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Phytochemical Analysis and Biological Activity Test and Functional Group Detection of Eucalyptus *Globulus*

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Abstract

This study was designed to explore phytochemical screening and antimicrobial activity of the Eucalyptus globulus. Extraction of phytochemical was performed by Soxhlet apparatus using ethanol and hot water extraction using water. The phytochemical screening of ethanolic extract showed the presence of alkaloids, carbohydrates, saponins, phenols, tannins, flavonoids, terpenoids, proteins & amino acids, steroids, volatile oils, and aqueous extract showed the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, flavonoids, proteins & amino acids, gums and mucilage, and steroids. Agar well diffusion method was used for the antimicrobial activity test. Both ethanolic and aqueous extracts of E. globulus are resistant to the E. coli, Staphylococcus sp., and Bacillus species except ethanolic extract shows activeness against Staphylococcus sp. & Bacillus species. Both ethanolic and aqueous extracts of E. globulus show inactivity against Aspergillus species. This study concludes the phytochemicals derived from given plants has great potential to treat life-threatening disease. The FTIR studies show that plant extracts consist of amine, carboxylic acid, alcohol & nitro compounds as the most abundant component.

Keywords Eucalyptus globulus, Staphylococcus species, Bacillus species, Aspergillus species.

1. Introduction

Nepal is a botanically enriched country due to its wide range of topographic features and climatic variations (Karki, *et al*., 2015). So far, 6076 species of flowering plants have been identified in Nepal (Press, *et al*., 2000). Of these, around 2000 species are used as medicinal herbal plants (Gaire and Subedi, 2011).

The use of plant and plant parts as medicine has been practiced since the ancient period (from Ayurveda to Patanjali). Medicinal plants show various therapeutic properties due to the presence of bioactive phytochemical constituents that produce definite physiological action in the body (Shanmugapriya and Maneemegalai, 2017). Most important bioactive phytochemical constituents include alkaloids, flavonoids, glycosides, tannins, steroids, terpenoids, mucilage and gums, essential oil, etc (Beegum, *et al*., 2014). These constituents are effective in treating cancer and other harmful diseases and have antioxidant properties as well (Naczk and Shahidi, 2006).

Eucalyptus globulus is commonly known as blue gum and is recognized as Nilgiri in Nepal. It is being rapidly planted in the terai region of eastern Nepal. It is a native plant to Australia. It is a flowering medium to large-sized evergreen and broadleaf tree belonging to the family Myrtaceae. It has a large therapeutic value as it possesses various bioingredients (Hayat, *et al.,* 2015).

2. Materials and methods

About 230 g of fresh leaves of *Eucalyptus globulus* was collected from Bharatpur-9, Kandaghari Chitwan. The leaves were well cleaned with distilled water to remove dust particles, cut into small pieces, and left for drying in shade for 20 days. The bulk powder of leaves was obtained by grinding in a mortar with a pestle. Then such powder size was further reduced to uniform-sized fine powder by using an electric mixer. The extraction was carried out by using ethanol and water. For ethanol extraction around 48 g of the powdered sample was packed in a cotton cloth. The cotton cloth was then inserted into the Soxhlet apparatus and extraction was done by using 500 ml ethanol as a solvent extraction was continued for 43 hours. Then the ethanolic extract was collected in a round bottom flask and poured into a clean and dry beaker. For water extraction around 50 g of the powdered sample was taken in a beaker and distilled water was added to it and boiled the mixture was for 8 hours.

Then the ethanolic extract is directly collected to the beaker through a round bottom flask and water extract is filtered using filter paper and collected in the beaker.

Both the extracts were dried by using a digital water bath maintaining 55 ^{O}C temperature and the percentage yield of extracts was calculated. Then the dried extracts were used for phytochemical screening. The phytochemical screening was performed based on journal articles (Kancherla, *et al*., 2019, Choudhary and Pawar, 2014 and Dahiru *et al*., 2006).

3. Phytochemical analysis

3.1. Alkaloids test (Kancherla, *et al*., 2019):

For the alkaloid test, the extract solution was treated with dil. HCl and filtered out using filter paper. Then the clear filtrate solution was taken for testing.

The alkaloids test was done by using the following methods.

Mayer's test: Mayer's reagent (Potassium Mercuric Iodide solution) was prepared by mixing 1.36 g of Mercuric chloride and 5 g of potassium iodide in 100 ml of water. Then 1 ml of such reagent was treated with 1 ml of filtrate solution and the formation of a whitish or cream-colored precipitate was observed.

Wagner's test: Wagner's reagent (Iodo-Potassium Iodide) was prepared by dissolving 2 g of iodine and 6 g of Potassium iodide in 100 ml of water. Then 1 ml of such reagent was treated with 1 ml of filtrate solution and the formation of a reddish-brown precipitate was observed.

Hager's test: 3 ml of saturated-aqueous solution of Picric acid (Hager's reagent) was treated with 1 ml of filtrate solution and the yellow-colored precipitate was observed.

3.2. Carbohydrates test (Kancherla, *et al*., 2019):

First of all, the extract solution was prepared by dissolving it in the solvent by which the extraction was carried out. Then this solution was filtered and the filtrate was taken on each type of the following test.

Fehling's test: 1 ml of filtrate was treated with a mixture of Fehling's solution A & B and heated in a water bath for 5 min. The formation or no formation of red or yellowish-red precipitate was observed.

Benedict's solution test: 1 ml of filtrate was treated with Benedict's reagent and heated in a water bath for 5 min. Then, whether or not a red precipitate formed was observed.

3.3. Glycosides test (Kancherla, *et al*., 2019)**:**

The glycosides test was done by the following method.

Liebermann's test: 2 ml of extract solution was treated with a mixture of 2 ml acetic acid and 2 ml chloroform. Then, whether or not violet to blue to green coloration is seen was observed.

Legal test: 1 ml of extract solution was treated with a few ml of pyridine, 2 ml of nitroprusside, and 2 drops of 20% NaOH solution. Then, whether or not red coloration is seen was observed.

4.4. Saponins test (Kancherla, *et al*., 2019)**:**

1 ml of extract solution was taken in a conical flask, diluted with 20 ml distilled water, and shaken for 5 minutes for observation of stable foam, the process is known as the Foam test.

3.5. Phenols test (Kancherla, *et al*., 2019)**:**

About 1 ml of aqueous or alcoholic solution of extract was treated with 2-3 ml of ferric chloride solution. The appearance of a blue, green, violet or red coloration was observed for the detection of phenols.

3.6. Tannins test (Kancherla, *et al*., 2019)**:**

5% ferric chloride solution was added drop by drop to 2-3 ml of the extract solution. Hydrolyzable tannins gave bluish-black color and condensed tannins gave brownish-green color.

3.7. Flavonoids test (Kancherla, *et al*., 2019)**:**

For the flavonoids test extract solution was prepared to depend on the solvent used in extraction. The solution was filtered out and only filtrate was taken for the following test.

Shinoda test: 1 ml of the filtrate was treated with a few drops of conc. HCl and Mg Pisces were added to this mixture. The flavonoids gave red, pink, or purple color.

Alkaline reagent test: 2% NaOH was added to 1 ml filtrate drop wisely. The flavonoid gave yellow color which disappeared when di. HCl was added.

3.8. Terpenoids test (Kancherla, *et al*., 2019)**:**

1 ml of extract solution was treated with 5 ml chloroform. Then 3 ml. conc. H_2SO_4 was

added to the mixture from the side of the test tube. The mixture was separated into 2 layers. The formation of a blue (green fluorescence) colored lower layer indicated the presence of terpenoids.

3.9. Proteins and amino acids test (Kancherla, *et al*., 2019)**:**
A little fraction of the extract solution was treated with a few drops of conc. HNO_3 and heated for about 5 minutes in a water bath. The appearance of yellow or orange color indicated the presence of protein and amino acids.

3.10. Gums and mucilage test (Choudhary and Pawar, 2014)**:**
A few ml of extract solution was mixed with Molisch's reagent and Conc. sulphuric acid. The appearance of a red-violet ring at the junction of two liquids indicates the presence of gums and mucilage.

3.11. Steroids test (Kancherla, *et al*., 2019) **:**
1 ml of extract solution was treated with 5 ml chloroform. Then 3 ml. conc. H_2SO_4 was added to the mixture from the side of the test tube. The mixture was separated into 2 layers. The formation a of red-colored upper layer indicated the presence of steroids.

3.12. Volatile oils test (Dahiru *et al*.,2006).**:**
2 ml of the extract was shaken with 1 ml of NaOH and a small quantity of dil. HCl. The formation of a white precipitate indicates the presence of volatile oil.

4. Antibacterial and antifungal study
Ethanolic extract solution having a concentration of 10mg/ml. was made by dissolving ethanolic extract in 10% DMSO. A water extract solution having a concentration of 10 mg/ml was made by dissolving water extract in distilled water. To ensure proper mixing, the solutions were kept in the centrifuge. The antibacterial and antifungal activity test of the plant extract was done by the use of three bacterial species namely *Bacillus* sp. & *Staphylococcus* species (gram-positive), *E. coli* (gram-negative), and one fungal species namely *Aspergillus* species. All the procedures followed were done with the help of a journal (Belhaj, *et al*., 2020). The bacterial broth culture of *E. coli*, *Bacillus* sp., and *Staphylococcus* sp. were swabbed on the three MHA plates, and the mold of Aspergillus sp. was swabbed on one PDA plate. After that 3 wells (bore) of 8mm were made on each agar plate. About 1000 µL of extract-water solutions and extract-DMSO solutions were poured into the 2 wells on each plate. 1000 µL DMSO (10%) was poured into the third well as a negative control. Such MHA plates were then incubated for 24 hours and the PDA plate was incubated for 72 hours all at 37°C and zones of inhibition were measured.

FTIR studies

FTIR analysis of an ethanolic extract *of E. globulus* was carried out by placing a sample of dry extract in the FTIR sample compartment and results for spectrum were calculated then peak tables were checked. The interpretation of the result was carried out using literature of IR tables (Burns and Ciurczak, 2007)

5. Results and discussion

Plant extract yield percentage

Table 1: Percentage Yield

Extract types	Ethanolic extract	Water Extract
Crude Weight	48 g	50 g
Extract Weight	9.03 g	11.07 g
Yield Percentage	18.81%	22.15%

Phytochemical analysis test

Table 2: Phytochemical test

S.N.	Phytochemical test	Result	
		Ethanolic extract	Water extract
1	Alkaloids test	+	+
2	Carbohydrates test	+	+
3	Glycosides test	-	+
4	Saponins test	+	+
5	Phenols test	+	+
6	Tannins test	+	+
7	Flavonoids test	+	+
8	Terpenoids test	+	-
9	Proteins and Amino acids test	+	+
10	Gums and Mucilage test	-	+
11	Steroids test	+	+
12	Volatile oils test	+	-

Antibacterial & antifungal activity test

Fig. 1: ZOI of *Bacillus sp.* *E. coli,* *Staphylococcus* sp. *Aspergillus* sp.

Table 3: Zone of inhibition of *Bacillus species, E. coli, Staphylococcus* species, and *Aspergillus* species (in diameter)

Organisms	***Bacillus sp.***		***E. coli***		***Staphylococcus* sp.**		***Aspergillus* sp.**	
Extraction Solvent	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water
Media	MHA	MHA	MHA	MHA	MHA	MHA	PDA	PDA
Solvent	DMSO	Water	DMSO	Water	DMSO	Water	DMSO	Water
Control	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
Obtained ZOI	11mm	8mm	8mm	8mm	10mm	8mm	8mm	8mm
Results	Active	Resist	Resist	Resist	Active	Resist	Inactive	Inactive

FTIR studies

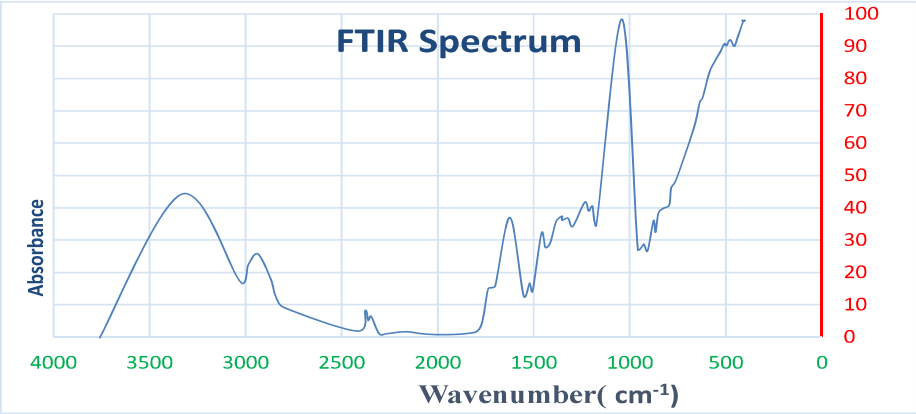

Fig. 2: FTIR interferogram of ethanolic extract from leaves of *E. globulus*

Table 4: FTIR spectra analysis of an ethanolic extract of *Eucalyptus globulus*

SN	**Wave number in cm^{-1}**	**Remarks**
1	1180—1190	Tertiary alcohol
2	1290-1320	Aromatic amine
3	1300-1350	Phenol
4	1400-1450	O-H stretching carboxylic acid
5	1620	α,β unsaturated ketone
6	2920	Alkane
7	3300-3350	Amine

The phytochemical screening of the ethanolic extract of *Eucalyptus globulus* revealed the presence of alkaloids, carbohydrates, saponin, phenols, tannins, flavonoids, terpenoids, proteins & amino acids, steroids, and volatile oils whereas glycosides and gums &

mucilage are undetected in this test. The phytochemical screening of water extract of *Eucalyptus globulus* revealed the presence of alkaloids, carbohydrates, glycosides, saponin, phenols, tannins, flavonoids, proteins & amino acids, gums & mucilage, and steroids whereas terpenoids and volatile oils are undetected in this test.

Both the ethanolic extract and water extract of *Eucalyptus globulus* showed resistivity against *E.coli* with a diameter of 8 mm and showed inactiveness against *Aspergillus* species with a diameter of 8 mm.

The ethanolic extract of *Eucalyptus globulus* showed a mild inhibiting effect against *Staphylococcus* species with a diameter of 10 mm and showed a mild inhibiting effect against *Bacillus* species with a diameter of 11 mm.

The water extract of *Eucalyptus globulus* showed resistivity against *Staphylococcus* species with a diameter of 8mm while this extract showed inactiveness against *Aspergillus* species with a diameter of 8mm.

The FTIR interferogram of the ethanolic extract of *E. globulus* revealed the overlapping of different spectrums of various components. The strong and broad peak at the range of 3300-3350 cm^{-1} was due to amine groups. The sharp and medium peak at 2920 cm^{-1} was due to the presence of alkane. The sharp and strong peak at 1620 cm^{-1} was due to α,β unsaturated ketone. The sharp and medium peak at 1400-1450 cm^{-1} was due to the presence of O-H bonding of carboxylic acid. The weak and broad peak at 1300-1350 cm^{-1} was due to the phenol. The weak and sharp peak at 1290-1320 cm^{-1} was due to aromatic amine. The sharp and medium peak at 1180-1190 cm^{-1} was due to the presence of tertiary alcohol.

6. Conclusion and recommendations

Conclusion

The phytochemical analysis and biological activity of the *Eucalyptus globulus* indicate the presence of various phytochemicals and a high pharmacological value of the plant. The phytochemicals identified from the different tests of plant extract provide strong support for this plant to have the potential to act as a source of a useful drug. The result shows the presence of different compounds that are vital for better physiological and metabolic activities of the body. The study revealed the effectiveness of plants against different bacteria and fungi. Moreover, this plant can also be used to cure improper functions of the body like diabetes, eye disease, fever, etc.

Based on the results of this study, it can be concluded that this plant can be considered an ideal candidate for a holistic medical application.

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