academicJournals

Vol. 14(3), pp. 202-205, 21 January, 2015
DOI: 10.5897/AJB2013.13237
Article Number: 1E11AEC49717
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Phytochemical screening and antimicrobial activities of the leaf extract of *Entandrophragama angolense*

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Received 3 September, 2013; Accepted 12 January, 2015

The phytochemical screening and antimicrobial activities of the leaf extract of *Entandrophragma angolense* were investigated using the agar well diffusion method. The methanolic extracts at crude level were shown *in vitro* to inhibit *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Streptococcus cremoris* with diameter zones of inhibition ranging from 6 to 14 mm. Minimum inhibitory concentration was 3.13 and 6.25 mg/ml for Gram positive and negative bacterial strains, respectively. Minimum bactericidal concentration (MBC) was 6.25 and 12.5 mg/ml for Gram positive and negative bacterial strains, respectively. The leaf extract also had bioactive compounds such as tannins, alkaloids, saponin and cardiac glycoside which may be responsible for the biological properties of this plant. The study confirms the antibacterial potential of *E. angolense.*

Key words: *Entandrophragma angolense,* antimcrobial activity, phytocheamical.

INTRODUCTION

Entandrophragma angolense (Welw) is a large forest tree recognized by its large fruits which split from the base. The bark too is distinctive as it is very smooth. *E. angolerise* is used as a folklore medicine: as an antimalaria, antiulcer, haematinic and for treatment of other gastrointestinal disorders. The antiulcer activity of the plant has been investigated and the compound responsible for this activity was found to be methyl angolensate. It is of interest to note that most plant extracts have been reported to possess inhibitory substances and lethal activities on some pathogenic microorganisms *in vitro* (Oluma and Elaigwu, 2006).

Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases (Vijaya and Ananthan, 2001). The studies of medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solutions to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against these drugs. Thus, the need to search for new and more potent antimicrobial compounds of natural origin to combat the activities of these pathogens form the basis for this study.

MATERIALS AND METHODS

The experimental plant leaves were collected from Ogbese, Ondo State, Nigeria and was identified in the Herbarium of Department of Biological sciences, University of Abuja, Abuja. FCT, Nigeria.

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Table 1. Phytochemical screening of the methanolic leaf extract of *E. angolense.*

Bioactive constituent	Observation
Alkaloids	+
Saponins	+
Tannins	+
Phlobatanins	-
Anthraquinones	-
Cardiac glycosides	+

+ = Present; - = absent.

Table 2. Means zone of inhibition of the methanolic leaf extract of *E. angolense* for organisms.

Test microorganism	Extract at 100 mg/ml (mm)	Extract at 50 mg/ml (mm)	Chloramphenicol at 100 mg/ml (mm)	Chloramphenicol at 50 mg/ml (mm)
Staphylococcus aureus	14.10	11.20	17.40	9.30
Streptococcus cremoris	11.30	9.20	13.00	8.40
Escherichia coli	12.40	10.80	15.50	10.20
Pseudomonas aeruginosa	9.70	7.80	14.50	10.40

Extraction of leaves

The procedure was as described by Odebiyi and Sofowora (1979). 100 g of the powdered leaves were extracted with 95% boiling methanol using a soxhlet extractor. The extract was filtered and evaporated to dryness using a rotary eraporator to give a dark green gummy residue.

Phytochemical screening of extract

This was as by Odebiyi and Sofowora (1979), Solomon-Wisdom et al. (2011); Solomon-Wisdom and Shittu (2010). The leaf extract was screened for saponin, alkaloids, tannins, phlobatanins anthroquinones and cardiac glycosides.

Test organisms

The test organisms namely *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Streptococcus cremoris* were collected from Microbiology Laboratory of University of Abuja, Teaching Hospital, Gwagwalada Abuja, Nigeria. The stock cultures of bacteria were maintained on nutrient agar slants.

Antibacterial assay

This was as described by Odama et al. (1986). An aliquot of 0.1 ml of 1% barium chloride was added to 9.9 ml of H_2SO_4 to give a Mctarland turbidity standard suspension No 1. This turbidity approximates bacterial density of about $3x10^8$ organisms per ml. About 0.2 ml of the standardized suspension of the bacterium test growth in nutrient broth was pipetted into Muller Hinton Agar plates and the extract was used. The plates were incubated at 37°C for 24 h and the zones of inhibition were then measured to the nearest millimeter using a ruler (Erickson and Sherris, 1971). The minimum inhibitory concentration (MIC) was determined using the agar incorporated method as described by Abdulrahman (1986). This was done by using 0.2 ml of the standardized bacterial density of 3 x 10^8 organisms per ml. The inoculums were pipetted on the Muller Hinton Agar incorporated with the extracts at various concentrations and incubated at 37°C for 24 h. Following the incubation, the growth of the organism on the agar plates with different concentration of the extracts were observed and measured.

RESULTS AND DISCUSSION

The result of phytochemical screening shows the presence of alkaloids, saponins, tannins and cardiac glycosides, however, phlobatanins and anthraquinones are absent in the leaf extract.

The entire organisms were sensitive to the extract with Gram positive organisms having higher inhibitory zones with *S. aureus* having the highest zone inhibition of 14.10 mm at 100 mg/ml of the methanolic extract of *E. angolense* and for the Gram negative the highest zone of inhibition was recorded in *E. coli* (12.40 mm at 10 mg/ml), while the least zone of inhibition was recorded in *P. aeruginosa* which had the zone of inhibition of 7.80 mm at 100 mg/ml (Table 1).

The antibacterial activity results of *E. angolense* showed good effect against the Gram positive and negative microorganisms. Table 2 shows that MIC of methanolic leaf extracts of *E. angolense* for Gram positive organism is 3.13 mg/ml while for the Gram negative organism, it is 6.25 mg/ml.

Table 3 shows the MIC of the methanolic leaf extract of *E. angolense* against all organisms tested. MIC for the Gram positive organism was 3.13 mg/ml while for the

Table 3. Minimum inhibitory concentration of the methanolic leaf extract of *E. angolense*.

Test microorganism	Concentration of extract (mg/ml)									
	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.195
Staphylococcus aureus	+	+	+	+	+	+	-	-	-	-
Streptococcus cremoris	+	+	+	+	+	+	-	-	-	-
Escherichia coli	+	+	+	+	+	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	-	-	-	-	-

+ = Inhibition; - = no inhibition.

Table 4. Minimum bactericidal concentration of the methanolic leaf extract of *E. angolense*.

Test microorganism	Concentration of extract (mg/ml)					
	100	50	25	12.5	6.25	3.13
Staphylococcus aureus	+	+	+	+	+	-
Streptococcus cremoris	+	+	+	+	+	-
Escherichia coli	+	+	+	+	-	-
Pseudomonas aeruginosa	+	+	+	+	-	-

+ = Inhibition; - = no inhibition.

Gram negative organism, it was 6.25 mg/ml. Table 4 shows the MBC of Gram positive organism to be 6.25 mg/ml and that of Gram negative to be 12.5 mg/ml.

Table 4 shows the minimum bactericidal concentration (MBC) of the methanolic leaf extract of *E. angolense* for both Gram positive and negative microorganisms; the MBC of *E. angolense* for Gram positive organisms was 6.25 mg/ml, while for Gram negative microorganisms (*E. coli* and *P. aeruginosa*), it was 12.5 mg/ml.

Phytochemicals have received increasing attention because of interesting new discoveries considering their biological activities (Solomon-Wisdom and Shittu, 2010). In most developing countries of the world, plants are the main medical sources used in treating infectious diseases. The various photochemical compounds detected are known to exhibit medicinal activity as well as physiological activity (Sofowora, 1992). Plants are important source of potentially useful structures for the development of new chemotherapeutic agent. They have an almost limitless ability to synthesize aromatic substances, most of which are phenols or other oxygen substituted derivatives (Geissman, 1963).

In the present study, methanolic extracts showed a great amount of phytochemicals which are of medicinal importance to human (Liu, 2004; Solomon-Wisdom et al., 2011). They are routinely used in medicine because of their profound biological activities. These compounds served as natural antibiotics, which help the body to fight infectious and microbial invasions (Sodipo et al., 2000, 2002; Aliyu et al., 2011; Shittu and Ogbaje, 2002). When the activity of the methanolic leaf extract was compared with that of the standard antibiotics at the same concentration, it was observed that the leaf extract compared favourably with those of standard antibiotics.

Conclusion

The findings of the bioactivity of the leaf extract of *E. angolense* are beneficial as it indicates the emergence of new antibiotics with such a wide spectrum of activity. The results obtained show clear cut idea on the traditional uses of the plants. Plants that produce antimicrobials have enormous therapeutic potential as they serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Further research is however still necessary to determine the identity of the antibacterial compounds in these plant parts and also to determine their full spectrum of efficacy.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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