkaloids

Approximately 0.2g of leaf extract of *E. globules* was warmed with 2% H_2SO_4 (2.0mL) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

The leaf extract of *E. globules* in **s**mall quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins.

Test for Glycosides

About 0.6g of leaf extract of *E. globulus* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Saponins

About 0.2g of leaf extract of *E. globules* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2g of leaf extract of *E. globules* was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of leaf extract of *E. globulus* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3g of leaf extract of *E. globules* was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Test for Proteins and Amino acids

To the 0.3g of leaf extract of *E. globules* few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue colouration indicate the presence of proteins.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the leaf extract of *E. globules* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725 nm increases linearly with the concentration of phenolics in the reaction medium (Singleton *et al.,* 1999). The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in the leaf extract of *E. globules* (Ordonez *et al.,* 2006). The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Tannins

The tannin concentration in the leaf extract of *E. globules* was determined following a modified version of the vanillin-HCl method (Chanwitheesuk *et al.,* 2005). The concentration of tannins was expressed in terms of mg gallic acid equivalent/g of extract powder.

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay (Blois, 1958; Uddin *et al.,* 2012). Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in methanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation.

Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.*, Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

Results and Discussion

The major phytochemicals found in leaf extract of *E. globules* were found to beflavonoids, glycosides, proteins & amino acids, saponins, steroids, phenolic compounds/tannins, and terpenoids. Whereas, the phytochemicals alkaloids were found to be absent in leaf extract of *E. globules* (Table 1).

The results of quantitative estimation of phytochemicals in leaf extract of *E. globulus* was represented in Table 2. Results revealed that flavonoid quantity of leaf extract of *E. globules* was found to be highest (11.46 GAE) followed by total phenols (5.12 GAE), and Tannins (1.26 GAE).

The results of antioxidant activities of leaf extract of *E. globulus* determined by DPPH free radical scavenging method was represented in Table 3.Results delineated that the IC_{50} values exhibited by leaf extract of *E. globules* was found to be 210 µg/mL.

Active research has been driven in recent years on plant-based products due to their biologically beneficial effects emanating from antioxidant activities of phenolic phytochemicals. The plant products over synthetic compound in the treatment of diseases are needed because of no deleterious effects on man. India is a home to a variety of traditional medicine system that relay to a very large extent on native plant species for their raw drug materials.

Therefore, there is a need to look backwards towards folk medicines which can serve as novel therapeutic agent. These free radical intermediates and ROS escape from the site of reaction and act on various biological molecules such as lipids, nucleic acids, proteins and carbohydrates thus causing deleterious changes in their structure and function and finally leading to cell death (Fridovich, 1978). Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Plant *Eucalyptus globulus* play a pivotal role in folk medicine. Therefore, in the present study we aimed for qualitative and quantitative phytochemical analysis and evaluation of antioxidant activities of leaf extract of *E. globulus*.

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Our study results on the qualitative phytochemical analysis of the leaf extract of *E. globulus* delineated the presence of phytochemical constituents such as flavonoids, glycosides, proteins & amino acids, saponins, steroids, phenolic compounds/tannins, and terpenoids. In addition, quantitative estimation of phytochemicals in leaf extract of *E. globules* revealed that flavonoid quantity found to be highest followed by total phenols, and tannins. These findings were comparable with that of literature findings wherein Ben Hassine *et al.*, (2012) reported the presence of phenol content in methanolic extract of *E. globulus*.

According to Amabye *et al.*, (2016) phytochemical composition of the same plant could vary from place to place due to geographical location, climatic conditions and soil condition of a particular area. This may explain why it could be possible to have differences in chemical composition of the same plant of study in other areas.

The phenolic compounds contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. These results gives a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals.

Table.1 Qualitative photochemical analysis of leaf extract of *E. globules*

Phytochemicals	Leaf Extract of *E. globulus*
Alkaloids	-
Flavonoids	+
Glycosides	+
Proteins and Amino acids	+
Saponins	+
Steroids	+
Phenolic compounds	+
Tannins	+
Terpenoids	+

Table.2 Quantitative phytochemical analysis of leaf extract of *E. globules*

Phytochemicals	Leaf Extract of *E. globulus*
Total phenolics	5.12 GAE
Total flavonoids	11.46 GAE
Tannins	1.26 GAE

Table.3 Antioxidant activities of leaf extract of *E. globules*

S. No.	Leaf Extract of *E. globulus*	IC_{50} (µg/mL)
1	Leaf extract	210.00

Tannin content in leaf extract of *E. globulus* in our study was found to be 1.26 mg gallic acid equivalent/g of extract powder. Tannins are another major group of polyphenols in our diets and usually subdivided into two groups: (i) hydrolysable tannins and (ii) condensed tannins. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases.

DPPH is a stable radical that has been used to evaluate the antioxidant activity of leaf extract of *E. globulus*. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to α, α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The activity of extracts is attributed to their hydrogen donating ability. Increasing the number of hydroxyl or catechol groups increases radical scavenging activity. In presence of other H-donating groups (sulfhydryl, amide) in molecule also accelerates this activity. The free radical scavenging activity by DPPH method was observed in leaf extract of *E. globulus* in our study. The IC_{50} value, which is the amount of extract needed to scavenge 50 % of DPPH radical for the leaf extract of *E. globulus* was found to be 210 µg/mL. In accordance with our study findings Pathak and Kumar (2015) reported high antioxidant activity in *E. globulus* extract.

The results obtained in the present study are encouraging as this study evidenced the vide variety of secondary metabolites present in

the leaf extract of *E. globules* and demonstrated considerable antioxidant properties. Hence this study supplies as evidence-based study for leaf extract of *E. globules* could be exploited in the management of various ailments.

This preliminary study confirms that the leaf extract of *E. globules* has wide variety of secondary metabolites. Biological activity such as antioxidant properties of leaf extract of *E. globules* depicted that *E. globulus* leaves could be potential drug agent of folk medicines. However further studies are need to be conducted to elucidate the mechanism of action and phytochemical responsible for biological activities of *E. globulus*.

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