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Preliminary phytochemical screening of secondary metabolites from *Eucalyptus grandis*'s leaves

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Abstract
Eucalyptus grandis is one of the useful plants which is easily available and showed various pharmacological activities. These activities depend upon the presence of secondary metabolites such as alkaloids, flavonoids, saponins tannins etc. Four solvents i.e. petroleum ether, acetone, methanol, and distilled water were used for the extraction of secondary metabolites from green leaves of *Eucalyptus grandis*. In the solvents of acetone and methanol, alkaloid glycoside, phenol, lignin, sterols and tannins were present but saponins are present only in extraction of methanol. Glycosides and tannins are present in each extraction which were petroleum ether, acetone, methanol and distilled water.

Keywords: secondary metabolites, *Eucalyptus grandis*, saponins

Introduction
Ayurveda is the oldest method for the treatment of various diseases in which plant and herbs extracts are used. These plant and herbs are using for the treatment and curing of various diseases like asthma, diabetes, malaria etc. Plants are a part of life which is directly effective for each organism. Plants have two types of metabolites that are primary and secondary where Primary metabolites directly affect to plant activity while secondary metabolites are supporting part of primary metabolites. The metabolites quality and quantity indicate the effectivity of plants' part against various organisms. Thus, these types of plants are known as medicinal plant of medicines in the rural areas of developing countries (Chitme *et. al.* 2003) [6]. Many people have used medicinal plants and herbal medicines for the treatment of various diseases since ancient time (Kim, 2005, Saad *et. al.* 2017) [12]. These secondary metabolites have various pharmacological activities. *Eucalyptus* is the most important plant which belongs to Myrtaceae family and more than 800 species distributed in different regions around the world (Hassine *et. al.* 2013) [8]. In the previous study, *Eucalyptus* leaves have shown various activities such as antioxidant, antiseptic and anti-inflammatory. Its essential oil showed herbicidal (Setia *et. al.* 2013) [24], insecticidal (Rudin, 2005; Park and Shin, 2005) [21, 18], anthelmintics (Bennet and Bryant, 1996) [4], anti-tumor (Takasaki *et. al.* 1995) [26] and anti-leech (Kirton, 2005) [11].

Material and Methods
Collection of Plant Material
The green leaves of *Eucalyptus grandis* were selected for the phytochemical screening of metabolic contents. These parts were collected from Mandsaur, District Mandsaur. Mandsaur forms the northern projection of Madhya Pradesh. It lies between the parallels of latitude 23^0 45' 50" North and 25^0 2' 55" North, and between the meridians of longitude 74^0 42' 30" East and 75^0 50' 20" East.

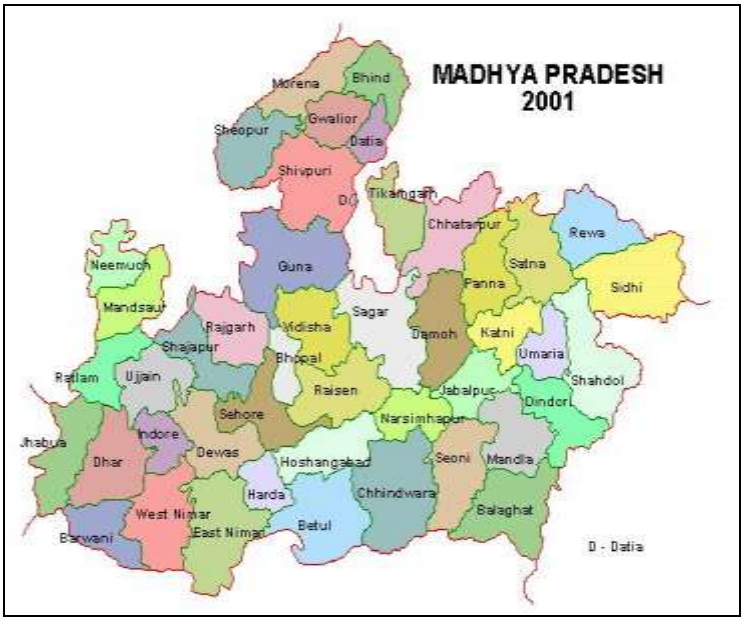

Fig 1: Map of Madhya Pradesh

Identification of Plant and Preliminary Screening of Secondary Metabolites

The green leaves of *Eucalyptus grandis* were collected from Mandsaur Madhya Pradesh and the plant was identified by Dr. S.N. Mishra, Principal Scientist, All India Coordinated Research Project on Medicinal and Aromatic Plants, College of Horticulture, Mandsaur, affiliated to R.V.S.K.V. Vishwavidyalaya, Gwalior (M.P.). The green leaves of *Eucalyptus grandis* were crushed using mixer grinder, and kept to cold percolation process for 48 hours with petroleum ether, acetone, methanol and distilled water. After it, the extracts were filtered and used for preliminary phytochemical screening (Shashank Bhatt *et. al.*, 2012) [25].

Preliminary Screening of Secondary Metabolites

After the cold percolation process of *Eucalyptus grandis*, the extracts were filtered and used for preliminary phytochemical screening such as alkaloids (Iodine, Dragendorff's test and Wagner test), flavonoids (Pew's, Shinoda and NaOH tests), glycosides (Keller-Killani, Conc. H_2SO_4, and Molisch tests), lignin (Labat and Lignin tests), phenols (Ellagic acid and Phenol tests), saponins (Foam and Haemolysis test), sterols (Salkowski test), tannins (Gelatin tests) were carried out (Shashank Bhatt *et. al.*, 2012) [25].

Preliminary Screening of Phytochemical Test

Phytochemical Screening

The filtrate obtained was subjected to preliminary phytochemical screening.

Test for Alkaloids

Iodine Test: Took 3 ml of test solution into test tube and added few drops of dilute iodine solution. Blue colour appeared; but disappeared on boiling and reappeared on cooling (Khandewal K.R., 2008).

Wagner's Test: Took 2 to 3 ml extract solution in test tube and added few drops of Wagner's reagent. Formation of reddish-brown precipitate into solution that indicates the presence of alkaloids (Kokate C. K. *et.al;* 2001) [13].

Dragendorff's Tests: Took 2 to 3 ml extract in test tube and added few drops of Dragendorff's reagent. Orange brown precipitate produced that indicates the presence of alkaloids (Kokate C. K. *et.al;* 2001) [13].

Test for Flavonoids

Pew's Tests: Zinc powder was added into 2-3 ml. extract, followed by drop wise addition of con. HCl. Formation of purple red or cherry colour indicates the presence of flavonoids (Peach K., Tracey MV. 1956) [19].

Shinoda Tests:- Took 2-3 ml. sample extract in test tube and added few fragments of magnesium metal into it, followed by dropwise addition of conc. HCl. Formation of magenta colour indicates the presence of flavonoids (Kokate C. K. *et.al;* 2001) [13].

NaOH Tests: Took 2-3 ml. of ample extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids (Khandewal K.R., 2008).

Test for Glycosides

Keller-Killani Test: Glacial acetic acid was added into 2 ml. sample extract and one drop 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown color appears at the junction of the two liquid layers and the upper layer of bluish green indicates the presence of glycosides (Kokate C. K. *et.al;* 2001) [13].

Glycosides test: 1 ml water was added into small amount of sample extract and shaked well. Then aqueous solution of NaOH was added. The appearance of yellow colour indicates the presence of glycosides (Treare GE, Evans WC. 1985) [27].

Concentrate H_2SO_4 Test: 2ml. glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added into 5ml sample extract, the appearance of brown ring indicates the presence of glycosides (Khandewal K.R., 2008).

Molisch's Test: 2 drops of Molisch's regent was added into 1 ml sample extract, and 2 ml of concentrate H_2SO_4 was added into above solution. Formation of violet ring at the junction indicates the presence of glycosides (Kokate C. K. *et. al;* 2001) [13].

Test for Phenols

Ellagic Acid Test: Took 2 ml sample extract was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or niger brown precipitate occurred in the extract. It indicates the presence of phenols solution (Gibbs R.D., 1974) [7].

Phenol Tests: 0.5 ml of $FeCl_3$ (w/v) solution was added into 2 ml of test solution, formation of an intense colour that indicates the presence of phenols (Gibbs R.D., 1974) [7].

Test for Lignins

Lignin test: 2 ml of 2% (w/v) furfuraldehyde was added into the test solution. Formation of red colour indicates the presence of lignin (Gibbs R.D., 1974) [7].

Labat test: 2 ml test solution was mixed with gallic acid; it developed olive green colour that indicates the positive reaction for lignins (Gibbs R.D., 1974) [7].

Test for saponins

Foam Test: 2 ml sample extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam, indicates the presence of saponins (Kokate C. K. *et al;* 2001) [13].

Haemolysis Tests: Took one drop of extract on the glass slide and added one drop of blood. Both are mixed with wood stick and observed. Hemolytic zone appeared (Kokate C.K., 1994) [14].

Test for Sterols

Salkowski's Test: 2ml sample extract was taken into a test tube and added 2 ml chloroform. Then conc. H_2SO_4 was added into test tube side by side. The layer of red chloroform and acid shows greenish yellow fluorescence. It indicates the presence of sterols (Kokate C. K. *et.al;* 2001) [13].

Test for Tannins

Gelatin Test: 1 ml Gelatin (gelatin dissolves in warm water

immediately) solution was added into 2 ml sample extract. Formation of white precipitate indicates the presence of tannins (Treare GE, Evans WC. 1985) [27].

Result and Discussion

The cold percolation method is one of the effective methods for the extraction of primary and secondary metabolites. Four solvents i.e. petroleum ether, acetone, methanol, and distilled water were used for the extraction of secondary metabolites from green leaves of *Eucalyptus grandis*. The result of phytochemical screening of *Eucalyptus grandis*' leaves was mentioned in Table-1. In the solvents of acetone and methanol, alkaloid glycoside, phenol, lignin, sterols and tannins were present but saponins are present only in extraction of methanol. Glycosides and tannins are present in each extraction which were petroleum ether, acetone, methanol and distilled water. Alkaloids, flavonoids, lignins, saponins and sterols absent in the extraction of petroleum ether and distilled water while phenol was present in the extraction of distilled water. Phenol contents absent in the extract of petroleum ether. Acetone and methanol are an effective solvent that have the capacity to extraction of secondary metabolites in 48 hrs while petroleum ether and distilled water is mild solvent for extraction of secondary metabolites from green leaves of *E. grandis* plant.

Each secondary metabolic content such as alkaloids, flavonoids, saponins, tannins etc. has specific pharmacological activity such as anti-diabetic, anti-inflammatory, anti-cancerous etc. The flavonoids showed ant-allergic, antimicrobial and anticancer activity while tannins showed general antimicrobial and antioxidant activities (Rievere *et. al.*, 2009) [20]. Tannins may have potential value such as cytotoxic and antineoplastic agents (Aguinaldo *et. al.*, 2005) [2]. Saponins are highly effective against fungus (Aboada and Efuwape, 2001; Mohanta *et. al.*, 2007) [1, 17] that have different types of activity against different pathogens. Therefore, it can be used in the treatment of diseases. It is a bioactive antibacterial agent of plants (Mandal *et. al.* 2005: Manjunatha, 2006) [15, 16]. Steroids possess insecticidal and antimicrobial properties hence that are generally used in herbal medicines and cosmetic products (Callow; 1936) [5]. Phenolic compounds have shown anti-oxidative, antidiabetic, anticarcinogenic, antimutagenic and anti-inflammatory (Arts and Hollman; 2005, Scalbert *et. al.;* 2005) [3, 23].

Table 1: *Eucalyptus grandis* Plant Seeds Phytochemical Analysis

Solvent	Petroleum Ether	Acetone	Methanol	Distilled Water
Alkaloids				
Iodine test	-ve	+ve	+ve	-ve
Dragndorff test	-ve	+ve	+ve	-ve
Wagner test	-ve	+ve	+ve	-ve
Hanger's test	-ve	+ve	+ve	-ve
Flavonoids				
Shinoda test	-ve	-ve	-ve	- ve
NaOH test	-ve	-ve	-ve	-ve
Glycosides				
Keller Killani test	+ve	+ve	+ve	+ve
Conc. H_2SO_4 test	+ve	+ve	+ve	+ve
Molish test	-ve	+ve	+ve	+ve
Phenol				
Ellagic test	-ve	+ve	+ve	+ve
Phenol test	-ve	+ve	+ve	+ve
Lignins				
Lignin test	-ve	+ve	+ve	-ve
Labat test	-ve	+ve	+ve	-ve
Saponins				
Foam test	-ve	-ve	+ve	-ve
Haemolysis test	-ve	-ve	+ve	-ve
Sterols				
Salkowaski test	-ve	+ve	+ve	-ve
Liebermann-Burchard Test	-ve	+ve	+ve	-ve
Tannins				
Gelatin test	+ve	+ve	+ve	+ve

(+) - Indicate the Presence of Content (-) - Indicate the absence of Content

Conclusion

Cold percolation method is one of most important method for the extraction of secondary metabolites from plant's parts. The green leaves of *Eucalyptus grandis* was used for the extraction of secondary metabolites alkaloid glycoside, phenol, lignin, sterols and tannins were present but saponins are present only in extraction of methanol. Glycosides and tannins are present in each extraction which were petroleum ether, acetone, methanol and distilled water. These contents have specific activity against specific diseases. In the four solvents, the methanol has good property for extraction of metabolic contents.

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