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The Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* (Myrtaceae)

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Abstract: Extracts of leaves of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* were screened phytochemically for the presence of secondary metabolites and for *in vitro* antibacterial properties. Methanol and dichloromethane extracts of leaves of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* were studied for their antibacterial activity against 8 clinically isolated organisms of gastrointestinal origin viz., *Klebsiella species* UCH 2101, *Proteus mirabilis* UCH 2102, *Proteus mirabilis* UCH 2204, *Salmonella typhi* UCH 2201, *Escherichia coli* CHO 3101, *Escherichia coli* UCH 2103, *Pseudomonas aeruginosa* CHO 3102 and *Pseudomonas aeruginosa* UCH 2203. The result of the phytochemical screening showed that both extracts contained tannins, saponins, cardiac glycosides but in addition to these, *E. torelliana* was found to contain anthraquinones. Both extracts were also found to inhibit all the isolates at 10 mg mL^{-1} concentration. The diameter of zones of inhibition exhibited by the extracts was between 10 and 22 mm. The methanol extracts compared favorably with gentamycin used as a standard control. The minimum inhibitory concentrations determined by the agar dilution method were between 0.04 and 10 mg mL^{-1}. The results obtained from this study reveals that extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* possess antibacterial activities against enteric pathogens and the extracts may be a potential source of new antimicrobials against enteric organisms.

Key words: *Eucalyptus camaldulensis*, *Eucalyptus torelliana*, phytochemical screening, antibacterial activity, minimum inhibitory concentration

INTRODUCTION

Eucalyptus species belong to the order myrtales and myrtaceae. It is a large genus of aromatic trees indigenous to Australia, Tasmania and the neighbouring islands but today can be found growing in subtropical regions of the world. The genus consists of about 700 species of evergreen trees and shrubs (Adeniyi *et al*., 2006).

The oils of the eucalyptus plant are frequently used as a remedy for cold and cough. They are used in pharmaceuticals such as cough syrups, lozenges, nasal drops and mouthwash. The leaves main medicinal ingredient, cineole, has demonstrated expectorant and nasal decongestant activities (Schulz *et al*., 1998). It may also provide potent antiseptic properties (Chao and Young, 1998). Eucalyptus is an ingredient in over-the-counter pharmaceuticals as temporary relief of minor aches and pains of muscles and for temporary relief of nasal congestion and coughs associated with cold.

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The three main sources of eucalyptus supplements are the common eucalyptus (*Eucalyptus globulus*), *Eucalyptus polybractea* and *Eucalyptus smithii* (Gruenwald *et al*., 1998). The leaf extracts of *E. globulus*, *E. maculata* and *E. viminalis* have been reported to significantly inhibit six Gram-positive bacteria (Takahashi *et al*., 2004).

The fungicidal effect of *E. camaldulensis* against dermatophytic fungal isolates has also been reported (Essien and Akpan, 2004; Mehraban, 2005).

The increasing resistance of most synthetically derived antimicrobial agents is of utmost concern. The search for suitable medicinal plants with potent active principles against microorganisms becomes imperative. Since the antimicrobial activity of eucalyptus has been established from previous studies, the main objective of this study is to examine the antimicrobial activities of the methanolic and dichloromethane leaf extracts of *E. camaldulensis* and *E. torelliana* against pathogenic enteric isolates.

MATERIALS AND METHODS

Plant Material

This study was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria in August 2003.

Leaves of *E. camaldulensis* and *E. torelliana* were collected from the Department of Forestry, University of Ibadan and authenticated at the Herbarium of the Department of Botany and Microbiology, University of Ibadan. A voucher specimen was deposited at the Herbarium for reference purposes.

Plant Extraction

Dried powdered leaves of *E. camaldulensis* and *E. torelliana* were extracted with methanol by soxhlet extraction. A part of the methanol extract was then partitioned into dichloromethane. The extracts were allowed to evaporate to dryness and stored in airtight bottles until ready for use.

Phytochemical Screening

Qualitative phytochemical screening of both plants was carried out using the methods of Harbone (1984) and Trease and Evans (1989) to test for the presence of these secondary metabolites: alkaloids, saponins, anthraquinones, tannins and cardiac glycosides.

Microorganisms

The organisms used in this study were *Klebsiella* sp., UCH 2101, *Proteus mirabilis* UCH 2102, *Proteus mirabilis* UCH 2204, *Salmonella typhi* UCH 2201, *Escherichia coli* CHO 3101, *Escherichia coli* UCH 2103, *Pseudomonas aeruginosa* CHO 3102 and *Pseudomonas aeruginosa* UCH 2203. The organisms were clinical isolates obtained from the University College Hospital, Ibadan, Nigeria and the Catholic Hospital, Oluyoro, Ibadan, Nigeria.

Determination of Antibacterial Activity

The antibacterial activity of the extracts was determined using the agar well diffusion technique (Adeniyi *et al*., 1996). Sensitivity test agar plates were seeded with 0.1 mL of an overnight culture of each bacterial isolate (equivalent to 10^7-10^8 cfu mL^{-1}). The seeded plates were allowed to set and a standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.1 mL of each extract at a concentration of 10 mg mL^{-1}. The antibiotic gentamycin at 5 μg mL^{-1} was used as positive control. The plates were incubated at 37°C for 24 h after which the diameter of zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration of the extract was carried out using the agar well dilution method (Rusell and Furr, 1972).

Different concentrations of the extracts were prepared to give a final concentration in the range of 5.0, 2.5, 1.25, 0.625, 0.313, 0.157, 0.079 and 0.04 mg mL^{-1}. Two milliliter of each dilution of the extract was mixed with 18 mL of Mueller Hinton agar, poured into Petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. The concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the extracts.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of the extracts of *E. camaldulensis* and *E. torelliana* indicated that both plants had tannins, saponnins and cardiac glycosides (Table 1). However *E. torelliana* had anthraquinones which were absent in *E. camaldulensis*.

Antibacterial Activities of the Extracts of *E. camaldulensis* and *E. torelliana*

The results of the antimicrobial activity of the extracts against the test organisms revealed that the methanolic extracts of the leaves of the two plants inhibited the growth of all the test organisms. The dichloromethane fractions also showed good activity on the isolates. However the dichloromethane fractions of both plants as well as gentamycin used as positive control were inactive against *E. coli* 2103 (Table 2).

The Minimum Inhibitory Concentration (MIC) of the Methanol and Dichloromethane Extracts

The MICs of the extracts of *E. camaldulensis* and *E. torelliana* ranged between 0.04 and 10 mg mL^{-1}. The MICs for *Klebsiella* sp., *Proteus mirabilis* and *Salmonella typhi* were however lower than that for *Escherichia coli* and *Pseudomonas aeruginosa* (Table 3).

Table 1: Phytochemical components of the leaf extracts of *E. camaldulensis* and *E. torelliana*

Phytochemical components	*E. camaldulensis*	*E. torelliana*
Alkaloids	-	-
Tannins	+	+
Saponins	+	+
Anthraquinones	-	+
Cardiac glycosides	+	+

Note: + = Present; - = Absent

Table 2: Antibacterial activities of the leaf extracts of *E. camaldulensis* and *E. torelliana* at 10 mg mL^{-1}

	E. camaldulensis		*E. torelliana*		
Organisms	Methanol crude	Dichlorom ethane	Methano crude	Dichlorom ethane	Positive control
Klebsiella sp., UCH 2101	15	17	13	15	24
P. mirabilis UCH 2102	13	12	14	15	22
P. mirabilis UCH 2204	13	13	16	14	26
S. typhi UCH 2201	20	19	16	22	27
E. coli CHO 3101	17	12	19	17	17
E. coli UCH 2103	13	-	14	-	-
P. aeruginosa CHO3102	12	10	14	15	10
P. aeruginosa UCH 2203	17	14	15	14	24

Resistance = No zone of inhibition, Positive control-Gentamycin (5 μg mL^{-1})

Table 3: Minimum Inhibitory Concentration (MIC) of the leaf extracts of *E. camaldulensis* and *E. torelliana*

	E. camaldulensis		*E. torelliana*	
Organisms	ME (mg mL^{-1})	DE (mg mL^{-1})	ME (mg mL^{-1})	DE (mg mL^{-1})
Klebsiella sp., UCH 2101	1.25	0.625	0.625	0.625
P. mirabilis UCH 2102	0.04	0.625	1.25	0.157
P. mirabilis UCH 2204	1.25	1.250	1.25	0.625
S. typhi UCH 2201	0.04	0.157	0.04	0.079
E. coli UCH 3101	10.00	10.000	5.00	5.000
E. coli UCH 2103	5.00	10.000	5.00	5.000
P. aeruginosa CHO 3102	2.50	5.000	5.00	5.000
P. aeruginosa UCH 2203	0.313	1.250	2.50	0.6125

ME: Methanol Extract; DE: Dichloromethane Extract

Previous research into the phytochemistry of the leaves of *E. camaldulensis* and *E. torelliana* revealed the presence of tannins, saponins and cardiac glycosides and in addition to these, *E. torelliana* contained anthraquinones. Ahmad *et al.* (1998) and Shariff (2001) have independently reported the presence of these components in members of the family Myrtaceae to which the plants used in this study belong. Also, Babayi *et al.* (2004) had reported that the phytochemical analysis of the crude extracts of Eucalyptus species revealed the presence of saponin, saponin glycosides, steroid, cardiac glycosides, tannins, volatile oils, phenols and balsam gum. Thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components.

One of the molecular actions of tannins is to form complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation resulting in the inhibition of cell protein synthesis (Stern *et al*., 1996). The presence of tannins therefore plays a significant role in the antimicrobial activity of the extracts. The antimicrobial activity of *E. torelliana* could partly be explained by the presence of anthraquinones. The bacteriostatic and bactericidal activities of anthraquinone from *Cassia italica*, have been established (Kazmi *et al*., 1994).

This study showed that *E. camaldulensis* and *E. torelliana* were effective inhibitors of microbial growth as they showed varying degrees of activity against the test organisms. There were no significant differences in the activity of the methanol extracts and dichloromethane fractions and this shows that both extracts can be exploited as antimicrobial agents. The leaf extracts of both plants also showed higher activities against *Salmonella typhi* compared with all other test bacteria. This is in support of the antimicrobial activity of *E. citriodora* against *S. typhi* as reported by Akin-Osanaiye *et al*., 2007. The Minimum Inhibitory Concentration (MIC) for *Klebsiella*, *Proteus mirabilis* and *Salmonella typhi* (between 0.04 and 1.25 mg mL^{-1}) were lower than that of *Escherichia coli* and *Pseudomonas aeruginosa* (between 0.625 and 10 mg mL^{-1}). This means that the extracts of the two plants are very potent against *Klebsiella*, *Proteus mirabilis* and *Salmonella typhi* but higher doses of the antimicrobial agents will be required in infections caused by *Escherichia coli* and *Pseudomonas aeruginosa*.

In conclusion, all the crude extracts and dichloromethane fractions of both plants have antimicrobial activities and thus confirmed the historical use of eucalyptus oil as an antibacterial agent (Kumar, 1988). The results of this study therefore form a good basis for selection of *E. camaldulensis* and *E. torelliana* for further phytochemical and pharmacological investigation for their use as possible antimicrobial agents in the treatment of gastrointestinal infections.

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