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Research article

ANTIMICROBIAL ACTIVITY OF *EUCALYPTUS CAMALDULENSIS* AND *CALOTROPIS GIGANTEA* AGAINST VARIOUS PATHOGENS

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ABSTRACT: *Eucalyptus camaldulensis and Calotropis gigantea* are common weed and known for various medicinal properties. The aim of the present study was to screen leaves of Eucalyptus camaldulensis and roots of *Calotropis gigantea* for the antimicrobial activity against clinical isolates of bacteria. The leaf and root extract were obtained by the organic solvents methanol. The methanolic extract of the *E. camaldulensis and C. gigantea* was studied for its antagonistic activity against *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Salmonella paratyphi.* The results obtained from this study inferred that the leaf extract of *Eucalyptus camaldulensis* was effectively inhibited the growth of test organism, while Calotropis gigantae did not show the activity which is in combination with E. camaldulensis shows the more activity against all pathogens.

Key words: *Eucalyptus camaldulensis,* Calotropis gigantae, Antimicrobial activity, MIC (Minimum Inhibitory Concentration), Leaf and Root Extract**,** Well diffusion method.

INTRODUCTION

Presently, there is a wide range of antimicrobial drugs derived from microbial and synthetic source available for treatment of infectious diseases at least for in developed countries and the urban elicits of developed countries (El-Mahamood Muhammad A *et al.*, 2010). Potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal shrubs and is appropriately called the botanical garden of the world (Murugan T *et al.*, 2012). In recent times, plants are being extensively explored for harbouring medicinal properties. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development. Plants are reported to have antimicrobial, anticancer, anti-inflammatory, anti-diabetic, haemolytic, antioxidant, larvicidal properties etc (Gaurav K *et al.*, 2010). Over three quarters of the world population relies mainly on plant extract for health care. The drugs are derived either from the whole plant or from different organs like leaves, stem, root bark, flower, seed etc. Some drugs are prepared from excretory plant products such as gum, resins, and latex. Plant especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity or reduced toxicity. Current study was focused to investigate the antibacterial activity of the crude leaves extract of *C. gigantea and Eucalyptus camaldulensis* against clinical isolates of bacteria.

Calotropis gigantean commonly known as Mudar Yercum belongs to the family *Asclepiadaeae* a shrub about 6 M high is widely distributed in Eastern and southern parts of India, Ceylon, Eastern Asia and other parts of tropics. Tribal people were using this plan parts to cure several illnesses such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea and mental disorders. *C. gigantea* is scientifically reported for its anti-*Candida* activity, cytotoxic activity, antipyretic activity and wound healing activity, The central part of flower is used to make sweetmeat, Inner part of flower used for flavoring, bark in small doses, dried and powered used as alternative and tonic, Root bark reported to promotes secretion and used for skin diseases, enlargement of abdominal viscera, intestinal worms, coughs, ascitesanas area (http://en.wikipedia.org/wiki/clasification of Calotropis). In India, pulverized root made into ointment, used in treatment of old ulcers. Root bark is used for chronic rheumatism, Leprosy, syphilis, cachexia, idiopathic ulceration, dysentery, diarrhea and chronic rheumatism. Leaves warmed and moistened with oil, used as dry fomentation for abdominal pains; also used as rubifacient (Ashraful A. M. *et al.*, 2008).

In India, fresh leaves or dried juice from the root bark taken internally as alternative, juice of young buds used for earache. For toothaches, milky juice mixed with salt. Flowers used for cough, asthma, cataract and loss of appetite. Dry leaf powder used for treating wounds and boils. Leaves to be effective on elephantiasis. Similarly *Eucalyptus* is one of the such medicinal plants belonging to the family 'Myrtaccae' which is frequently seen occupying open istle spaces and grassland, roadsides, along riverbanks, and wet lands .They are widely cultivated in tropical areas. Of the more than 700 species that comprise this genus, most are endemic to Australia. They have also been widely introduced into drier subtropical and tropical regions in areas as diverse as Africa, the Middle East, India, USA and South America. *Eucalyptus* spp. Exhibit excellent performance on alluvial soils and thus can be planted on a large scale for social forestry. In India, the tree is widely used in traditional medicine to treat a variety of disease including colds, Asthma, Cough, Diarrhea and Dysentery, Sore throat, Hemorrhagelaryngalgia, Spasm, Tracheae, Vermifuge. The leaf paste or juice is applied to the for-head to relive headaches and common cold and also used in relief from Muscles and joints pain ointment containing *Eucalyptus* leaves are also applied to the nose and chest to relief congestion (http://en.wikipedia.org/wiki/ clasification of *Eucalyptus*). The *Eucalyptus* used to treat Gastrointestinal symptoms like, arrest bleeding, open wounds and cuts as well as the drinking of the decoction for relief of aches and if pain in tooth. In treatment with enteric infection including diarrhea and dysentery, constipations and other, much problems like asthma, oral thrush, sores, skin, wound, bronchitis, athletes foot . In Japan, health teas are prepared from *Eucalyptus* which is rich in water soluble polysaccharide Tannins, K, Na, Ca. An anti-oxidant for food and cosmetic use also manufacturing from leaves. Methanolic extract of the leaves exhibits significant insect repellent activity against Blue nuzzle. *Eucalyptus* is used internally among other complaints for asthma, whooping, cough, liver, gallbladder problems, urinary tract infection, rheumatic disease and gonorrhea. In several studies extract of *Eucalyptus* leaves reduces the blood sugar level in mice with diabetes mellitus. *Eucalyptus* oil is present in mainly products including liquid and ointments, commercial cough drop, syrups, vaporizer, fluids, liminents, tooth paste, mouth itches. In skin care it can be used in cases of chicken pox, colds, flu and measles.

MATERIALS AND METHOD

Collection of the plant material

The leaves of *Eucalyptus camaldulensis* and Roots of *Calotropis gigantean* plant have been used for the present study. The part of both plant were collected from Maharajbagh nursery Agriculture collage, Nagpur, India, in the month of October 2012. The plant samples were brought to the Research Laboratory, Post Graduate Teaching Department of Microbiology Rashtrasanth Tukadoji Maharaj, Nagpur University, Nagpur.

Processing of the plant

The collected fresh Leaves and Roots of plant were washed thoroughly with distilled water at least three times to remove the debris. The leaves were shade dried at room temperature, and the Roots were dried in presence of Sunlight. Both the Leaves and Roots were grinded separately using mechanical grinder and stored at 4ºC until further use.

Method of extraction

The extraction was carried out in methanol, 10 gm of dried leaf powder were accurately weighed and dissolved in 100 ml of appropriate solvents in an air tight cork bottle and sealed with aluminum foil and labelled accordingly which was then loaded on an orbit shaker at a speed of 120 rpm for 24 hours. The mixture was filtered using Whatman filter paper. The filtrate was concentrated using rotary evaporator and dried. Dried extract was collected in an air tight container and stored at 4°C.

Organisms used

The following eight clinical isolates of bacteria were used for the study: *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Salmonella paratyphi.* All these cultures were maintained on nutrient agar plates at 4°C.

Positive and Negative Control

Penicillin, Amikacin, Gentamycin, Norfloxacin, Ciprofloxacin were used as a positive and negative control against all test organisms by agar well diffusion method.

RESULTS AND DISCUSSION

Screening for Antibacterial Activity

Eucalyptus camaldulensis:

Part used-	Leaves
Extracted with-	Methanol
Antimicrobial activities tested-	Antibacterial

Antimicrobial susceptibility pattern was studied by Kirby-Bauer well diffusion technique. This technique was developed by Bauer *et.al* (1996). It is most convenient and widely used method for routine antimicrobial susceptibility testing. Pure culture was used as inoculums, the growth of test organism was transferred into about 5ml Nutrient broth which were incubated at 30^0C or 18 hrs till moderate turbidity develops. The bacterial culture was adjusted and swab inoculated on Mueller Hinton Agar (MHA) plates. Allow inoculums to dry for 5 min. The wells were prepared on MHA plates with the help of 8 mm Cork borer. Add 10ul of extract in well labeled as extract and Add 10ul of antibiotic suspension in wells labeled by particular names. Plates were then kept in refrigerator for 1hr for proper diffusion of suspension. Incubate the plates at 37ºC for 24 hrs. Measure the zone diameter showing complete inhibition and recorded it in mm.

Result Reported*:* Methanolic extract of Eucalyptus gives the equal or greater zone of inhibition (Sensitivity) against all test organisms in comparison with positive and negative control**.**

Table 1. Comparative antibacterial study of *E. camaldulensis* with some standard drugs

S.No	Name of Organisms	Antibiotics (Positive and Negative control) Zone of Inhibition[b]					*E. camaldulensis* (Zone of Inhibition[b])
		Penicillin	Amikacin	Gentamycin	Norfloxacin	Ciprofloxacin	
1	*E. coli*	NA	22	22	35	36	19
2	*S. aureus*	15	NA	NA	NA	NA	20
3	*P. vulgaris*	NA	25	22	34	38	18
4	*S. typhi*	NA	18	17	31	39	18
5	*P. aeruginosa*	NA	21	19	30	37	22
6	*B. subtilis*	NA	25	33	21	31	20
7	*K. pneumonia*	19	20	NA	NA	NA	19
8	*S. paratyphi*	NA	21	19	29	33	19

b = average zone of inhibition in mm, Concentration = 10 ug/ml
NA = Not active

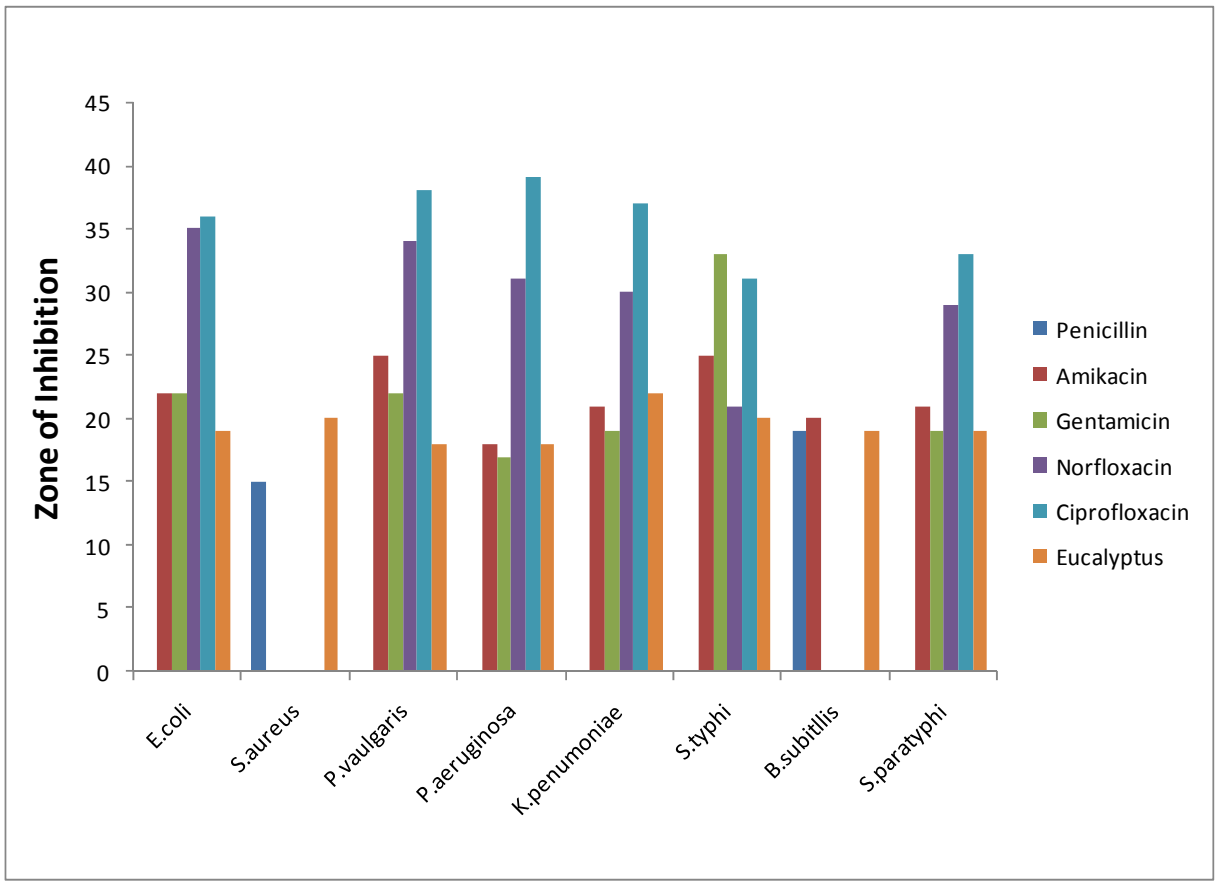

Fig 1. Antibacterial Activity of *Eucalyptus camaldulensis*

Calotropis gignatea

Part used- Leaves
Extracted with- Methanol
Antimicrobial activities tested- Antibacterial

Antimicrobial susceptibility pattern was studied by Kirby-Bauer well diffusion technique, details as in the (3.a.1).

Result Reported: Methanolic extract of Calotropis does not given the zone of inhibition (Sensitivity) against all test organisms in comparison with positive and negative control.

Combine antimicrobial activity of E. camaldulensis And C. gignatea.

Part used- Leaves and Roots
Extracted with- Methanol
Antimicrobial activities tested- Antibacterial

Antimicrobial susceptibility pattern was studied by Kirby-Bauer well diffusion technique, details as in the (3.a.1). Methanolic extract of both the samples were taken in 1:1 proportion.

Result Reported: The extract was reported to inhibit the growth of *E. coli, S. aureus, P. vulgaris, S. typhi, P. aeruginosa, B. subtilis, K. pneumonia, S. paratyphi* was found to exhibit strong inhibitory effects. *Eucalyptus* showed the zone of inhibition against all the micro-organisms, each organism have the different zone of inhibition. On the other hand Calotropis spp. does not have any antimicrobial activity, which in combination with *Eucalyptus* enhances the zone diameter.

Table 2. Antimicrobial Activity of combine plant study

S. No	Name of Organisms	*Eucalyptus*	Calotropis	Combine
1	*E.coli*	19	NA	23
2	*S.aureus*	20	NA	22
3	*P.vaulgaris*	18	NA	20
4	*P.aeruginosa*	18	NA	23
5	*K.penumoniae*	22	NA	23
6	*S .typhi*	20	NA	22
7	*B. subtillis*	19	NA	22
8	*S.paratyphi*	19	NA	22

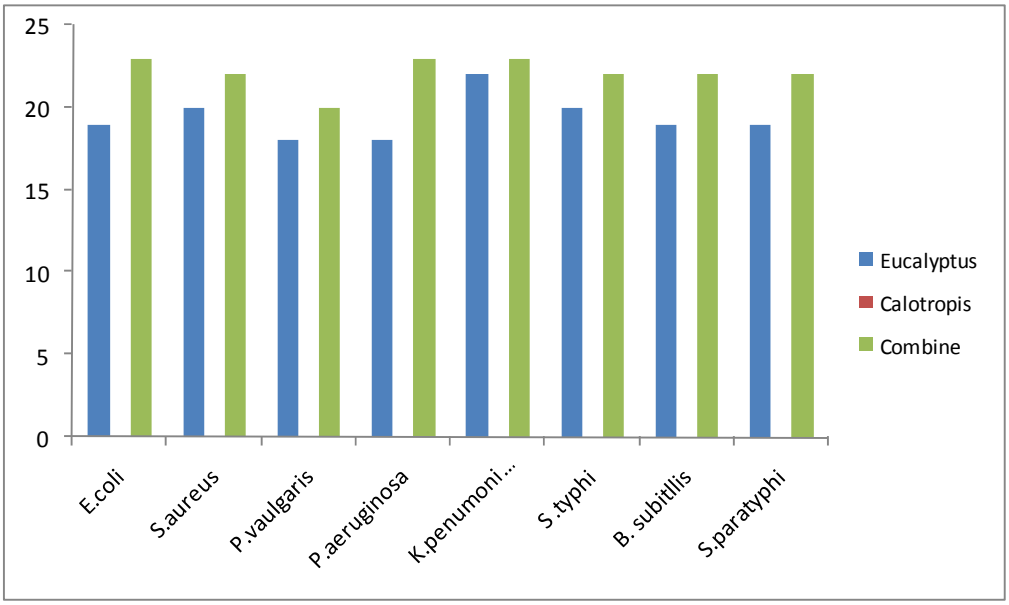

Fig 2. Antibacterial Activity of *E. camaldulensis* And *C. gignatea*

Determination of minimum inhibitory concentration (MIC)

The MIC determination was performed for the two extracts *viz*., leaf and Root extracts of methanol by modified agar well diffusion method. Two fold serial dilution of the stock solution was prepared in sterilized distilled water to make a concentration range from 0.5 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml. The bacterial suspensions were seeded on MHA plates using a sterilized cotton swab. In each of these plates six wells were cut out using a standard cork borer (6 mm). Using a micropipette, 100 µl of each dilution was added in to wells. All the plates were incubated at 37 ºC for 24 hours.

Result Reported: Antimicrobial activity of the leaf extract was evaluated by measuring the zone of inhibition.

Table 3. MIC of *Eucalyptus* against different organisms

S.No	Name of organisms	100%	75%	50%	25%	10%	5%
1	*E.coli*	11	10	<10	<10	<10	<10
2	*S.aureus*	12	11	11	11	10	10
3	*P.vaulgaris*	11	11	10	10	<10	<10
4	*P.aeruginosa*	11	11	10	10	10	<10
5	*K.penumoniae*	11	11	10	10	<10	<10
6	*S .typhi*	11	10	10	<10	<10	<10
7	*B. subtillis*	22	21	14	12	12	11
8	*S.paratyphi*	12	11	10	10	10	<10

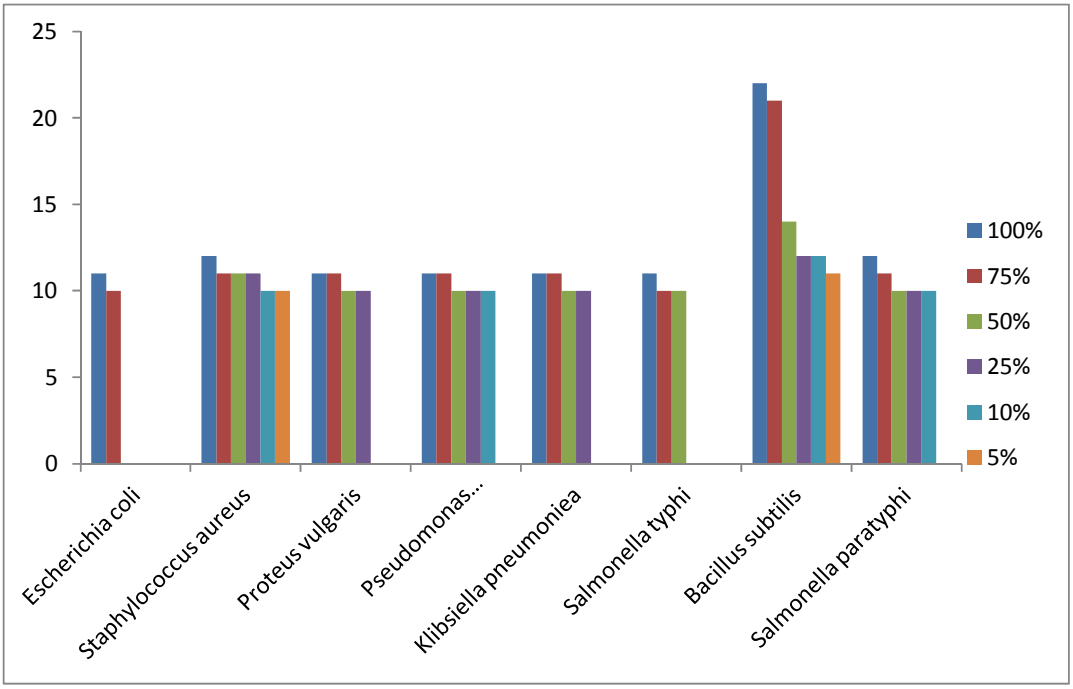

Fig.3 MIC of *Eucalyptus* against different organisms

Table 4. MIC of combine plant extract.

S.No.	Name of organisms	100%	75%	50%	25%	10%	5%
1	*E.coli*	14	13	12	11	10	10
2	*S.aureus*	20	17	16	11	10	10
3	*P.vaulgaris*	17	17	15	14	13	11
4	*P.aeruginosa*	26	16	13	12	11	10
5	*K.penumoniae*	11	10	10	10	10	10
6	*S .typhi*	18	14	14	12	11	10
7	*B. subtillis*	25	21	18	14	13	11
8	*S.paratyphi*	21	18	17	14	12	10

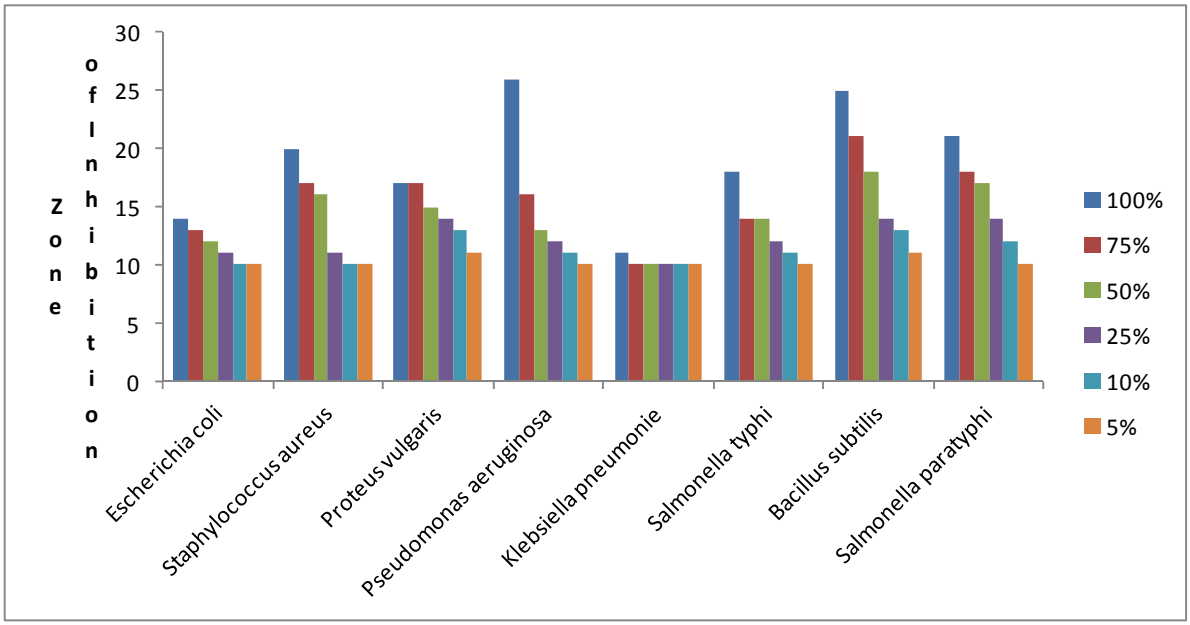

Fig.4 MIC of combine plant extract.

Medicinal plants are being probed as an alternate source to get therapeutic compounds based on their medicinal properties. *C. gigantea* is easily available in most of the agricultural and non-agricultural fields and the usage of this plant for medicinal purpose was reported by several researchers.

Ethno botanical approach is one of the common methods that are employed in choosing the plants for pharmacological study (Cox P A *et al.*, 1994). India is one of the twelve mega biodiversity centres having more than 45,000 plant species. Its diversity is unmatched due to the presence of sixteen different agro climatic zones, 10 vegetative zone and 15 biotic provinces. Use of plants as a source of medicine has been inherited and is an important component of the health care system. Approximately 20% of the plants found in the world have been submitted to Pharmacological or biological tests (Suffredini J B *et al.*, 2004). Continuous effort to find new antibacterial compounds. Considering the rich diversity of plants necessary to screen plants for their antibacterial active. The plant *Eucalyptus* is the principle source of medicinal *Eucalyptus* oil,the oil is also called as "Nilgiri Taila" because of its area of cultivation in India .The oil is widely extracted in the hills of Udagamand(nilgiris south india)The Nilgiri oil is being used by the medicinal department of the Government of Chennai for several years in cases of upper respiratory tract infection and has proved quite satisfactory (Takahashi T *et al.*, 2004). The large zone of growth inhibition exibited by the crude extract against the pathogenic bacteria used in this study justify the use of *Eucalyptus* in traditional medicine to treatopen wounds,boils, anda variety of enteric diseases. While the calotropis is also the principle source of medicinal use.The flowers and leaves of calottropis(akonda) is use for worship of God(lord Hanuman). It is the example of Entomophily pollination and pollination is achieved with the help of bees .In large doses, Arka (rui) is known to act as a purgative and an emetic. The Methanolic extract were obtained from the leaves of Eucalyptus Camaldulensis and roots of Calotropis gigantae tested against different bacterial pathogens (Kareem S O *et al.*, 2008). The pathogen are commonly implicated in pus causing wounds & food poisoning.The methanolic fraction can be used as medicin in the treatment of wounds & food poisoning.

The plant extract of Eucalyptus showed the activity against all pathogens in methanolic solvent , But the Calotropis gigantea did not show activity against all organism in the same solvent. *K. penumoniae* provided wider zone of inhibition (22 mm) whereas *P. vulgaris* and *P. auroginosa* provided lower zone (18 mm respectively) against same methanolic leaf extract. As such in combination with both plant extract the maximum zone of inhibition were shown by *E. coli*, *K. penumoniae* & *P. auroginosa* (23 mm). Whereas, minimum zone of inhibition were shown by *P.* vulgaris (20 mm) respectively.

The minimum inhibitory (MIC) values of the extract showed that the highest activity was recorded against *B. subtilis* 22 mm (100%), 21mm (75%), 14 mm (50%), 12 mm (10%) , 11 mm (5%).

Whereas the MIC value of combined plant extract showed that the highest activity was recorded against *P. auroginosa* – 26 mm (100%). *B. subtilis* – 21mm (75%). *B. subtilis* – 18 mm (50%). *P. vulgaris, B. subtilis, S. paratyphi* – 14 mm (25%). *B. subtilis, P. vulgaris* – 13 mm (10%). & *P. vulgaris, B. subtilis* – 11 mm (5%).

In summary, these studies confirm and extend the previously reported antibacterial activities of E. camaldulensis and C. gigantae methanolic extracts (Cock I E *et al.,* 2008). Most previous studies of Eucalyptus antibacterial activity have reported on the antimicrobial activity of oils (Delaquis P J *et al.,* 2002 and Sartorelli P *et al.,* 2007) with variable results. The current report uses methanolic extracts to overcome the problems associated with the insolubility of oil components in agar gels. Both Gram-positive and Gram-negative bacteria were susceptible to E. camaldulensis and C.gigantae extracts. The broad range of microbial susceptibilities indicates the potential of these extracts as a surface disinfectant as well as for medicinal purposes and possibly as food additives to inhibit spoilage. However, further studies are needed before these extracts can be applied to these purposes. In particular, toxicity studies are needed to determine the suitability of these extracts for the use as antiseptic agents and as a food additive.

CONCLUSION

It was concluded that there is some factors present in *Calotropis gigantea* which in combination with Eucalyptus *camaldulensis* enhance the activity of *Eucalyptus* and gives the maximum zone of inhibition as campaire to *Eucalyptus* alone aaginst different pathogens, which justify the traditional use of the plant for infectious diseases.

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