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Comparative evaluation of *Ceiba pentandra* ethanolic leaf extract, stem bark extract and the combination thereof for *in vitro* bacterial growth inhibition

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ABSTRACT
In spite of the numerous reports on medicinal potentials of *Ceiba pentandra*, the medicinal values of extract from the combination of the leaf and stem bark has not been adequately exploited. The combination therapy may lead to additive or synergistic effect. The antibacterial activities of the ethanolic extract of leaf (ELE), stem bark (ESE) and their combination (CLSE) were evaluated *in vitro* using selected human pathogens such as *Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. Extracts were screened for phytochemicals and their activities evaluated *in vitro* using the Agar well diffusion method. Data obtained was analyzed statistically using paired test. $P \leq 0.05$ was considered significant. Preliminary phytochemical screening showed the presence reducing sugars, saponins, polyuronoids, polyphenols, tannins and phlobatanins in ELE. ESE was found to contain reducing sugars, phlobatanins and alkaloids. All these phytochemicals were found in CLSE. Results from antibacterial assay showed mean diameter of inhibition zone <12 mm for ELE at concentrations ranging between 30 - 50 mg/ml. At a similar concentration range, ESE showed mean diameter of zone of inhibition of <12 mm against all the organisms except *Klebsiella pneumonia* (13.00±0.66 mm at 50mg/ml). Similarly, the activity of CLSE was ≤ 12 mm against all test organisms except for *E. coli* (13.00±0.33 mm at 50mg/ml). The activity of the combined extract was not significantly different from those of the stem bark and leaf extracts ($P>0.05$). In conclusion, ethanolic extract of leaf and stem bark of *Ceiba pentandra* as well as their combination showed significant antibacterial activity. However, the combined extract did not show synergistic nor additive effects on the test organisms.

Keywords: *Ceiba pentandra*, Combination therapy, Phytochemistry, Antibacterial assay

1.0 INTRODUCTION

Incidence of increased bacterial resistance to conventional drugs is of great concern to the scientific community. Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different cultures and are a source of many potent and powerful drugs (Srivastava *et al*., 1996).

The use of medicinal plant is accepted as the most common form of traditional medicine in the world. In some traditional practices, extracts from a combination of different parts of a single plant or of different plants have been used for therapeutic purposes instead of single plant parts. Scientific reports have suggested synergistic and additive effects in many combination therapies coupled with other advantages such as low doses of the individual components in the mixture. Several reasons have been suggested for the improved activity arising from combination therapies. Formation of complexes and parallel modes of action of active components in mixtures are some of the basis for improved activity in combination therapies.

Many plants have been screened for their antimicrobial activities to provide scientific backing for their ethnomedicinal claims in the treatment of many infectious diseases of microbial origin and have been found promising (Doughari and Ioryue, 2009). Investigations have also presented a plethora of antibiotics afforded by lower plants such as fungi, yet microbial diseases are still on the rise in developing countries due to relative unavailability of medicines and the emergence of wide spread drug resistance (Okeke *et al*., 2005). Of great concerns are recent reports that seem to suggest that infectious diseases are actually on the increase in developed

countries, (Pinner *et al*., 1996). Thus, the search for antimicrobial compounds in higher plants is vigorously pursued by many phytochemical laboratories (Hamburger and Hostettmann, 1991).
Ceiba pentandra is among the many higher plants that have been identified and used for medicinal purposes in traditional practices across several cultures for the treatment of bacteria, fungal, parasitic and inflammatory disorders. There are little convergence in the traditional use of *C. pentandra* throughout West and Central Africa and North America as anti-inflammatory, analgesic, anti-bacteria, anti-cancer, anti-diabetic, antifungal, anti-malarial and antioxidant (Abosi *et al*., 2003; Phillipson *et al*., 1993).

Extracts of *C. pentandra* are reported to contain bioactive substances such as glycosides, tannins, tannins, saponins, sesquiterpene lactones, flavonoids, polyuronoids, reducing sugars, phlobatannins etc. (Adebayo-Tayo *et al.,* 2008; Fadeyi *et al.,* 1989). The following compounds have been isolated from the bark of this plant; vavain 3'-O-B-D-glucoside, and its aglycone, vavain; flavan-3-ol, (+)-catechin (Ylva *et al*., 1998), pentandrin and pentandrin glucoside and beta-sistosterol and 3-beta-D-glucopyranoside (Ngounou *et al.*, 2000). The stem bark of *Ceiba pentandra*, is used locally as myriad of effects on medical conditions such as treatment of wounds, cough, high blood pressures, diarrhoea, dysentery, yellow fever and tumours. Leaves are used as antidysentric, leucorrhoea, anemia and infertility. Several investigations also reveal that, the leaf and the stem bark extracts individually, are very effective against diabetes mellitus and malaria. *Ceiba pentandra* has also been used in wound healing (*Sandhya et al.,* 2011).

In spite of the several scientific reports on the antimicrobial activities of leaf and stem bark extracts of *C. pentandra*, there has been little information regarding medicinal potential of extract of the combination of leaf and stem bark. We therefore present a comparative evaluation of antibacterial activities of ethanolic leaf and stem bark extracts when used individually and that of their combination against selected pathogens. The various extracts will be screened for phytochemicals and their *in vitro* antibacterial activity compared.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents
All reagents used were of analytical grade and were obtained from Panreac, Spain. Reagents were used without further purification.

2.1.2 Equipment
Rotary evaporator (Eyela, Japan), Freeze dryer (Eyela, Japan), incubator (P SELECTA, Spain).

2.1.3 Plant material
Adequate quantities of fresh leaves and stem bark of *C. pentandra* were collected around a suburb of Navrongo in the Kassena-Nankana District of Ghana in the month of April and identified and authenticated by Dr. Walter Kpikpi of the Department of Applied Biology of University for Development Studies, Navrongo, Ghana.

2.1.4 Microorganisms
Four (4) different microbes of wild strains were purchased from Centre for Scientific Research in Herbal Medicine Mampong–Akuapem in the Eastern region of Ghana. The microbes were *E. coli, K. pneumonia, P. aeruginosa and S. aureus*.

2.1.5 Media
Muller Hinton Agar (OXOID, CMO 337) and Peptone Agar were used for the microbial activities.

2.2 Methods

2.2.1 Extraction from plant material
The samples were air-dried for about three (3) days. Samples were pulverized (coarse grinded). About 100 g of the samples were weighed on a chemical balance. For the combined sample, 100g of each of powdered leaf and stem bark were mixed prior to extraction. Each sample was cold- macerated in 100 mL of 95% ethanol for 24 hours at room temperature and then filtered. The various filtrates were concentrated using rotary evaporator at a reduced pressure.

2.2.2 Qualitative determination of phytochemicals
Phytochemical screening was undertaken using standard qualitative methods as described by Odebiyi *et al.,* (1990) and Fadeyi *et al.* (1989).

2.3 Microbiology

2.3.1 Sterilization

All micro plates used were sterilized at 160 ^{o}C for 3 hours. Also, the media and broth prepared were sterilized at 121^{o}C for three (3) hours minutes in an autoclave machine and cooled to about 60^{o}C. All materials used in the microbiological work were sterilized before and after usage.

2.3.2 Preparation of Muller Hinton agar

The agar was prepared using the manufacturer's description**.**

About 27.36 g of Muller Hinton agar was weighed into 720 ml of distilled water and heated and stirred to dissolve. The media was sterilized at 121^{o}C for three (3) hours and cooled to about 60^{o}C.

2.3.3 Peptone water

The peptone water was prepared by using the manufacturer's description

Peptone agar was used as broth for culturing of the microbes. About 5.1 g of peptone agar was weighed into 200 ml of distilled water and heated to dissolve. The mixture was sterilized at 121°C for three (3) hours and cooled to about 60°C. 10 ml of the broth was measured with pipette into 5 test tubes and incubated for 16 hours.

2.3.4 Culturing

Small amount of the microbes were picked and sub - cultured for 2 hours. After the 2 hours sub- culture, the micro organisms were introduced in to the media. *E. coli, K. Pneumonia, P. aeruginosa and S. aureus* were introduced on the Muller Hinton agar media in the micro plates. A 5 mm hole borer was used to bore holes in the 36 plates, in which extracts were introduced into (*Cruickshank et al.,* 1980).

2.3.5 Antimicrobial susceptibility test

The spreading method of *Cruickshank et al.,* 1980 and dose (agar) diffusion method were used. Twenty-four hours old cultures of the organisms to be tested were used. A loopful of the cultures were uniformly spread over the surface of sterile Muller Hinton Agar (MHA) for *E. coli, K. pneumonia, P. aeruginosa* and *S. aureus* with a sterile bent rod.

Various concentrations (30 mg/ml, 40 mg/ml and 50 mg/ml) of leaf, stem bark and the combination extract were prepared in 10 ml of Dimethylsulfoxide (DMSO). Chloramphenicol was also used as test control on the microbes.

About 100 µl of the prepared extracts were used to fill holes bored by 5mm cork borer in the inoculated agar. The plates were made in triplicate. All plates were incubated at 37^{o}C for 24 hours. Diameters of the zones of inhibition in the triplicate plates were measured by calculating the difference between cork borer (5mm) and the zone of inhibition (Hewett *et al.,* 1989; Adebayo-Tayo *et al.*, 2008).

2.4 Statistical analysis

Data are expressed as mean±SEM and were statistically analyzed using paired test. Statistical Product for Social Solution (SPSS) software was used to analyze the data from antimicrobial activities. *P*-values ≤ α (0.05) were considered significant.

3.0 RESULTS AND DISCUSSION

Results from phytochemical screening of the extracts of *Ceiba pentandra* revealed the presence of bioactive principles. Phlobatannins and reducing sugars were found to be present in all the extracts: ELE, ESE and CLSE. However, cynogenic glycosides and anthraquinones were absent in all extracts. All phytochemicals found to be present in either ELE or ESE were also present in CLSE (Table 1). ELE appeared to contain more of the phytochemicals tested than ESE. Results of phytochemical screening of leaf and stem bark extracts are consistent with previous investigations (Kubmarawa *et al*., 2007; Akaneme, 2008; Sule *et al.,* 2009). The bioactive compounds (alkaloids, phenolics and saponnins) are known to exhibit medicinal activity as well as physiological activity (Sofowora, 1993). Tannins, alkaloids, saponins and phlobotannins have been implicated as antibacterial agents (Enzo, 2007). In addition, alkaloids and saponins are also known to be effective against syphilis and other bacterial infections (Sofowora, 1993). The presence of these compounds in the various extracts supports their traditional use and forms the basis of the antibacterial activities demonstrated by the extracts. The various extracts demonstrated moderate broad spectrum antibacterial activity against the test organisms (Table 2). The results for the antibacterial assay showed mean diameter of inhibition zone less than 12

mm for ELE at concentrations ranging between 30 mg/L and 50 mg/L. At similar concentration range, ESE showed mean diameter of zone of inhibition less than 12 mm against *Pseudomonas aeruginosa*, *Escherichia* coli, *Staphylococcus aureus* and 13.00±0.66 mm for *Klebsiella pneumonia*. CLSE showed activity with mean diameter zone of inhibition ≤ 12 mm against *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* at concentration range between 30 mg/L and 50 mg/L and 13.00±0.33 mm for *Escherichia* coli at a concentration of 30 mg/L .The activity of the combined extract was not significantly different from those of the stem bark and leaf extracts (P>0.05). The standard drug (Chloramphenicol) demonstrated the strongest antibacterial activity amongst all the drugs used. The comparable antibacterial activity shown by the various extracts suggest that the active components may be similar or may have similar modes of actions. The result further reveals that no new stronger antibacterial agent was formed as a result of the combination. Thus, the combination therapy did not show additive nor synergistic effects on the test organisms.

4.0 CONCLUSION

Results from phytochemical screening of the ethanolic extract of leaf and stem bark of *C. pentandra* supports their ethnomedicinal claims as antibacterial agents since most of the phytochemicals present have been reported to be bioactive. The extract from combination of leaf and stem bark of *C. pentandra* contained the various phytochemicals identified in the ethanolic extract of leaf and the stem bark. In addition, results from phytochemical screening of the leaf and stem bark extracts are in agreement with previous reports (Ngounou *et al*.; 2000; Adebayo-Tayo *et al*., 2008; Fadeyi *et al*., 1989). The combined extract showed no superior antibacterial activity when compared with the ethanolic stem bark extract and the ethanolic leaf extract. There was no demonstration of synergism and additive effect in the activity of the combined extract.

Table 1: Phytochemical analysis of ethanolic extract of leaf, stem bark of *C. pentandra* and the combination thereof

Constituent	ELE	ESE	CLSE
Reducing Sugars	+	+	+
Saponins	+	_	+
Cynogenic Glycosides	–	_	_
Polyuronoids	+	_	+
Polyphenols	+	_	+
Tannins	+	_	+
Phlobatannins	+	+	+
Anthraquinone	–	_	_
Alkaloids	_	+	+

(+) = Presence of Phytochemical constituent **(-)** = Absence of Phytochemical constituents
ELE = ethanolic leaf extrac
ESE = ethanolic stem bark extract
CLSE = ethanolic extract from combination of leaf and stem bark

Table 2: Antibacterial activities of extracts of *Ceiba pentandra* leaf, stem bark and the combination

Mean diameter of zones of inhibition (mm)

organisms	ELE			ESE			CLSE			Control
	30 mg/ml	40 mg/ml	50 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	
K. pnuemonea	10.00±0.33	11.00±0.33	12.00±0.33	9.00±0.33	13.00±0.66	13.00±0.33	6.00±0.33	10.00±0.00	12.00±0.00	22.00±0.00
P. aeruginosa	10.00±0.00	7.00±0.33	8.00±0.00	7.00±0.66	10.00±0.66	11.00±0.66	10.00±0.33	10.00±0.00	10.00±0.00	22.00±0.00
E. coli	8.00±0.66	10.00±0.00	10.00±0.30	10.00±0.00	11.00±0.00	11.00±0.00	11.00±0.33	12.00±0.66	13.00±0.33	26.00±0.00
S. aureus	8.00±0.33	8.00±0.66	10.00±0.33	10.00±0.00	11.00±0.33	13.00±0.66	10.00±0.33	10.00±0.66	11.00±0.33	17.00±0.00

Data expressed as mean±SEM. (n = 3)

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