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Phytochemical analysis of *Eucalyptus* leaves extract

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Abstract
Eucalyptus has been used as a medicinal plant from ages because of its various properties. The phytochemical content of Eucalyptus leaves was analyzed by soxhlet extraction of the dried leaves using methanol and acetone. Maximum amount of phytochemicals present in eucalyptus leaves were retained in methanolic extract. The methanolic extract contained quinones, saponins, carbohydrates, tannins, phenols, flavonoids & fat while in the acetonic extract quinones, flavonoids & fat was present. The quantitative analysis of methanolic extract revealed an appreciable amount of antioxidant activity assessed by DPPH scavenging activity expressed in terms of ascorbic acid i.e. IC_{50} value of 444.6, the phenolic content was found to be 11.41% while the total flavonoid content was 35.88%.

Keywords: Eucalyptus, methanolic, Acetonic, phytochemical, DPPH, flavonoid, phenol

Introduction
Nature has been a source of medicinal agents for thousands of years. Recently, research is being done on medicinal plants worldwide. Plants had been used for the healing of diseases ages ago 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) [22]. More than 50% of these synthetic drugs are derivative of natural products. These natural products play a crucial role in drug development (Jeyachandran *et al*, 2007) [8]. 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. Certain plant extracts have shown antimicrobial properties against certain bacteria (Paster *et al*, 1990) [14] and have been studied to be utilized as agro chemical with favorable antimicrobial activity (Kim *et al*, 1996) [9].
The essential oils from the Myrtaceae family exhibit diverse biological activities like bacteriostatic, fungistatic and anti-inflammatory effects. Eucalyptus is one of the important genera of Myrtaceae family, a large genus of evergreen trees and shrubs containing about 700 species (Batish *et al*, 2008) [2]. Although most of the plants of this genera have their native origin in Australia and Tasmania (Oyedeji, 1999) [12], but these have been cultivated in many other countries, including Tunisia and are used for its various properties. Around 500 species of eucalyptus produce essential oil. Eucalyptus are highly valued for their wood and are also a good source of proteins, tannins, and dyes although eucalyptus oil being the most valuable product. The oil can be readily distilled from their leaves (Sartorelli, 2007; Trivedi and Hotchandani, 2004) [18, 24]. Eucalyptus oil is being extracted in many countries like China, India, South Africa, Portugal, Brazil and Tasmania on commercial scale. The other name of this plant is “forest red gum”, which was used traditionally for the treatment of wounds, boils and other ailments. The leaves can be obtained from trees grown solely for oil production. Eucalyptus has been known to be a good source of many natural substances exhibiting antagonistic activities against several microorganisms (Dwivedi and Dubey, 1986; Singh and Agrawal, 1988). Various volatile phytochemicals like isoprenoids are found in the leaves of eucalyptus which show a number of medicinal/antimicrobial properties (Olorundare *et al*, 1998) [11]. 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*, 2006).
Traditionally, eucalyptus leaves have been used to heal wounds and fungal infections. Eucalyptus leaves show many activities such as antioxidant, antiseptic and anti-inflammatory (Rai *et* al, 2013). Besides antimicrobial activity, the essential oil and its constituents also show herbicidal (Setia *et al*, 2007) [19], insecticidal (Rudin 2005; Park and Shin 2005) [17, 13], anthelmintics (Bennet and Bryant, 1996) [3], anti-tumor (Takasaki *et al*, 1995) [23] and

anti-leech (Kirton, 2005) [10] activity. Keeping in view of these properties of eucalyptus, present study was conducted with two objectives:

1) Extraction of eucalyptus oil from the leaves using methanol and acetone.
2) Phytochemical screening of different compounds present in leaves.

Material and Methods

Collection of plant material

Fresh leaves of eucalyptus were collected from the Environment Park, Patiala, Punjab (India). Fresh leaves were picked and sun dried for 20 days and was grinded to a fine powder. This fine powder was used for the extraction. 70 g of the dried leaves were used for the extraction. The extraction from the leaves was done with the help of soxhlet apparatus.

Extraction using soxhlet apparatus

Extraction was done using method described by William, 2007 [25]. The sun dried powdered plant material was extracted in the soxhlet apparatus with two different solvents like methanol and acetone. In the soxhlet apparatus 70 g of eucalyptus leaves powder were extracted with 400 ml of solvent, the boiling temperature was maintained at 67 ºC and 56 ºC 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 67 ºC to remove the solvent so as to obtain a semi solid extract.

Phytochemical screening of eucalyptus extract

Phytochemical screening of leaves extract was done by using both quantitative and qualitative methods. The procedures used to detect the presence of various phytochemical compounds are described below:

Qualitative methods

The phytochemical tests were performed by the method given by Harborne, 1973.

Foam test: Two ml of extract was dissolved in 3 ml distilled water and shaken vigorously. A stable top layer of foam was formed, indicating the presence of saponins in the sample.

Hansch test: Two ml of extract was taken in a test tube. One ml of concentrated H_2SO_4 was added from the side walls of the test tube and the formation of a brown ring suggested the presence of carbohydrates.

Tannin test: To 0.5 ml of extract solution one ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

Flavonoid test: Two ml of filtrate was taken. Five to six drops of concentrated HCl and a few magnesium filings were added to it. Appearance of red color indicates the presence of flavonoids.

Phenol test: In two ml of extract, a pinch of ferric chloride was added. Appearance of green color indicates the presence of phenols.

Protein test: Two ml of extract was taken, and one to two drops of nitric acid was added. Development of yellow color indicates the presence of proteins.

Quinone test: Two ml of extract was taken, few drops of concentrated H_2SO_4 were added and appearance of red color indicates the presence of quinones.

Fat test: The extract was tapped on the filter paper. Appearance of oil on the filter paper showed the presence of fat in the extract of eucalyptus.

Quantitative methods

Determination phenolic contents

The determination of total phenolic content was done using the procedure given by Singleton *et al*, 1999 [21]. Different concentrations of sample were pipetted out from 0.2 to one ml in test tubes. The final volume was made in each test tube to three ml with the help of distilled water. 0.5 ml of folin-ciocalteau reagent was added to test tubes and incubated at room temperature for 3 min then two ml of sodium carbonate was added to it and mixed it thoroughly. Test tubes were kept in boiling water bath for one min. Cooled the test tubes under tap water. The absorbance was measured at 720 nm. The reference standard was taken as gallic acid.

Determination of antioxidant activity

The antioxidant activity was determined by in vitro method (DPPH free radical scavenging assay method of William *et al*, 1995) [26]. The extract of eucalyptus and standard was taken. The different aliquot pipetted out with different concentrations. The final volume was made to 1 ml with distilled water. 50 µl of above each concentration was pipetted out into the second set of test tubes. 5 ml of 0.004% DPPH was added. Tubes were incubated for 30 min at room temperature. The absorbance was measured at 517 nm. The percentage of DPPH scavenging by plant extracts was obtained in terms of ascorbic acid. Percentage of DPPH scavenging was calculated as:

% DPPH inhibition= (Abs of Blank– Abs of Sample)/ Abs of Blank * 100

Determination of flavonoids

Determination of flavonoids was done by procedure given by Zhuang *et al*, 1992 [27]. The extract and standard was taken. The different aliquots pipetted out with different concentrations. Then five ml distilled water and three ml $AlCl_3$ (1:10, w/v) were added. After six min, two ml CH_3-COOK (1M) was added and the total volume was made up to 10 ml and absorbance was measured against a blank at 415 nm. Quercetin served as the standard compound for the preparation of calibration curve.

Results and Discussion

Phytochemical screening of Eucalyptus extract

Qualitative screening

The methanolic extract obtained from the eucalyptus leaves was screened for various phytochemicals. Qualitative phytochemical screening of methanol extract of eucalyptus leaves demonstrated the presence of saponins, quinone, carbohydrate, tannin, phenol, fat, flavonoid while protein was absent (Table 1). Babayi *et al*, 2004 [1] demonstrated the presence of saponins, tannins and fat in methanolic extract of eucalyptus leaves except quinone, flavonoids and phenols.

Rai *et al*, 2013 demonstrated the presence of flavonoid, tannin and phenol in methanolic extract. The acetone extract obtained from the eucalyptus leaves were screened for phytochemicals. Qualitative phytochemical screening of acetone extract of eucalyptus leaves demonstrated the presence of saponins, carbohydrate, tannin, and phenol while quinone, fat, protein and flavonoid were absent. Hassine *et al*, 2012 [6] demonstrated the presence of tannin and phenol in acetone extract of eucalyptus except saponins, carbohydrates. Jahan *et al*, 2011 [7] reported the presence of saponins in acetone extract.

Table1: Phytochemical screening of eucalyptus extract

S. No	Phytochemical constituents	Methanolic extract of Eucalyptus	Acetonic extract of Eucalyptus
1.	Quinones	+	-
2	Saponins	+	+
3	Hansch carbohydrates	+	+
4	Tannins	+	+
5	Phenols	+	+
6	Flavanoids	+	-
7	Proteins	-	-
8	Fat	+	-

Positive (+) Negative (-)

It is clear from the above table that maximum number of phytochemicals from eucalyptus leaf retained in the methanolic extract than that of the Acetonic extracts. So it was further used for the quantitative determination of phenolic, antioxidant and flavonoid content in eucalyptus extract.

Quantitative screening

Estimation of total phenolic contents in eucalyptus extract

Total phenolic contents were determined by taking eucalyptus extract OD at 650 nm and comparing it with standard Gallic acid solution. The total phenolic content of 10 ppm methanolic extract of eucalyptus was found 11.41% (Figure 1). Similarly Hassine *et al* in 2012 [6] reported the presence of phenol content in methanol extract of eucalyptus.

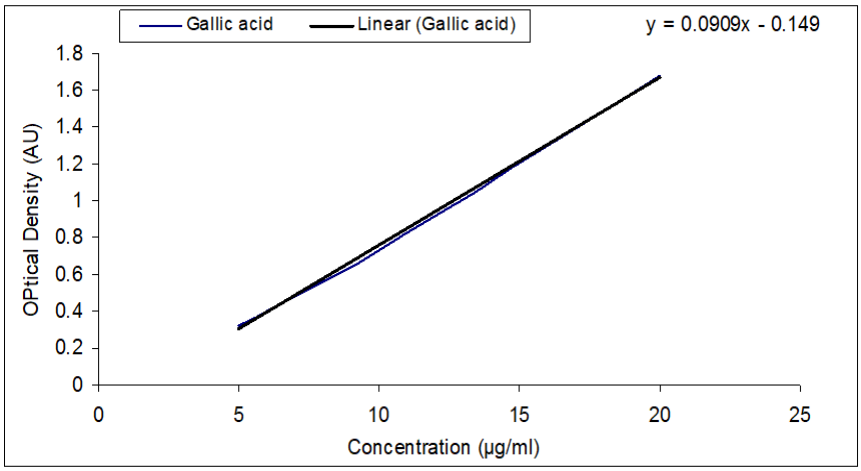

Fig 1: Standard curve for total phenol content

Estimation of antioxidant activity in eucalyptus extract

The antioxidant activity of eucalyptus extract was determined by DPPH method. The IC_{50} value (antioxidant activity) of methanolic extract at 200 ppm was found 444.6 while for Ascorbic acid was found to be 38.56 (Figure 2). Similarly Pathak and Kumar (2015) [15] reported high antioxidant activity in eucalyptus extract.

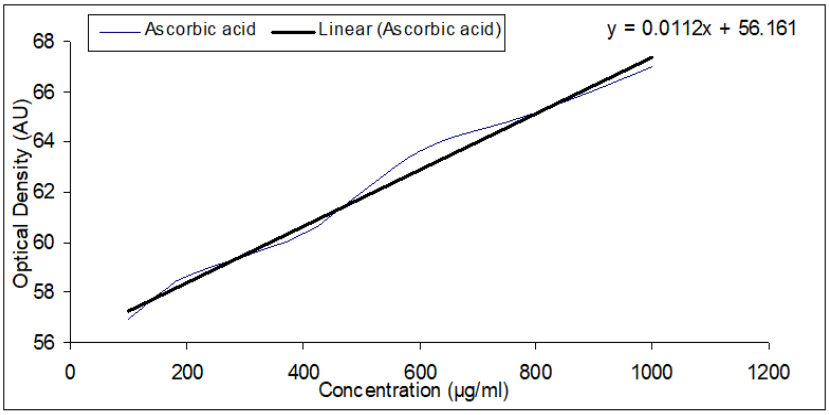

Fig 2: Standard curve for antioxidant activity

Estimation of total flavonoid content in eucalyptus extract
The flavonoid content was estimated by aluminum chloride method. The absorbance was recorded at 415 nm. Quercetin was used as reference standard. The total flavonoid content was found 35.88% in methanolic extract (Figure 3). Similarly Pathak and Kumar (2015) [15] reported presence of flavonoid in eucalyptus extract.

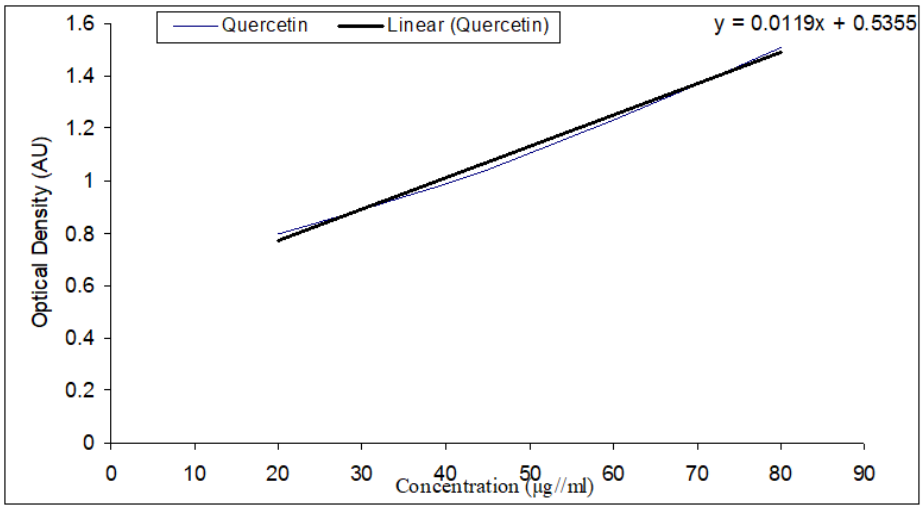

Fig 3: Standard curve for total flavonoid content

Conclusion
Medicinal plants are useful and economically essential. Eucalyptus is a plant of medicinal importance and used worldwide due to its activity against microorganism and fungus. The extracts obtained by eucalyptus plant showed significant antimicrobial and antioxidant activities. Dried leaves of eucalyptus were used to obtain extract using soxhlet apparatus. Two extracts were obtained i.e. methanolic extract and acetone extract from eucalyptus leaves. The qualitative and quantitative phytochemical analysis of the extract were done, which showed the presence of saponins, quinone, carbohydrate, tannin, phenol, fat, flavonoid and absence of protein in methanolic extract of eucalyptus while in acetonic extract saponins, carbohydrate, tannin, phenol were present and quinone, fat, protein and flavonoid were absent. Maximum amount of phytochemicals in eucalyptus got retained in the methanolic extract. The quantitative analysis showed that eucalyptus 11.41% of phenol content, 35.88% flavonoid content and an antioxidant activity of 444.60.

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