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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *EUCALYPTUS* SP LEAF EXTRACT AGAINST CLINICAL PATHOGENS

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ABSTRACT

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. Infectious diseases are world's most important reason of untimely death, killing 50,000 people each day. Resistance to antimicrobial agents is rising in a wide diversity of pathogens and numerous drug resistances are becoming common in diverse organisms. The plant extracts have been developed and proposed for use as antimicrobial substances. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. The present study was aimed to evaluate the antibacterial potential of methanol extract of *Eucalyptus globulus* against bacterial pathogens and phytochemical analysis was done.

INTRODUCTION

In the production of drugs, the role of plants is very important. There is a lot of drugs are produced from the plants and its various parts (Fabricant and Farnsworth 2001, Farnsworth et al., 19858). Eucalyptus is a large genus of evergreen aromatic tress, rarely shrubs (mallees), indigenous to Australia, Tasmania, New Guinea and the neighboring islands, where they constitute a large portion of the forest vegetation and giving it a characteristic appearance. Various species of *Eucalyptus sp.* are cultivated (*Eucalyptus citriodora, Eucalyptus cladocalyx, Eucalyptus consideniana, Eucalyptus cypellocarpa, Eucalyptus dives, Eucalyptus gigantea,* ***Eucalyptus globulus,*** *Eucalyptus gomphocephala, Eucalyptus grandis, Eucalyptus gunnii, Eucalyptus incrassate, Eucalyptus kino, Eucalyptus largeflorens, Eucalyptus lesouefii, Eucalyptus macrocarpa, Eucalyptus macrorhyncha, Eucalyptus maculata, Eucalyptus marginata, Eucalyptus melanophloia, Eucalyptus melliodora*) particularly in sub-tropical and warm temperate regions, on account of their economic value (Sastri 2002).

Taxonomical Classification

Kingdom : Plantae
Subkingdom : Tracheobionta
Super division : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Rosidae
Order : Myrtales
Family : Myrtaceae
Genus : *Eucalyptus*
Species : globulus

Figure 1: *Eucalyptus* sp, tree and its leaves

Phytochemical and pharmacological aspects

There is a various chemicals present in eucalypyus sp have lot of applications in pharmacology, the chemical present in eucalyptus like Sideroxylonal C inhibits human plasminogen activator inhibitor type-1 without any significant effect on human tissue plasminogen activator (Neve J *et al 1999*). Euglobal-Am-II isolated from leaves of Eucalyptus *amplifolia*, exhibit significant inhibitory effects on Epstein-Barr virus (EBV) (Takasaki et al 1995). Bark of *Eucalyptus camaldulensis* is commonly used a chewing stick. Bark extract of *Eucalyptus camaldulensis* shows inhibition zones of comparable magnitude with those of the standard antimicrobial agents (Khan et al 2000). *Eucalyptus camaldulensis*, possesses an anti-nociceptive effect against both acetic acid-induced writhing and hot plate-induced thermal stimulation (Atta AH, and Alkofahi 1998). The extracts obtained by ethanol digestion and by supercritical fluid extraction (SFE; CO2 with 15% ethanol) of leaves from *Eucalvptus camaldulensis* var. *brevirostris* trees show the most promising antioxidative activities. The main two compounds of the SFE extract with antioxidative activity are 5-hydroxy-7, 4'-dimethoxy flavone and 5-hydroxy-7, 4'-dimethoxy-8-methyl flavone. Gallic and ellagic acid are found to be the prevailing antioxidants in the ethanolic extract (El-Ghorab et al 2003). Extracts obtained from *Eucalyptus camaldulensis* has significant cytotoxic activity against human ECV-304 cells (Al-Fatimi et al 2005). Using acetic acid-induced writhes in mice and hot plate thermal stimulation in rats, it has been prooved that the essential oil of *Eucalyptus citriodora* induced analgesic effects in both models, suggesting peripheral and central actions. The volatile oil extracted from the leaves of *Eucalyptus citriodora* showed a wide spectrum of antifungal activity. *Eucalyptus* oil, camphor and menthol and thymol oil are the most efficacious component against the fungal pathogens such as *Tricophyton rubru*,, *Trichophyton mentagrophytes*, *Microsporum canis*, *Epidermophyton floccosum* and *Epidermophyton stockdale* (Ramsewak et al 2003). Essential oil from the *Eucalyptus citriodora* produced anti-inflammatory effects, Anti inflammatory activity of *Eucalyptus citriodora* demonstrated by inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan, and vascular permeability induced by carrageenan and histamine. *Eucalyptus* essential oil and monoterpenes are efficient inhibitors of bone resorption in the rat (Muhlbauer et al 2003). A lemon *Eucalyptus* extract (Citriodiol) has been shown to be a natural repellent against mosquitoes, stable flies, and midges (Gardulf et al

2004). It kill the *Ixodes ricinus* which can transmit several microorganisms, out of which *Borrelia burgdorferi* and tick-borne encephalitis (TBE) virus are the most important pathogens in humans. Freshly prepared camphor oil from *Eucalyptus globulus* with or without glycerol dilutions gave complete cure of human facial demodicidosis with concentrations of 100%, 75% and 50% (Morsy et al 2002). *Eucalyptus globulus* leaf extracts and oil showed antifungal property as they progressively inhibited the growth of *Malassezia furfur* on Sabouraud's destrose agar medium (Vijayakumar et al 2006). Hexane extract of leaves, ethanol extract of fruits and leaves of *Eucalyptus globulus* inhibited IgE dependent histamine release from RBL-2H3 cells (Ikawati et al 2001). The present study was aimed to evaluated the antibacterial potential of Aqueous, Acetone, Methanol and Ethanol extract of *Eucalyptus globulus* against bacterial pathogens such as *Bacillus subtilis, Escherichia coli, Salmonella typhi, Klebsiella pneumonia*e and *Staphylococcus aureus* and phytochemical analysis was done.

MATERIALS AND METHODS

Collection and Drying of plant materials

Fresh leaves of were collected *Eucalyptus globulus* from Vellore in Tamil Nadu. The leaves were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of plant extract

5 g of powdered leaves were extracted successively with 100 ml of methanol, water, Acetone and ethanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

Test for Alkaloids (Mayer's Test)

The extract of *Eucalyptus* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a

few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated Sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer.

Test for Terpenoid and Steroid

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids.

Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavonoid.

Test for Reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Triterpenes

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and observed for red colour formation.

Test for Phenolic Compounds (Ferric chloride test)

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

Test for Saponins

2g of the powered sample was boiled in 20 ml of distilled water in a water bath. 10ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent broth.

The following was mixed with 3 drops of Olive oil and shaken vigorously and then observed for the formation of emulsion.

Determination of antibacterial activity (Agar well Diffusion)

Test microorganisms

Five pathogenic bacteria, *viz*., *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* and *Staphylococcus aureus* were used during the present study and were obtained from MTCC, Chandigarh. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4°C.

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards.

Determination of antibacterial activity (Agar well Diffusion)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Methanol extracts with same concentrations of 100 mg/ml were used. The plates were assessed by measuring the diameter of the zone of inhibition (in mm).

RESULT AND DISCUSSION

Phytochemicals of Eucalyptus

Phytochemical analysis	Aqueous	Methanol	Acetone	Ethanol
1.Alkaloids	Absent	Absent	Absent	Absent
2.GLYCOSIDES	2+	1+	1+	1+
3.SAPONINS	Absent	Absent	Present	Absent
4.PHENOL	1+	Present	1+	Present
5.FLAVONOIDS	Absent	Absent	Absent	Absent
6.PROTEINS AND AMINO ACIDS	1+	Absent	1+	Absent
7.DITERPENS	1+	Absent	1+	Absent
8. STEROIDS	Present	Present	Present	Present

Antimicrobial activity of Eucalyptus sp extracts against various pathogens

Plants are the major sources producing lot of natural products to humanbeings health impacts (Dahanukar et al., 200). The present study aimed at testing the antibacterial activity of *Eucalyptus* leaves against five human pathogens and the findings were summarized. The extracts of *Eucalyptus* were tested against pathogenic bacteria like *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* and *Staphylococcus aureus* by agar well diffusion method. The methanol extract of *Eucalyptus* (100mg/ml) showed maximum zone of inhibition (22mm) against *Pseudomonas aeurginosa. Streptococcus pyogens* showed (16mm) less zone of inhibition (Table 1 - 5). Antibacterial activity of *Eucalyptus* plant leaves is due to the presence of phytochemical compounds like phenolic compounds, tannins, steroids, flavonoids, saponins (Table 1-5). The phytochemicals of Eucalyptus sp playing vigorously against the bacterial pathogens (Takarada et al., 2004, Salari et al., 2006)

Table (1 – 5) antimicrobial activity of *Eucalyptus globulus* extracts against

(a) Bacillus subtilis

Solvents	Zone of inhibition
Methanol	**15.00±0.30**
Aqueous	**14.25±0.33**
Acetone	**12.43±0.18**
Ethanol	**16.00±0.30**

(b) Escherichia coli

Solvents	Zone of inhibition
Methanol	**16.43±0.18**
Aqueous	**15.00±0.30**
Acetone	**11.25±0.33**
Ethanol	**17.43±0.18**

(c) Salmonella typhi

Solvents	Zone of inhibition
Methanol	**14.43±0.18**
Aqueous	**15.00±0.30**
Acetone	**10.25±0.33**
Ethanol	**12.00±0.30**

(d) Staphylococcus aureus

Solvents	Zone of inhibition
Methanol	**11.43±0.18**
Aqueous	**10.00±0.30**
Acetone	**8.25±0.33**
Ethanol	**13.26±0.13**

(e) Klebsiella pneumonia

Solvents	Zone of inhibition
Methanol	**11.26±0.13**
Aqueous	**17.00±0.30**
Acetone	**15.25±0.33**
Ethanol	**12.00±0.30**

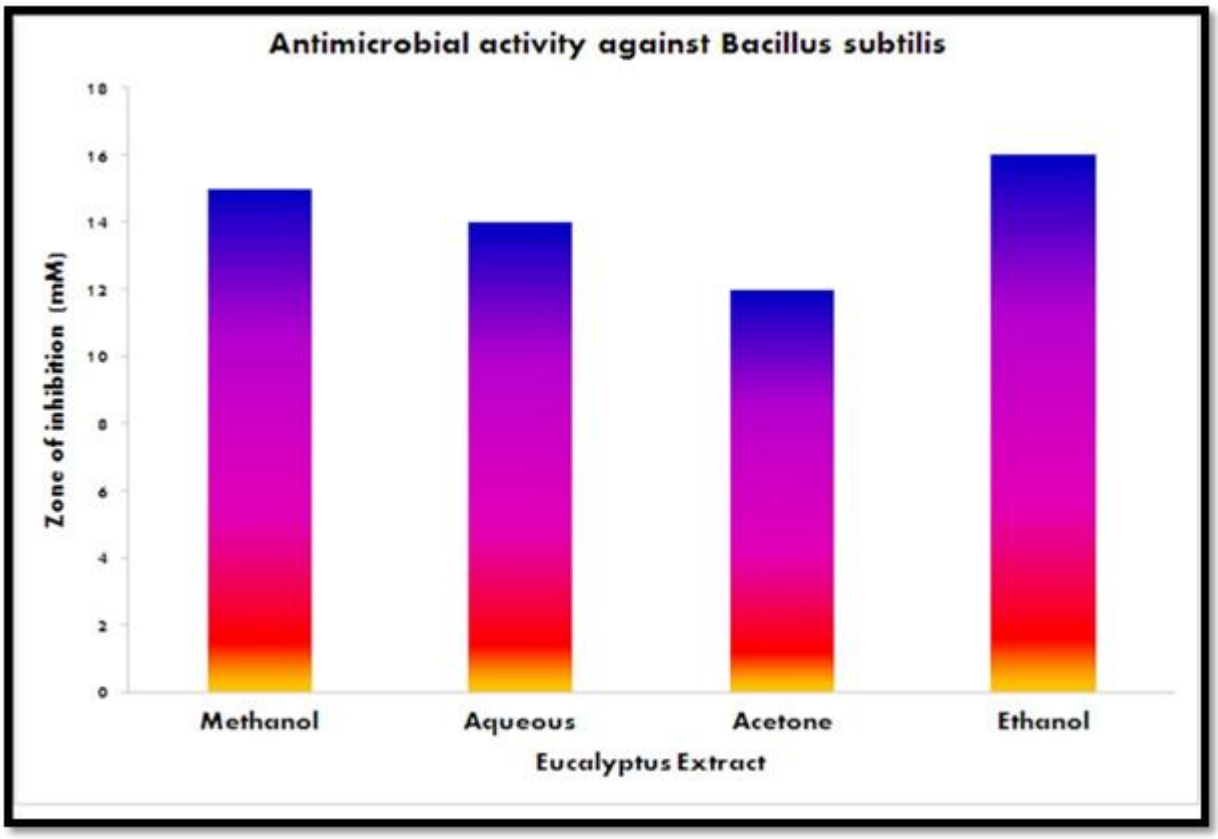

Figure : 2 Antimicrobial ativity of *Eucalyptus globulus* against *Bacillus subtilis*

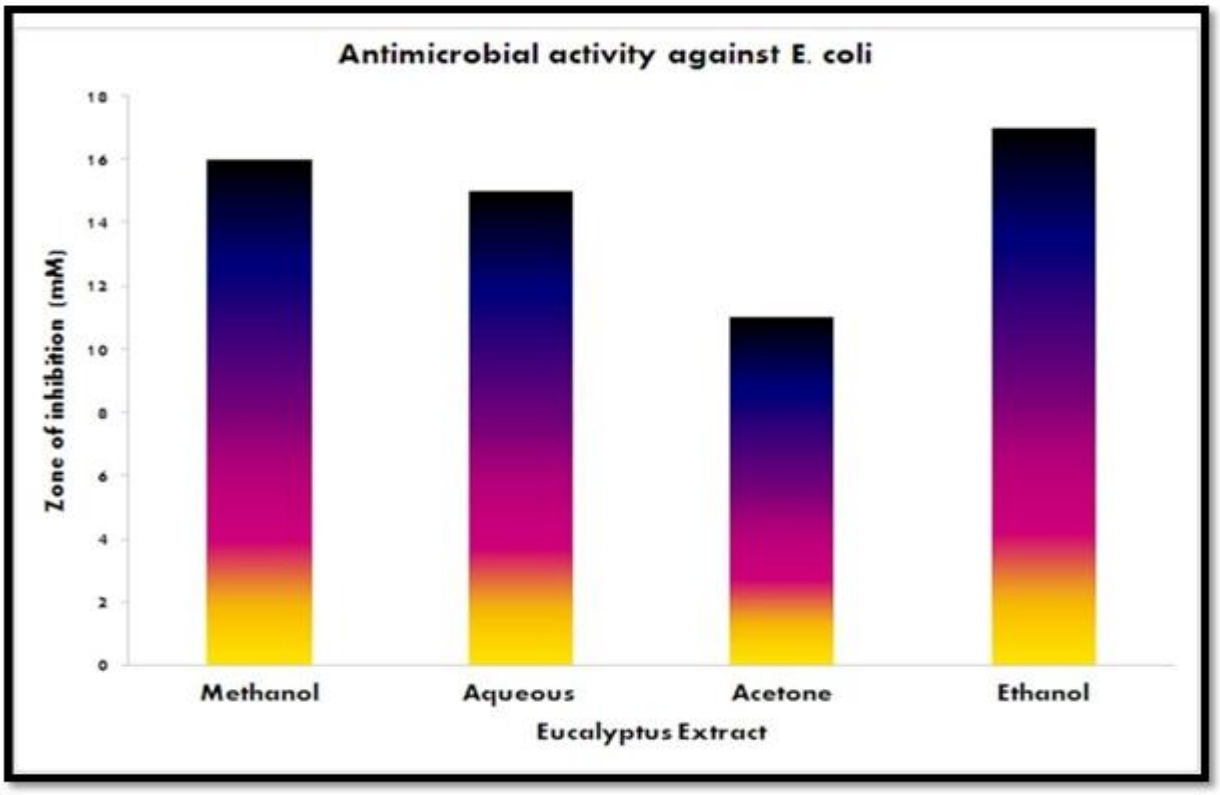

Figure : 3 Antimicrobial ativity of *Eucalyptus globulus* against *E. coli*

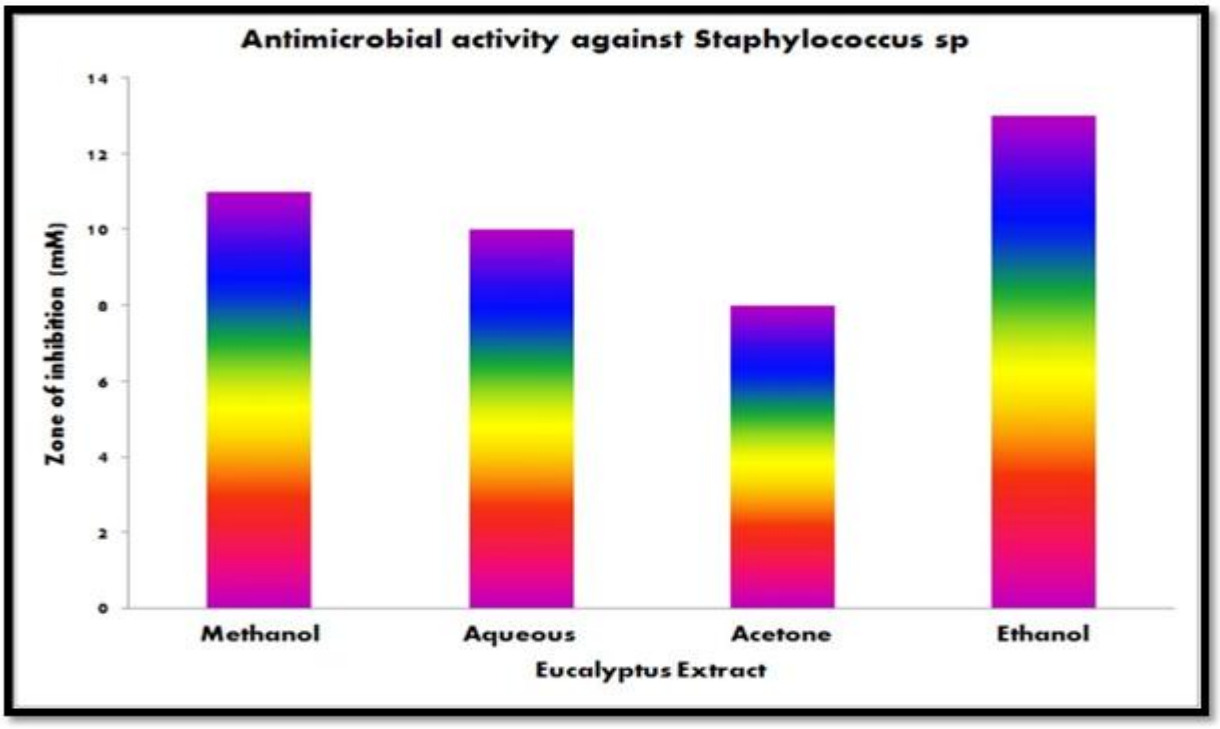

Figure: 4 Antimicrobial ativity of *Eucalyptus globulus* against *Staphylococcus sp*

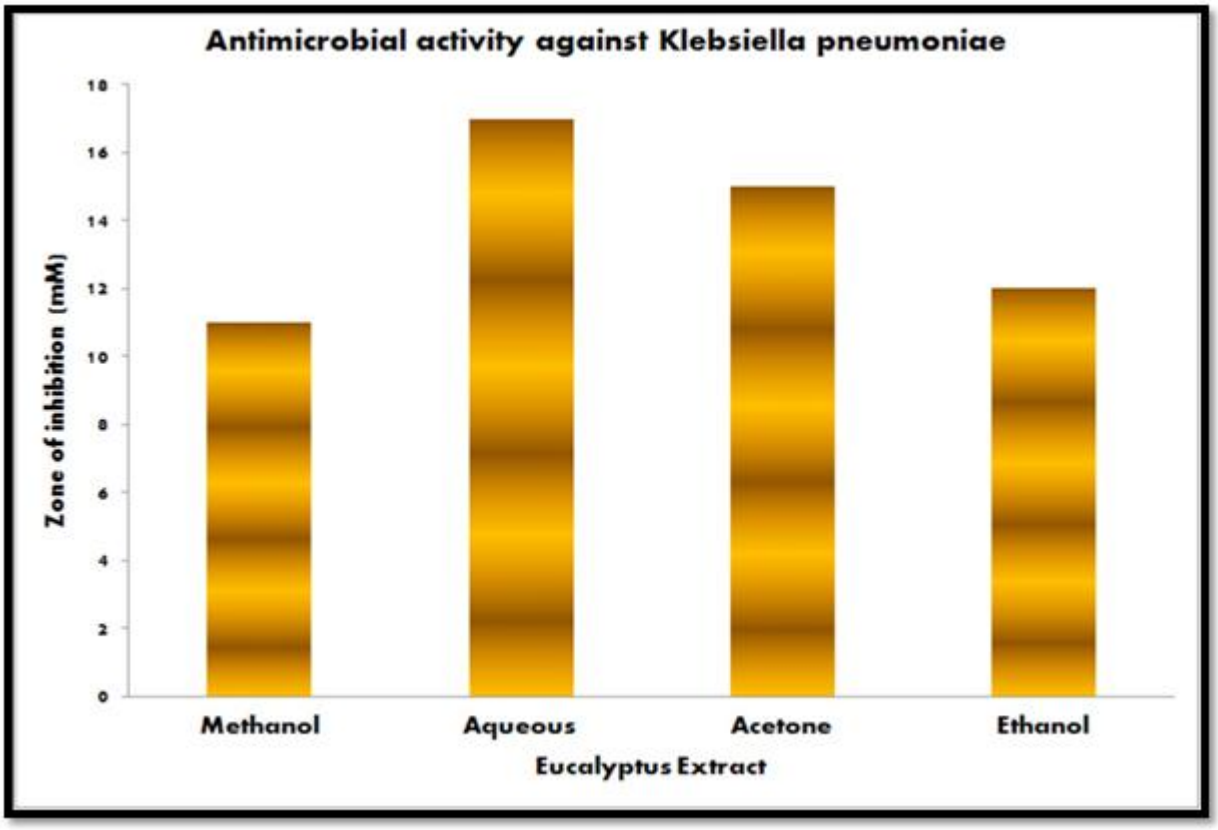

Figure: 5 Antimicrobial ativity of *Eucalyptus globulus* against *Klebsiella pneumoniae*

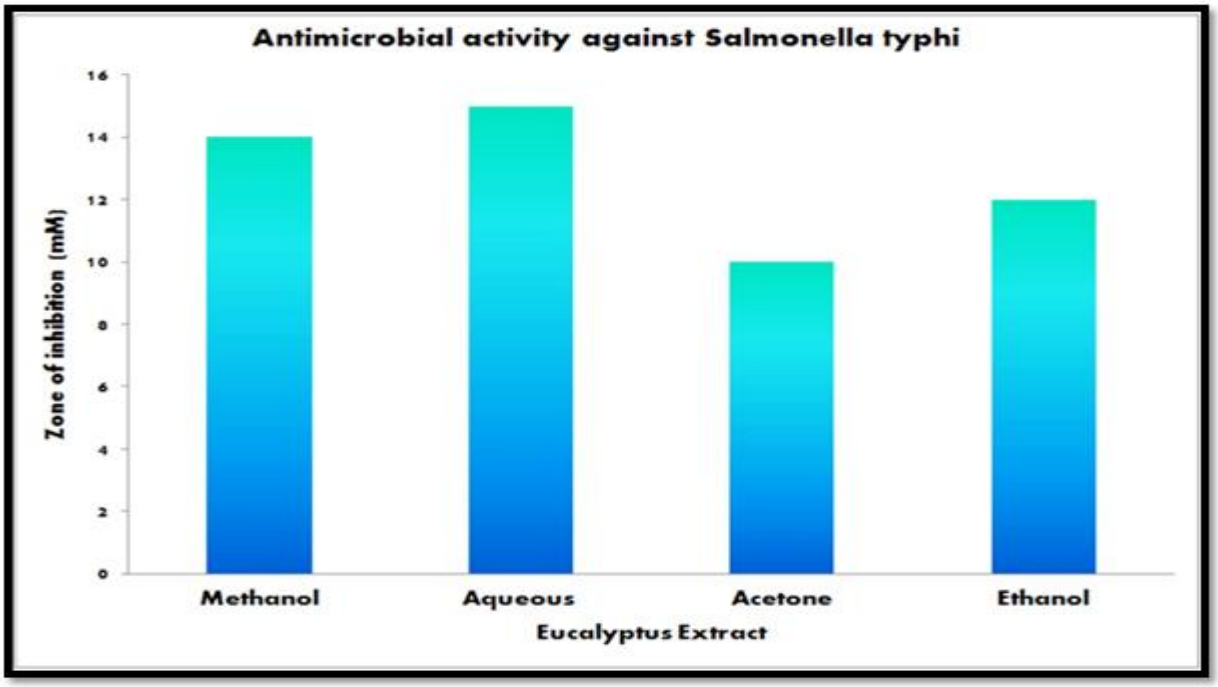

Figure: 6 Antimicrobial ativity of *Eucalyptus globulus* against *Salmonella typhi*

CONCLUSION

The microbial fighting is mounting day by day and the viewpoint for the use of antimicrobial drugs in the prospect is still uncertain. Therefore, way to be taken to decrease this problem, for example, to control the use of antibiotic, build up research to enhance understand the genetic mechanisms of resistance, and to continue studies to develop new drugs either synthetic or natural. The final goal is to present suitable and well-organized antimicrobial drugs to the patient3. Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. Phytochemical and antibacterial activity of *Eucalyptus globulus* extract showed that it is mainly due to the presence of phytochemical compounds such like tannins, saponins, glycosides, triterpenes and tripenoid. The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs.

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