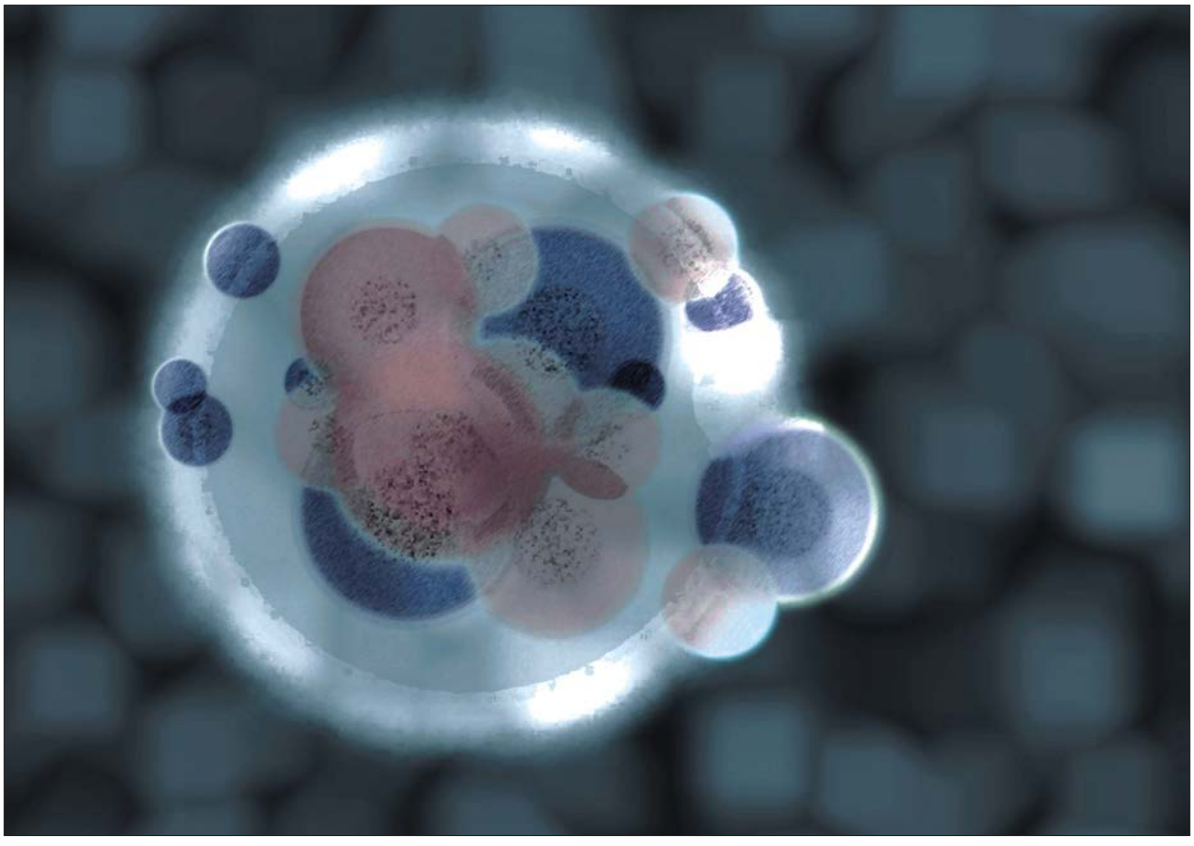

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Antimicrobial and Gastroprotective Activities of *Eucalyptus camaldulensis (Myrtaceae)* Crude Extracts

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Abstract: The crude extracts of *Eucalyptus camaldulensis* were investigated for their *in vitro* antimicrobial and gastro-protective activities in albino rats. The antimicrobial activity was investigated by screening the crude plant extract for activity against *Candida albicans* and clinically isolated gentamycin resistant wound bacteria viz: *Staphylococcus aureus* UCH 2600, *Escherichia coli* UCH 2554, *Pseudomonas aeruginosa* UCH 2780 and *Proteus mirabilis* UCH 2773. These microorgamisms were susceptible to the crude extracts at a 10 mg mL^{-1} concentration. The gastroprotective activity of the methanol extract of *Eucalyptus camaldulensis* (MEEC) was investigated in rats. Gastro protection was evaluated against gastric mucosal damage induced by ethanol/HCl mixture. The HCl/Ethanol mixture (1.5 mL of 0.15 N HCl in 70% ethanol) caused severe gastric damage with ulcer index 2.7±0.33. Pre-treatment of animals with crude extract of *Eucalyptus camaldulensis* leaf 200 and 1000 mg kg^{-1} orally for 1 h significantly reduced the formation of ulcer by the HCl/ethanol mixture with preventive ratios of 56 and 89%, respectively. Ranitidine afforded 92.5% protection. The results suggest that crude extracts of *Eucalyptus camaldulensis* has both antimicrobial and anti-ulcer properties.

Key words: *Eucalyptus camaldulensis*, antimicrobial property, anti-ulcer property, albino rats

INTRODUCTION

The use of herbs as medicines is widespread throughout the world and has been in existence for several thousand years before the development and spread of modern scientific medicine.

Eucalyptus camaldulensis (family *Myrtaceae*; common name: River red gum) is one of the species of *Eucalyptus* introduced into Nigeria. The *Eucalyptus* has been used in folklore medicine as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. The inhalation of the decoction vapour of the leaves is used for catarrh and nasal congestion (Kokwako, 1976). Essentials oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorant and cough stimulant. These oils were stated to have antitubercular properties (Oyedeji *et al*., 1999). Poultice of the leaves is applied over ulcers and wounds (Gill, 1992). There is however no previous study on the antimicrobial activity of crude extract of *E. camaldulensis*. The study is therefore aimed at justifying the ethnopharmacological antimicrobial activity of the plant in relation to wound healing as well as the anti-ulcer effect of the plant.

MATERIALS AND METHODS

Plant material: *Eucalyptus camaldulensis* leaves and stem bark were collected and authenticated at the Department of Botany, University of Ibadan and Forest Research Institute of Nigeria (FRIN) Herbarium. Voucher specimen was deposited at FRIN with the herbarium number [FHI 104908]. The samples were air-dried and pulverized before use for the study.

Preparation of plant extracts: Two kilograms each of pulverized plant samples were subjected to soxhlet extraction with methanol as extraction solvent. The extracts were later partitioned into petroleum ether and dichloromethane having kept part of the extracts as the methanol crude extract. Extracts were filtered, evaporated to dryness *in-vacuo*, weighed and stores at -4°C until needed.

Phytochemical screening: The pulverized samples of the leaves and stem bark of *Eucalyptus camaldulensis* was examined for the presence of anthraquinones, tannins, saponins, alkaloids and cardenolides. The procedure used was as previously described by Harborne (1973).

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Table 1: List of microorganisms

Organisms	Antibiogram
Staphylococcus aureus	CRO^S, AUG^S, ERY^S, CIP^S, CHL^S
UCH 2600	$GENT^R$, CXC^R, TET^R, COT^R, AMX^R
Escherichia coli	CAZ^S, CRO^S, CXM^S, $GENT^R$, OFLR
UCH 2554	AUG^R, AMX^R, TET^S, CIP^R
Pseudomonas aeruginosa	CRO^S, CAZ^S, OFL^R, $GENT^R$
UCH 2780	SP^R, CIP^R
Proteus mirabilis	CRO^S, CIP^R, $GENT^R$, OFL^R
UCH 2773	SP^R, PEF^R
Candida albicans	

S = Sensitive; R = Resistance

Microorganisms: The microorganisms used were clinical strains of bacteria isolated from infected wounds and *Candida albicans*. Biochemical tests were carried out to confirm that the organisms were pure culture. The antimicrobial sensitivity test was also carried out for each of the bacterial isolates (Table 1).

Media: The media used were nutrient broth and nutrient agar both of which are product of BIOTEC as well as sensitivity test agar which is a product of Lab M.

Animals: A total of 24 adult albino rats (150-250 g) were used for the study. They were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan where they were fed with standard laboratory rat diet and water. The animals were well ventilated and maintained at room temperature at about 27°C until the time of experiment.

Determination of antimicrobial activity: This was carried out using the agar cup diffusion technique described by Adeniyi *et al.* (1996) and Cotter and Adley (2001). A 1 mL of a 1:100 dilution of an overnight culture of each bacterial isolates (equivalent to 10^7-10^8cfu mL^{-1}) was used to seed sterile molten sensitivity test agar medium maintained at 45°C. Sabouraud dextrose agar plate was also seeded with the fungus. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm diameter was used to cut equidistant wells on the surface of the agar into which was added 0.1 mL solution of each extract reconstituted with methanol at concentrations of 10 and 20 mg mL^{-1}. The bacteria-seeded agar plates were incubated at 37°C for 24 h while the fungus seeded agar plates were incubated at room temperature for 72 h after which diameters of zones of inhibition were measured. Since each of the methanol crude, petroleum ether, dichloromethane and methanol residue extracts was re-suspended in 40% methanol before being tested, methanol (40%) was included in each plate as a solvent control while ciprofloxacin (10 μg mL^{-1}) and tioconazole [1%w/v] were included as positive controls. All the assays were carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC): This was carried out using the agar dilution method (Lajubutu *et al.*, 1995). This was carried out for the methanol crude and methanol residue extracts of the stem bark. Different concentrations of the extracts were prepared to final concentrations in the range of 10 to 0.039 mg mL^{-1}. A 2 mL of the extract was mixed with 18mLs of molten nutrient agar and poured into sterile petri dish allowing the agar to set. The surface of the set agar was allowed to dry before streaking with overnight broth cultures of microorganisms. Plates were incubated at 37°C for 24 h for bacteria and at room temperature for 72 h for the fungus and examined for the presence or absence of growth. The lowest concentration preventing growth was taken as the MIC of the extract.

Induction of gastric mucosal damage in rats: The gastro protective activity of the plant leaf crude extract was investigated in rats by induction of gastric mucosal damage with ethanol/HCl mixture. The animals were fasted for 24 h before the experiment.

At the commencement of the experiment the animals were divided into four groups of six animals in each group. Group A (control) animals were given 10 mL kg^{-1} distilled water, group B-200 mg kg^{-1} leaf crude extract, group C-1000 mg kg^{-1} leaf crude extract while group D animals were given Ranitidine 50 mg kg^{-1} by oral intubation.

After animal treatments, all the animals were subjected to surgery under sodium pentobarbitone anaesthesia according to Brodie (1966). The 4 h gastric juice collection was drained into a graduated test tube and centrifuged at 2000 rpm for 10 min. The supernatant volume and pH were recorded. The total acid content of the gastric juice was also determined by titration to pH 7.0 with 0.05N NaOH, using phenolphthalein as indicator. Gastric lesions were evaluated by examining the inner surface with a magnifying lens. Mucosal lesions were independently scored (in mm) by two observers. The severity of gastric damage was determined by measuring each lesion along its greatest length. In the case of petechial haemorrhages, five such lesions were taken as the equivalent of 1 mm (Koo, 1994). Index of ulceration was calculated as the total lesion lengths divided by the number in each group (Cho and Ogle, 1978). The preventive ratio was calculated as $(1-I_t/I_c) \times 100$, where I_t is the ulcer index for the treatment group and I_c is the ulcer index for the untreated group (Ibironke *et al.*, 1997).

RESULTS

Phytochemical screening of the plant leaf and stem bark revealed the presence of tannin, saponin and cardenolide. Alkaloids and anthraquinones were absent.

Table 2: Antimicrobial screening of the extracts of *Eucalyptus camaldulensis*

Organisms	Extracts/Diameter Zone of Inhibition (mm)* Leaf								Stem Bark								Control		
	MeOH crude		Pet. Ether		Dichl.		MeOH residue		MeOH crude		Pet. Ether		Dichl.		MeOH residue		Negative control	Ciprofloxacin (10 µg/Ml)	Tioconazole (1%)
	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20			
S. aureus 2600	17	22	14	18	-	-	16	24	15	17	17	19	14	16	16	24	-	-	NT
E. coli 2554	13	16	11	11	10	14	14	16	15	17	12	14	15	16	12	14	-	-	NT
Ps. aeruginosa 2780	15	17	-	-	12	14	13	14	15	18	-	-	-	10	14	18	-	-	NT
Pr. Mirabilis 2773	12	14	-	-	12	13	12	12	12	14	-	-	13	14	11	14	-	-	NT
Candida albicans	12	15	12	14	10	14	12	18	14	15	-	-	-	10	14	15	-	18	15

Diameter of cork borer = 8 mm; Concentration of extract in mg mL^{-1} * Mean of triplicate experiment±0.2-0.4 mm, Resistant (-) i.e. no zone of inhibition. MeOH-Methanol; Pet. Ether-Petroleum ether, Dichl.-Dichloromethane, Negative control-40% methanol, Positives controls-Ciprofloxacin (10 µg mL^{-1}) Tioconazole [1%w/v]

Table 3: Minimum Inhibitory Concentration (MIC) of the methanol crude and methanol residue extracts of the stem bark of *Eucalyptus camaldulensis*

Organisms	MeOH crude (mg mL^{-1})	MeOH residue (mg mL^{-1})	Ciprofloxacin 10 µg mL^{-1}	Trosyd
S. aureus UCH 2600	0.625	0.313	R*	NT
E. coli UCH 2554	5.00	10.00	R	NT
Ps. aeruginosa UCH 2780	0.625	0.313	R	NT
Pr. mirabilis UCH 2773	0.625	0.625	R	NT
Candida albicans	0.157	0.313	NT	S

*R = resistance; NT = Not tested, S = Sensitive

Table 4: Effects of *Eucalyptus camaldulensis* leaf crude extract and ranitidine on ethanol/HCl induced gastric mucous secretion and ulceration in rats (mean±SE)

Treatments	No. of animals	Ulcer index	Mucus content (g)	pH
Control (10 mL kg^{-1})	6	2.7±0.33	0.25±0.033	3.15±0.096
E. cam (200 mg kg^{-1}) leaf	6	1.2±0.17	0.12±0.013	3.12±0.023
E. cam (1000 mg kg^{-1}) leaf	6	0.3±0.18*	0.25±0.029	3.43±0.244
Ranitidine (50 mg kg^{-1})	6	0.2± 0.00**	0.02±0.004*	3.05±0.021

Significant, student t-test: * $p<0.05$, ** $p<0.01$ compared to control

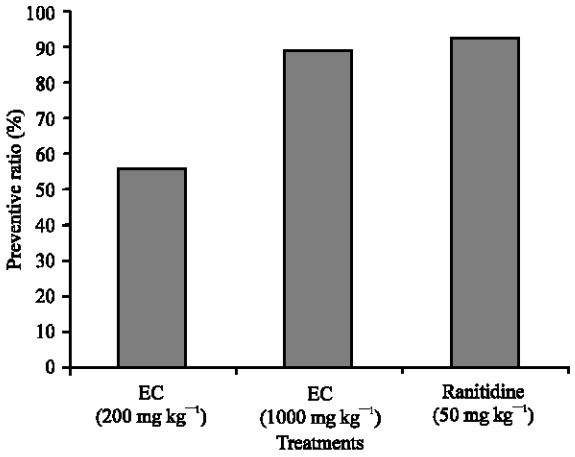

Fig. 1: Preventive ratios of methanol extracts of *Eucalyptus camaldulensis* (MEEC) and Ranitdine on HCl/Ethanol induced gastric ulceration

Results obtained from the antimicrobial screening of the leaf and stem bark (Table 2) revealed that the methanol crude and methanol residue extracts of the plant has a broad spectrum activity against all the tested microorganisms at 10 mg mL^{-1}, followed by the dichloromethane fraction. The least activity was demonstrated by the petroleum ether.

The Minimum Inhibitory Concentration (MIC) of the methanol stem bark of the plant ranges between 0.157 to 10 mg mL^{-1} when compared to control drugs Ciprofloxacin and tioconazole (Table 3).

The result of the ethanol/HCl induced gastric ulceration in extract pre-treated rats is shown in Table 4. The crude extract of the leaf reduced gastric mucosal damage and this reduction as indicated by a reduction in the ulcer index was found to be dose-dependent (Table 4). The preventive ratio of the extracts on ulcer is shown in Fig. 1.

DISCUSSION

Many microorganisms have been found to infect wounds; the most commonly implicated microorganism is *Staphylococcus aureus*. Others are *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* species, *Klebsiella* species and *Candida albicans* (Sleigh and Timbury, 1981; Melnick and Adelberg, 1988). And since most people occasionally sustain wound which sometimes become infected by microorganisms, treatment of such infected wound is therefore necessary (Nester *et al*., 2001). Therapeutic agents used in the treatment of wound are widely varied depending on the infecting microorganism. However these microorganisms have been found to

develop resistance to most of these drugs. This therefore necessitates that other therapeutic agents to which these resistant strains of microorganisms are susceptible be sourced.

Results obtained from antimicrobial screening of plant crude extracts showed the susceptibility of the tested gentamycin-resistant wound organisms to the crude extracts. The antimicrobial activity shown by the plant extract against the wound organisms can be attributed to the presence of tannin in the plant. Tannins have been used in medicine to aid the healing of wounds and burns. The mechanism of activity of tannins is based on their ability to bind to proteins of exposed tissues, thus precipitating the proteins. This then forms a firm antiseptic protective coat (preventing infection of the wounds and burns) under which the regeneration of new tissues take place in the process of which leads to wound healing (Osol and Hoover, 1970; Tyler *et al*., 1981).

Peptic ulcer disease is an important gastrointestinal disorder affecting up to 10% of the population with sufficient severity to prompt victims to seek medical attention. It is a typically recurrent condition with 50-90% of duodenal ulcer patients having a recurrence within a year (Munson, 1996).

The goals in the treatment of peptic ulcers are (i) to enhance ulcer healing (ii) to relieve pain and (iii) prevent recurrence. These are normally accomplished by (a) decreasing the secretory activity of the stomach with the use of histamine H_2-receptor antagonists (such as cimetidine, ranitidine) or acid/proton pump inhibitors (e.g. omeprazole), (b) neutralizing the hydrochloric acid of the stomach by the frequent administration of antacids and (c) by protecting the gastric mucosa with the administration of prostaglandins e.g. misoprostol (Bernard, 1985). It is well established that gastric acid secretion plays a role in gastric ulcer and that anti-ulcerogenic drugs accelerate healing by reducing the acid secretion (Okcu *et al*., 1992; Ibironke *et al*., 1997; Schmann and Switzerland, 1998). The observed reduction in acid output and increase in pH of gastric juice by MEEC in the present study may therefore be related to its acid-lowering effect which compare favourably with that of ranitidine.

In conclusion, the results presented indicate that crude extracts of *Eucalyptus camaldulensis* has both antimicrobial and gastroprotective activities and may represent a therapeutic opportunity for the treatment of infected wounds and ulcers. Isolation of the components producing these effects is therefore important to enable the elucidation of the chemical structure and confirmation of the mechanism of action.

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