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Full Length Research Paper

Evaluation of antimicrobial potentials of stem bark extracts of *Erythrina senegalensis* DC

James Hamuel Doughari

Department of Microbiology, School of Pure and Applied Sciences, Federal University of Technology, P. M. B. 2076, Yola, 640002 Adamawa State, Nigeria. E-mail: jameshamuel@yahoo.com. Tel: +234 (0) 8083819054, +27 (0) 733 55 0274.

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Antimicrobial activity of organic (methanol and chloroform) and aqueous stem back extracts of *Erytrina senegalensis* against some pathogenic microorganisms was investigated using the filter paper disc diffusion method. Phytochemical studies revealed the presence of saponins, tannins, glycosides, phenols and alkaloids. The extracts demonstrated antimicrobial activity against both bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*) and fungi (*Aspergillus flavus, Aspergillus fumigatus*, *Candida albicans, Penicillium notatum*). For the bacteria, the highest activity (14 mm zone diameter of inhibition) was demonstrated against *E. coli* and the lowest activity (4 mm zone diameter of inhibition) against *S. aureus* and *P. aeruginosa,* while for the test fungi, the highest activity of 8 and 6 mm (zone diameter of inhibition) was demonstrated against *C. albicans* and *A. flavus* respectively, and the lowest activity of 4 mm against *P. notatum.* The methanol extracts demonstrated the highest activity while, the aqueous extracts demonstrated the lowest activity against all the test organisms. The activity of the extracts increased with increase in temperature (4 - 100ºC) and acidic pH, but decreased as the pH was adjusted toward alkalinity (pH 8 - 10). The MIC (7.5 - 30 mg/ml) and MMC (8.0 - 30.0) for bacteria, and MIC (7.5 - 40) and MMC (8.0 - 30.0) shows that *E. senegalensis* stem bark, if further purified can be used to source novel antibiotic substances for drug development against infections such as typhoid fever, urinary tract and wound infections, dysentery and mycotic infections.

Key words: Antimicrobial activity, antibiotic substance, bacterial infections, disc diffusion method, *Erythrina senegalensis,* phytochemicals, MIC, MMC, mycotic infections.

INTRODUCTION

Plant materials have remained central to tradomedical practices and have remained useful sources of new drugs. Although orthodox medical practice is generally acceptable, alternative healthcare is still relied on all over the world (Ozulua and Alonge, 2008). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of compounds for pharmaceutical purposes has gradually increased in different parts of the world. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use drugs, which has compounds derived from medicinal plants, while 80% of developing countries rely directly on crude concoctions, infusions, decoctions or poultices of plants in traditional medicine for their health remedies. Ethnobotanical studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional African medicines (Sofowora, 1981; Adesina and Sofowora, 1992; Mann et al., 2008). Globally, only a small proportion, out of the several thousand plant species has been investigated both phytochemically and pharmacologically, when one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident (Hostettmann et al., 1995; Banso and Adeyemo, 2007). Plants should therefore be investigated to better understand their properties, safety and efficiency (Nascimento et al., 2000). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in

therapeutic treatments. *Erythrina senegalensis* DC (Fabaceae), a plant specially introduced from Asia and wide spread in tropical and in grasslands, is commonly cultivated in fuel plantations and elsewhere in and around the towns and villages. Commonly called coral flower and parrot tree (English), echichi meaning "title" (Igbo, South Eastern Nigeria), ologun sheshe (Western Nigeria) Murjiya, Showoh or Ankai (Hausa, Tiv and Jukun respectively, Northern Nigeria) and Nte´ (Mali, West Africa), (Blench, 2007; Udem et al., 2009), the tree is up to 14 m high, bark gray and rough, the leaves 8 - 12 pairs on leaflets are green, and 3.5 - 6 cm long by 12 - 25 m broad. The flowers, which occur at most seasons, are red about 3.4 cm across in stout racemes up to 10 cm long arranged in panicles at the ends of the branches. In Gambia and Senegal the sap from the crushed leaves is applied to wounds for two or three days to promote healing. In Ghana and Nigeria the pounded bark and leaves are taken by women in soup against barrenness and in Mali, different parts of the plant have found application in the treatment of bronchial infections, cough, and throat inflammation, malaria, jaundice, infections, gastrointestinal disorders including diarrhea, amenorrhea, dysmenorrhoea, jaundice, sterility, onchocerchiasis, body pain and wound infections (Togola et al., 2008). The powdered bark and leaves are used in the form of soup to treat female infertility (oral information). The root infusion is used in Nigeria as a toothache remedy and in Ivory Coast for general disease treatment (Udem et al., 2009). In Mali, ethanol extracts of the plants was found to contain active bacteriocides against *Staphylococcus aureus, Enteracocus faccalis, Bacillus subtilis* and *Streptococcus pyogenes*. Despite its widespread multifunctional medicinal application, there is dearth in medicinal research documentation of the plant. This work was therefore designed to evaluate the antimicrobial potentials of this plant against some pathogenic bacteria and fungi and to determine the phytochemical constitution of the stem bark extracts.

MATERIALS AND METHODS

Test organisms

The test bacteria *S. aureus* (SA12MBFTY), *S. pyogenes* (SP006MBFTY), *Escherichia coli* (EC22MBFTY), *Salmonella typhi* (ST007MBFTY), *Pseudomonas aeruginosa* (PA 008 MBFTY) and *Candida albicans* (CA 006 MBFTY) were clinical isolates obtained from the Microbiology Laboratory of the Specialist Hospital Yola, Nigeria. While the fungi *Aspergillus flavus* (KB03SC), *Aspergillus fumigatus* (KB04SC), *Penicilum notatum* (PN 004 MBFTY) were laboratory isolates obtained from the Microbiology Laboratory, Department of Microbiology, Federal University of Technology, Yola, Nigeria. The clinical isolates, after further biochemical characterization (Prescott et al., 2002) and subculturing unto Nutrient agar (NA), were preserved onto slants of NA at 4°C until required. All the fungi [after microscopic characterization using lactophenol (Prescott et al., 2002)] were subcultured unto plates of Sabouraud Dextrose agar (SDA) and preserved at room temperature until use.

Collection and preparation of plant samples

Fresh stem bark of *E. senegalensis* were collected from Avri Ward in Wukari Town, Wukari Local Government Area of Taraba State, Nigeria and were identified and authenticated by Mr. D. A. Jauro of the Forestry Department, Federal University of Technology, Yola, Nigeria. The plant parts were chopped into pieces and dried at room temperature to constant weight for 5 days. The dried plant parts were coarsely pounded using pestle and mortar and further reduced to powder using an electric blender. The powdered plant part was used for extraction purposes.

Extraction of phytoconstituents

The method of Odebiyi and Sofowora (1987) was used for this purpose. Twenty grammes (20 g) of the plant powder was soaked separately into 100 ml of distilled water, methanol, and chloroform for 72 h with stirring at 24 h interval. The mixture was then filtered using a clean white cloth and then using Whitman No 1 filter paper. The filtrates were then concentrated under vacuum at 40°C and the concentrated extracts stored in brown bottles until use.

Phytochemical analysis

The stem bark extract was then analyzed for the presence of phytochemical components using the method described by Sofowara (1993).

Assay of Antimicrobial Activity

The antimicrobial activity was determined by the paper disc diffusion method (Doughari and Nuya, 2008; Doughari et al., 2008) with slight modification. Sterilized filter papers (2 mm diameter) were soaked in different concentrations of extracts (10 - 50 mg/disc) and then allowed to dry. To test for susceptibility, the bacterial isolates (0.5 ml McFarland turbidity standard) were seeded on to sterile Mueller Hinton Agar (MHA) plates and spread out using a sterile glass rod in order to achieve confluent growth. The plates were left on the table for 5 min to dry. Sterile filter paper discs soaked in various concentrations (10, 20, 30, 40, 50 mg/disc) of the extract solution were placed on each of the MHA plates earlier seeded with the different test bacteria. For fungi, the sterilized filter papers (6 mm diameter) impregnated with different concentrations of the extracts (10, 20, 30, 40, 50 mg/disc) were placed on Potato Dextrose Agar plates (PDA, oxoid) previously inoculated with fungal spores [10^6 spores/ml suspension from Potato Dextrose Broth (PDB, Oxoid), Oxoid]. Plates containing the antibiotic disc, ciprofloxacin (5 mg/m) (for bacteria) and griseofulvin (20 mg/ml) (for fungi) respectively were used as positive controls, while sterilized paper discs without extracts or antibiotics were used as negative controls for both bacteria and fungi. All the plates were then incubated at 37°C for 24 h (for bacteria and *C. albicans*) and at room temperature for 72 h (for the other filamentous fungi). The experiment was performed in triplicate. Following incubation, antimicrobial activity was determined by measurement of the zone diameters of inhibition (recorded as mean values) against the test organisms.

Effect of temperature and pH on antimicrobial activity of extracts

The method of Doughari et al. (2007) was used for this purpose with slight modifications in the extract concentration and temperature ranges used. To do this, 20 mg/ml concentration of

Table 1. Phytochemical analysis of stem bark extracts of *E. senegalensis*

Phytochemical components	Stem bark extracts
Saponins	-
Tannins	+
Glycosides	+
Phenols	-
Alkaloids	+
Cardiac glycosides	+

+ = Positive; = Negative.

extract was reconstituted in 1 ml distilled water and heated at various temperatures (4, 30, 60, 80 and 100°C) for 30 min. After the heating period, the extract suspension was incubated with 1 ml of test bacterial (0.5 McFarland turbidity standard) or fungal (10^6 spores/ml) suspension at 37ëC for 24 h and room temperature for 3 - 5 days respectively. After incubation, the antimicrobial activities of the heated and unheated samples were determined using the filter paper disc diffusion method earlier described. For the effect of pH, the extract (20 mg/ml) was soaked in dilute HCl or dilute NaOH as the case may be, at pH ranges of 2.5 to 8.5 for 30 min by adding the acid or base drop wisely (pH was determined at each stage using a pH meter). After the 30 min period of acid-base treatment, the extracts were again neutralized using 1 N HCl and 1 N NaOH solutions as the case may be and the antimicrobial activity against the test bacteria or fungi determined as earlier described.

Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

To determine the MIC, 0.5 ml of varying concentrations (10, 20, 30, and 40 mg/ml) of the extract was aseptically dispensed into four sets of clean sterile test tubes each containing 2 ml of Nutrient Broth (NB) or PDB. A loopful of each of the test bacteria (0.5 McFarland turbidity standard) or fungi (10^6 spores/ml) was introduced into each of the corresponding test tubes containing the NB (for the bacteria) or PDB (for the fungi) and the reconstituted extracts (0.5 ml). A set of test tubes containing broth only were seeded with the respective test organisms and set as control. All the test tubes were then incubated at 37ëC for 24 h (for bacteria and *C. albicans*) and at room temperature for 3 - 5 days (for fungi). After incubation, the concentration that showed no visible growth of the test organism was taken as the minimum inhibitory concentration (MIC) (Brock and Madigan, 2002; Doughari et al., 2008).

To determine the MMC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any visible growth and were inoculated onto sterile MHA or SDA plates, and incubated at 37°C for 24 h (for bacteria and *C. albicans*) and at room temperature for 3 - 5 days (for fungi) (Brock and Madigan, 2002). After incubation, the plates that showed no visible growth at any of the concentrations were regarded as the MMC.

RESULTS

Results of phytochemical analysis of *E. senegalensis* stem bark extracts showed the presence of saponins, tannins, glycosides, phenols and alkaloids (Table 1).

Figure 1 shows results of antimicrobial activity of *E. senegalensis.* Results showed that methanol extracts (40 mg/ml) demonstrated the highest activity against all the test bacteria compared to the fungi. For the bacteria, the activity ranged between 4 - 14 mm (zone diameter of inhibition) with the highest activity (14 mm) demonstrated against *E. coli* and the least activity (4 mm zone diameter of inhibition) against *S. aureus* and *P. aerugenosa.* For the test fungi the highest activity (8 mm zone diameter of inhibition) was demonstrated against *C. albicans* and 6 mm (zone diameter of inhibition) against *A. flavus* and *P. notatum.* The minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of extracts of *E. senegalensis* against the test organisms is shown in Table 2. For the bacteria, results showed that the least MIC values (7.5 - 8 mg/ml) and MMC (8 - 10 mg/ml) were demonstrated against *S. typhi* and *E. coli* and the highest MIC and MMC values (25 and 27.5 mg/ml respectively) were demonstrated against *S. pyogenes.* For the test fungi, the least MIC (10 mg/ml) and MMC (15 mg/ml) was demonstrated against *C. albicans* and the highest MIC (30 mg/ml) and MMC (32.5 mg/ml) were demonstrated against *A. fumigatus.* Ciprofloxacin and grseofulvin demonstrated the least MIC and MMC values (range of 0.625 - 7.5 mg/ml). Results of effect of pH on the antibacterial activity of the extracts (methanol) showed that the activity increased from 12 mm (zone diameter of inhibition) at pH 3.8 (untreated extracts) to 15 mm at pH 2.5 and 6 against *S. aureus*. As the pH was adjusted towards alkalinity (pH 8.5 and 10), the activity reduced to 10 and 6 mm respectively (Table 3). A similar trend was observed for all the other test bacteria and fungi. The effect of temperature (Table 4) however showed that the antimicrobial activity of the extracts increased with increase in temperature. For instance the activity of the extracts against *E. coli* and *C. albicans* at 30°C (untreated) was 14 and 8 mm (zone diameter of inhibition) respectively, but this increased to 16 and 14 mm respectively as the temperatures were increased to between 60 - 100°C.

DISCUSSION

Phytochemical screening of the extracts in this study revealed that *E. senegalensis* contained some active chemical compounds (saponins, tannins, glycosides, phenols and alkaloids) (Table 1). The presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs (Mann et al., 2008) and therefore explains its traditional use as health remedy. Secondary metabolites in plants confers them protection against bacterial, fungal and pesticidal attacks and thus are responsible for the exertion of antimicrobial activity against some microorganisms (Marjorie, 1999). The inhibitory activity exhibited by the secondary metabolites tends to agree

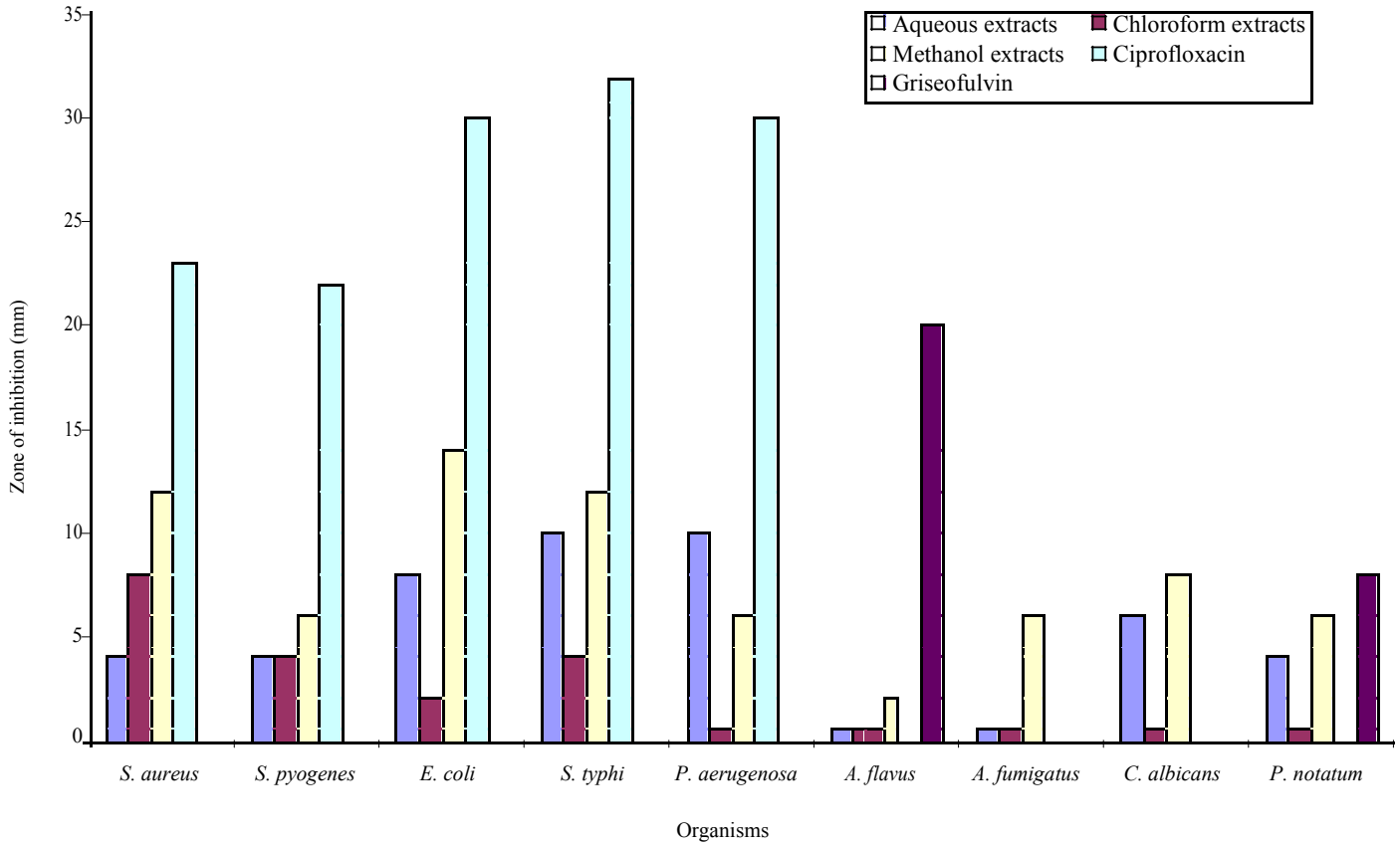

Figure 1. Antimicrobial activity of stem bark extracts (50 mg/ml) of *Erythrina senegalensis.*

Table 2. Minimum inhibitory and minimum microbicidal concentration of methanol extracts of *Erythrina senegalensis*.

Test organisms	ME extracts (mg/ml)		Ciprofloxacin (mg/ml)		Griseofulvin (mg/ml)	
	MIC	MMC	MIC	MMC	MIC	MMC
S. aureus (SA12MBFTY)	10.0	10.0	1.25	1.25	x	x
S. pyogenes (SP006MBFTY)	25.0	27.5	0.625	1.25	x	x
E. coli (EC22MBFTY)	8.0	8.0	2.5	2.5	x	x
S. typhi (ST007MBFTY)	7.5	8.0	2.5	5.0	x	x
P. aeruginosa (PA 008MBFTY)	7.5	10.0	5.0	5.0	x	x
A. flavus (KB03SC)	30.0	30.0	x	x	7.5	7.5
A. fumigatus (KB04SC)	30.0	32.5	x	x	40.0	40.0
C. albicans (CA 006 MBFTY)	10.0	15.0	x	x	40.0	40.0
P. notatum (PN 004 MBFTY)	22.5	22.5	x	x	20.0	22.5

ME = methanol extracts; x = not measured.

with various other previous reports (Leven et al., 1979; Scherbonvaski, 1971; Adebayo et al., 1983; Igbokwe et al., 2006) both of which linked the antibacterial properties of plants to the presence of secondary metabolites. The methanol extracts exerted antimicrobial activity against all the test bacteria (*S. aureus, S. pyogenes, E. coli, S. typhi* and *P. aeruginosa*) and fungi (*A. fumigatus, A. niger, A. flavus, C. albicans* and *P. notatum*), while the aqueous extracts did not exert any activity on *A. fumigatus* and *A. flavus.* These organisms are responsible for various types of infections; urinary tract infections (*S. aureus* and *E. coli*), dysentery (*E. coli*), wound infections (*P. aeruginosa*), typhoid fever (*S. typhi*), while candidiasis is caused by *C. albicans* and mycotic infections by *Aspergillus fumigatus* and *Aspergillus flavus*. Aqueous extracts demonstrated the least activity in this study

Table 3. Effect of pH on antimicrobial activity of methanol extracts of *Erythrina senegalensis.*

Test organisms	Zone of inhibition (mm)/pH				
	3.8*	**2.5**	**6.0**	**8.5**	**10.0**
S. aureus (SA12MBFTY)	12.0	15.0	15.0	10.0	6.0
S. pyogenes (SP006MBFTY)	6.0	9.0	10.0	8.0	6.0
E. coli (EC22MBFTY)	14.0	18.0	18.0	14.0	10.0
S. typhi (ST007MBFTY)	12.0	14.0	14.0	10.0	7.0
P. aeruginosa (PA 008 MBFTY)	6.0	6.0	7.0	6.0	4.0
A. flavus (KB03SC)	2.0	6.0	6.0	4.0	4.0
A. fumigatus (KB04SC)	6.0	10.0	8.0	10.0	6.0
C. albicans (CA 006 MBFTY)	8.0	11.0	10.0	8.0	6.0
P. notatum (PN 004 MBFTY)	6.0	9.0	9.0	6.0	4.0

*Non treated extracts.

Table 4. Effect of temperature on the antimicrobial activity of methanol extracts of *Erythrina senegalensis.*

Test organisms	Zone of inhibition (mm)/Temperature (°C)				
	30*	**4**	**60**	**80**	**100**
S. aureus (SA12MBFTY)	12.0	13.0	15.0	18.0	18.0
S. pyogenes (SP006MBFTY)	6.0	6.0	8.0	11.0	13.0
E. coli (EC22MBFTY)	14.0	14.0	16.0	15.0	16.0
S. typhi (ST007MBFTY)	12.0	13.0	14.0	15.0	18.0
P. aeruginosa (PA 008 MBFTY)	6.0	7.0	10.0	14.0	16.0
A. flavus (KB03SC)	2.0	3.0	5.0	9.0	12.0
A.fumigatus (KB04SC)	6.0	7.0	10.0	12.0	14.0
C. albicans (CA 006 MBFTY)	8.0	8.0	10.0	14.0	14.0
P. notatum (PN 004 MBFTY)	6.0	7.0	10.0	11.0	10.0

*Ambient temperature.

although water is used traditionally in the application of this plant as local health remedy. Use of the solvents accounted for increased extraction of the biologically active constituents thus displaying wider zone diameter of inhibition. Though the chloroform extracts demonstrated lower activities than the methanol extracts, their activities were still higher than those of the aqueous extracts. Different solvents have different polarities hence different degrees in solubility for the various phytoconstituents (Marjorie, 1999), thus accounting for this disparity in activity between the solvents used. The constituents might be more soluble in methanol than the other solvents therefore accounting for the high activity in this solvent compared to the others. The study also showed that the activity of the extracts is concentration dependent. Several factors ranging from concentration of antimicrobial agent, initial population density of the organisms, their growth rate and the rate of diffusion into the medium affects the activity of antimicrobials (Hugo and Russel, 1998; Prescott et al., 2002). The extracts were active against both the bacteria and fungi studied. The broad spectrum of activity exhibited shows that *E. senegalensis* has potential for novel drug source that will serve as chemotherapy for various infections. The MIC values were generally lower than the MBC values against the test organisms showing that the extract is bactericidal in action. Lower MIC and MBC values also indicates the high efficacy of the extracts. *C. albicans* was resistant to greseofulvin but susceptible to the methanol extracts of *E. senegalensis.* Selective pressure imposed by the use of antimicrobials in both human and veterinary medicine promotes the spread of multiple antimicrobial resistances resulting in the growing problem of infections that are difficult to treat (Carattoli, 2003). Resistance to some -lactam antibiotics, tetracyclines, chloramphenicol, or trimethoprim is reported with increasing frequency (Gallardo et al., 1999 and Velonakis et al., 2001). The adverse effect of antimicrobial resistance has typically been recognized as treatment failure; the disease caused by the pathogen can significantly worsen because of the antimicrobial drug used (Suh and Odeh, 2008). Antimicrobial resistance among *C. albicans* is on the rise and is a global concern. The efficacy of the extracts against the resistant bacteria and fungi demonstrated in this study is an indication that more effective antimicrobial substances could be sourced from the plant.

Demonstration of antibacterial activity by the plants in this study gives the basis for their traditional application as health remedies against diarrhea, wound and unitary tract infections. Results also showed that the activity of both the plants increased with increase in temperature (Table 1). Higher temperatures have been reported to increase solubility of solutes. This therefore means that more of the phytoconstituents were extracted at the higher temperature (100˚C) used in this study and also indicates heat stability. For the effect of pH, the antimicrobial activity increased at acidic pH (pH 2.5) and reduced when the pH was adjusted to alkaline (pH 8.0). This could mean that the phytoconstituents of this plant are labile at alkaline conditions. This also means that local practice of addition of potash to enhance infusion may be damaging to the antibacterial potency of the extracts. Stability of some plant extracts against varying temperatures and pH has earlier been reported (Doughari et al., 2007). Results of MIC and MMC investigation showed that the plants demonstrated slightly low values (MIC 20 mg/ml, MBC 30 mg/ml). Further purification may increase activity of the plant extracts.

Conclusion

The study has shown that the extracts of *E. senegalensis* possessed antimicrobial properties thus justifying its traditional usage as health remedy. The exhibition of antimicrobial activity by the plant against some pathogenic bacteria and fungi is an indication that the plant can be used for sourcing novel antimicrobial substances for drug development for the treatment of dysentery, typhoid fever, wound and unitary tract infections and mycotic diseases. The activity of this plant against a wider range of bacteria and fungi should be investigated. In addition, purification and toxicological studies should be carried out on the plant with a view to sourcing novel and safer antimicrobial substances for drug development for human consumption.

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