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Antibacterial and Gastroprotective Properties of *Eucalyptus torelliana* [Myrtaceae] Crude Extracts

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Abstract: The antibacterial and gastroprotective properties of crude extracts of *Eucalyptus torelliana* were investigated. Antibacterial activity was investigated by screening the crude extracts for activity against clinically isolated strains of wound bacteria viz., *Staphylococcus aureus* UCH 2010, *Pseudomonas aeruginosa* UCH 2125, *Escherichia coli* UCH 2007, *Klebsiella species* UCH 2694 and *Proteus mirabilis* CHO 2014. The dichloromethane crude extracts demonstrated highest antibacterial activity against all tested microorganisms at 10 mg mL^{-1} concentration. The gastroprotective effect of the crude extract of the leaf was investigated in albino rats. This was evaluated against gastric mucosal damage induced by ethanol/HCl mixture. Ethanol/HCl mixture (1.5 mL of 0.15 N HCl in 70% ethanol) caused severe gastric mucosal damage with ulcer index of 2.7±0.33. Pre-treatment of animals with crude extract of *Eucalyptus torelliana* leaf 200 and 1000 mg kg^{-1} orally for 1h significantly reduced the Pre-treatment of animals with 50 mg kg^{-1}. Ranitidine for 1 h reduced the reduced the formation of ulcer by the ethanol HCl mixture with preventive ratios 56 and 92.5%, respectively. Ranitidine (50 mg kg^{-1}) afforded 92.5% protection. The results therefore suggest that crude extracts of *Eucalyptus torelliana* possess both antibacterial and gastroprotective properties.

Key words: *Eucalyptus torelliana*, antibacterial property, gastroprotective property, rats

INTRODUCTION

The family *Myrtaceae* in the order *Myrtales* contains 90 genera and 3000 species consisting of trees or shrubs. Among the genera is the genus *Eucalyptus* which is a large genus of evergreen trees and shrubs containing about 700 species (Penford and Willis, 1961). The genus is exclusively Australian with only about 50 species introduced into Nigeria among which is *Eucalyptus torelliana.*

Ethnopharmacologically *Eucalyptus* has been reported to relieve catarrh and feverish condition. The essential oil from the leaves is applied or rubbed over the chest and throat. The leaves are also chewed for bad breath (Gill, 1992).

The poultice of the leaves is applied over ulcers and wounds while the oils were stated to have antitubercular properties (Oyedeji *et al*., 1999).

Since *Eucalyptus* species had been reported to have antibacterial activity and have been employed in the treatment of wounds and ulcers, there is therefore need to confirm these claims by investigating the antibacterial and gastroprotective properties of the plant, *Eucalyptus torelliana.*

MATERIALS AND METHODS

Eucalyptus torelliana leaves and stem bark were collected, authenticated at the University of Ibadan Herbarium, air-dried and pulverized before use for the study. Plant samples were subjected to soxhlet extraction with methanol as the 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 evaporated to dryness and weighed.

Phytochemical screening: This was carried out for the presence of secondary metabolites such as anthraquinones, alkaloids, tannins, saponins and cardenolide using standard procedures (Sofowora, 1982; Trease and Evans, 1989).

Microorganisms: The microorganisms used for the study were clinically isolated strains of wound bacteria. Biochemical tests were carried out to confirm that the bacteria were pure culture. The antimicrobial sensitivity test was also carried out for each of the bacterial isolates (Table 1).

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Table 1: List of microorganisms

Organism	Antibiogram
Staphylococcus aureus UCH 2010	ERY^S, CHL^S, FU^S, CRO^S, VA^S, GENT^S, UCH AUG^R, TET^R, COT^R, CXC^R, AMX^R
Pseudomonas aeruginosa UCH 2125	CAZ^S, CRO^S, OFL^S, SP^S, GENT^R
Escherichia coli UCH 200	CXM^S, CAZ^S, CRO^S, GENT^S, CIP^S, OFL^S, SP^S, AMX^R, AUG^R
Klebsiella sp. UCH 2694	CRO^S, CIP^R, GENT^R, AUG^R, AMX^R, COT^R, PEF^R
Proteus mirabilis CHO 2014	CRO^S, CAZ^S, NA^R, NIT^R, COT^R, TET^R, CHO STREP^R, AMP^R, COL^R, GENT^R, PEF^R, OFL^R

NOTE: CHO – Catholic Hospital, Oluyoro, Ibadan, Nigeria

Media and antimicrobial agents: The media used were Sensitivity Test Agar (STA), a product of Lab M, nutrient broth and nutrient agar which were products of Biotec. The antimicrobial agents used were Gentamycin at 5 μg mL^{-1} concentration as the positive control and 40% methanol as the negative control.

Animals: Adult albino rats (150 – 250 g) used for the study were obtained from the University of Ibadan animal house. They were housed in cages and fed with standard laboratory rat diet and water.

Determination of antibacterial activity: This was carried out using the agar diffusion method (Cotter and Adley, 2001). A 0.1 mL of a 1:100 dilution of an overnight culture of each bacterial isolate was used to seed terile molten sensitivity test agar medium maintained at 45°C. The 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 which on the surface of the agar, into each of which was added 0.1 mL solution of each extract reconstituted with 40% methanol to final concentrations of 10 and 20 mg mL^{-1}. Gentamycin at 5 μg mL^{-1} concentration served as the positive control while methanol (40% $^v/_v$) was used as the negative control. The agar plates were incubated at 37°C for 24 h after which diameter of zones of inhibition were measured.

Induction of gastric mucosal damage in rats: The gastroprotective 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 (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 0.05 N 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 revealed the presence of tannins, saponins, cardenolides and anthraquinones while alkaloid was absent. In the stem bark of the plant, tannins, saponins and cardenolides were present whereas alkaloids and anthraquinones were absent.

Results obtained from the antibacterial screening of crude extracts of plant leaf and stem bark (Table 2) showed that the dichloromethane crude extract demonstrated highest antibacterial activity against all tested microorganisms at a 100 mg mL^{-1} concentration, followed by the methanol residue extract at the same concentration. The antibacterial activity was found to increase with increase in concentration of the extracts as shown by the increase in the diameter of the zone of inhibition (Table 2).

The crude extracts of the leaf at 200 and 1000 mg kg^{-1} dose reduced gastric mucosal damage (Table 3). The reduction as shown by a reduction in the ulcer index was found to be dose-dependent. The preventive ratio of the extracts on ulcer is shown in Table 4.

Table 2: Antibacterial screening of the extracts of *Eucalyptus torelliana*

	Leaf								Stem bark									
	Meoh Crude		Pet. Ether		Dichl.		Meoh residue		Meoh crude		Pet. Ether		Dichl.		MEOH residue			
Organisms	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20	Negative control	Positive control
S. anreus 2010	-	17	-	-	13	18	11	16	13	13	-	-	13	14	15	12	-	16
E. coli 2007	-	16	-	-	14	16	12	14	15	16	-	-	14	16	14	16	-	18
Ps. aeruginosa 2125	12	14	-	-	13	18	12	17	19	17	-	14	14	17	15	17	-	-
Pr. mirabilis 2014	-	15	12	14	13	16	13	18	17	18	-	16	16	17	12	16	-	12
Klebsiella spp. 2694	-	16	-	-	15	19	13	18	16	18	-	-	15	13	14	14	-	-

Diameter of cork borer = 8 mm, Concentration of extract in mg mL^{-1}, Resistance i.e., no zone of inhibition., MeOH – Methanol; Pet. Ether – Petroleum ether, Dichl. – Dichloromethane, Negative control – 40% methanol, Positive control – Gentamycin (5 μg mL^{-1})

Table 3: Effects of *Eucalyptus torelliana* leaf crude extract and ranitidine on ethanol/HCl induced gastric mucous secretion and ulceration in rats (mean±SE)

Treatment	Number 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. tor. (200 mg kg^{-1}) leaf	6	1.2±0.17	0.18±0.026	3.19±0.108
E. tor. (1000 mg kg^{-1}) leaf	6	0.2±0.16*	0.14±0.028	2.97±0.024
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

Table 4: Preventive ratios of methanol extracts of *Eucalyptus torelliana* (MEET) and Ranitidine on HCl/Ethanol induced gastric ulceration

Treatment	Preventive ratio (%)
E. tor. (200 mg kg^{-1}) leaf	56.0
E. tor. (1000 mg kg^{-1}) leaf	92.5
Ranitidine (50 mg kg^{-1})	92.5

DISCUSSION

The antibacterial activity demonstrated by the crude extracts appeared to be broad spectrum since both gram-positive and gram-negative organisms were sensitive to the extracts. Since the tested microorganisms were all isolated from infected wounds, the result therefore justifies the use of the plant in the treatment of wounds and hence the antibacterial activity.

Peptic ulcer, an important gastro-intestinal disorder is a chronic inflammatory condition characterised by ulceration most commonly in the first few centimeters of the duodenum (duodenal ulcer) and along the lesser curvature of the stomach (gastric ulcer) caused by gastric acid (HCl) and pepsin (Marvin and John, 1989). It affects up to 10% of the population with sufficient severity to prompt victims to seek medical attention (Munson, 1996). It is typically a recurrent condition with 50-90% of duodenal ulcer patients having a recurrent within a year (Munson, 1996). It can either be acute or chronic.

The goals in the treatment of peptic ulcers are to relieve pain, enhance ulcer healing and prevent recurrence. These are normally accomplished by (i) neutralizing the hydrochloric acid in the stomach by the frequent administration of antacids; (ii) decreasing the secretory activity of the stomach with the use of histamine H_2-receptor antagonists (e.g., Cimetidine, Ramtidine) and (iii) by protecting the gastric mucosa with the administration of prostaglandius (e.g., Misoprostol (Jaup, 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 (Ibironke *et al*., 1997; Okcu *et al*., 1992; Schmann and Switzerland, 1998). The observed reduction in acid output and increase in pH of gastric juice by MEET in the present study may therefore be related to its acid-lowering effect..

The activity of the plant leaf extract can be compared to that of (a) Ramtidine, an anti-secretory agent since there is a reduction in the secretion of hydrochloric acid and consequently a reduction in the gastric mucosal damage (b) Misoprostol, a prostaglandins with a gastro-protective property which has been demonstrated against necrosis produced by irritant agents (Cho-Chi and Ogle, 1992; Lange *et al*., 1985).

The anti-secretory and gastro-protective activities demonstrated by the crude extract of the plant leaf were revealed by a significant reduction in the ulcer index when compared to the control.

These activities therefore justify the use of the plant in traditional medicine and suggest that extracts from the plant may represent a therapeutic opportunity in the treatment of peptic ulcers and wounds.

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