

Full Length Research Paper

Phytochemical and antimicrobial study on the leaf extracts of *Erythrophleum africanum* (Caesalpinaceae)

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The leaf of *Erythrophleum africanum* was exhaustively extracted with ethanol using cold maceration techniques. This was subsequently partitioned with petroleum ether, chloroform, ethylacetate and n-butanol. The agar diffusion method was used to determine the antimicrobial activity against the following micro-organisms *ethicillin resistant Staphylococcus*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus vulgaris*, *Candida albicans*, *Candida krusei* and *Candida tropicalis*. Minimum Inhibitory Concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined. The *in vitro* antimicrobial screening revealed that the extracts exhibited diverse activities against different microbes with zones of inhibition ranging from 12 to 36 mm, MIC ranging from 3.25 to 60 mg/ml and MBC/MFC of 3.25 to 60 mg/ml for sensitive organisms at the tested concentrations. The activities observed could be attributed to the presence of terpenoids, saponins, flavonoids, alkaloids and tannins. The results justify the ethnomedicinal use of this plant in the treatment of sores, boils, wounds, dysentery, diarrhea and sexually transmitted infections.

Key words: *Erythrophleum africanum*, phytochemistry, antimicrobial activity.

INTRODUCTION

Despite the tremendous progress in human medicine, infectious diseases caused by bacteria, fungi, virus and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicine and emergence of drug resistance (Zampini et al., 2009). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics (Okemo et al., 2003), has led to the search for new biologically active compounds from natural sources as new antimicrobial agent with the view to discover new chemical structures, which could overcome the above disadvantages (Bouamama et al., 2006; Meenakshi et al.,

2001). The development of resistance to current antibiotics by disease causing microbes has also reinforced research for discovery of new ones. Current trends in drug development process are focused on natural sources, especially of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. Therefore, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide (Osawa et al., 1990; Kunle and Egharevba, 2009; Sophon et al., 2002; Begum et al., 2002).

Erythrophleum africanum (African black wood), is an

endangered African medicinal plant. Studies have revealed that, it is extremely toxic to livestock all over the world especially to goats, sheep and cows. Several species which include *E. quineense*, *E. invoreense*, *E. lasicanthum* and *E. chlorostachys* are known to be poisonous (Watt and Breyer Brandwijk, 1963; Dalziel et al., 1959; Griffin et al., 1971). This plant is reputed for its uses as an ordeal poison for executing capital punishment for witches and also to kill or scare away stubborn pest from cultivated farm land (Dalma, 1970; Mattocks, 1987; Loder et al., 1974).

The leaf of *E. africanum* is used in the treatment of various ailments which include emetics, as an anti-inflammatory agent, as an analgesic and also in sore and wound dressing. It is also used to treat chicken pox and gangrenous sores. The leaf decoction of this plant is well known by the traditional healers in Congo, (Democratic Republic of Congo), Zaire, Eastern province of Cameroun and India who used it empirically for several ailments including cardiovascular disease, various inflammation, diabetes, simple goiter, dysentery, diarrhea and as an astringent (Dalziel, 1999). This plant is reported to contain flavonoids and anthocyanidins and as such was used as a tooth pick for oral hygiene (Nwude and Chineme, 1981; Watt and Bayer Bradwyle, 1962; Burkill, 1995). Some alkaloids (pyrolizine alkaloids, PAS) from the leaf of this plant have been implicated to be gastrointestinal tract irritants, cholinesterase inhibitors and also affect the nervous system by causing drowsiness, salivation, labored breathing, trembling, loss of consciousness, coma and death due to paralysis (Roberts and Wink, 1998; Ahmad et al., 1994).

In the northern part of Nigeria (Africa), Gwaska as it is called by the Hausas, the leaf decoction with natron is taken for the treatment of sexually transmitted disease, as an abortifacient agent and is also used in the treatment of leprosy (Jinju, 1990). The aqueous leaf extract of this plant is also used to cure cancer of the blood and mentally related sickness (Watt and Breyer Brandwijk, 1963; Dalziel, 1999; Jinju, 1990). Consequently, we decided to screen the leaf part of this plant with a view to validate the folkloric claim.

MATERIALS AND METHODS

Plant materials

Fresh plant material *E. africanum* was obtained from Nimbia forest in Sanga Local Government Area of Kaduna State, Nigeria. The plant was identified at the Herbarium Biological Science Department, Ahmadu Bello University Zaria, Nigeria. Voucher specimen was kept with No. 1047.

Extraction

The dried powdered leaf (500 g) of *E. africanum* was macerated with 96% ethanol four times for 6 h each at room temperature. The alcoholic extract was concentrated *in vacuo* to afford (47.8 g). This

was suspended in water and then partitioned successively with petroleum ether (3 × 2 L), chloroform (4 × 2 L), ethylacetate (2 × 1.5 L) and n-butanol (5 × 2 L). The partitioned portion of the extracts were concentrated using rotary evaporator to afford petroleum ether (2.542 g), chloroform soluble (4.834 g), ethylacetate soluble (2.113 g), n-butanol soluble (6.811 g) and aqueous residue (4.201 g). These extracts were subjected to phytochemical screening (Table 1) using standard protocols (Sofowora, 2008; Trease and Evans, 2002; Brain and Tuner, 1975). All the extracts were stored at room temperature until required.

Test microorganisms

The test microorganisms were isolates obtained from the Medical Microbiology Department, Ahmadu Bello University Teaching Hospital, Zaria. These include *Mithicillin R. staph.*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus vulgaris*, *Candida albicans*, *Candida krusei* and *Candida tropicalis*.

Antimicrobial assay

The minimum inhibitory concentration of the various leaf extracts against the test microorganisms were determined using broth dilution techniques (Vollkova et al., 2001; Sidney et al., 1978). Mueller-Hinton agar broth was prepared according to manufacturer's instruction using 10 ml of the broth to be dispensed into test tubes. The broth was sterilized at 121°C for 15 min after which the broth was allowed to cool. Test microbes were inoculated and incubated at 37°C for 6 h.

Mcfarland's turbidity scale number 0.5 was prepared to give a turbid suspension of the microorganisms. Dilution of the test microbes was done in normal saline until the turbidity matches with that of the Mcfarland's scale by visual comparison (concentration of about 1.5×10^8 cfu/ml). Two fold serial dilutions of the extract were made in the sterile broth to obtain the concentrations of 60, 30, 15, 7.5 and 3.25 mg/ml, respectively. The initial concentration was obtained by dissolving 0.6 g of the extract in the sterile broth. 0.1 ml of each test microbe in the normal saline was then inoculated into the different concentrations. After incubation at 37°C for 24 h, each test tube was observed for turbidity. The lowest concentration of the extract for which no turbidity was recorded was the minimum inhibition concentration (Osadebe and Okueze, 2004; Cowan, 1991).

MBC/MFC

The minimum bactericidal and fungicidal concentration of the extracts was determined (Table 4). Mueller-Hinton agar was prepared and sterilized at 121°C for 15 min poured into sterile petri dishes and allowed to cool and solidify. The content of the MIC in the serial dilution was then sub-cultured onto the medium. Incubation was made at 37°C for 24 h, after which the plates were observed for colony growth. The lowest concentrations of the extracts for which no microbial growth was observed were registered as the minimum bactericidal or minimum fungicidal concentration (Ogbulie et al., 2007).

RESULTS AND DISCUSSION

The sensitivity test or solvent used for the reconstitution of different extracts was carried out. It was observed that

Table 1. Phytochemical screening of the ethanolic leaf extract of *Erythrophleum africanum*.

Constituent	Test	Observation	Inference
Carbohydrate			
General test	Molisch	Red colour	+
Monosaccharide	Barfoed's	Red. ppt	+
Reducing sugar	Fehlings	Red. ppt	+
Tannins	Lead ethanoate	White ppt	+
	Methanol's	Red. ppt	+
	Iron (III) chloride	Blue-black	+
Saponins	Frothing	Persist frothing	+
Sterols	Liebermann, Burchad	Blue-green	+
	Salkowski	Red ring at interphase	+
Terpenoids	Liebermann, Burchard	Brown ring with brown interphase	+
Alkaloids	Dragendoff's	-	+
	Mayer's	-	-
	Wagner's	-	+
Flavonoids	Shinoda	-	+
	Ferric chloride	-	+
	Sulphuric acid	-	+
Cardiac glycoside	Keller-Kilanis	Reddish brown	+
	Legal's	Deep red colour	+

different concentrations of the extracts had different responses against the tested isolates. Increased concentrations of the extracts tend to increase the zone of growth inhibition of the sensitive micro-organisms (Table 3). The extract was found not to inhibit the growth of *S. feacalis*, *S. typhi* and *C. krusei*, respectively. The highest zone of growth inhibition of 36 mm diameter was exhibited by *S. aureus* while the lowest zone of growth inhibition was observed for *P. vulgaris* (12 mm diameter).

Results obtained in this study indicated that the various extracts of *E. africanum* inhibited the growth of some of the micro-organism except *S. feacalis*, *S. typhi* and *C. krusei*. This therefore shows that the extract contains substance(s) that can inhibit the growth of some micro-organisms. Other workers have also shown that the extracts of plants inhibited the growth of various micro-organisms at different concentrations (Akujobi et al., 2004; Nweze et al., 2004; Ntiejumokwu and Alemika, 1991; 2004; Osadebe and Ukwueze, 2004). The observed antimicrobial effects on the microorganism could be attributed to the presence of saponins, glycosides, flavonoids, tannins, alkaloids and terpenoids which have been shown to possess antimicrobial activities (Draughon, 2004; Cowan, 1999). The presence

of these metabolites suggests great potential for the use of the studied plant as a source of phytomedicines. The presence of alkaloids in plants is known for decreasing blood pressure and balancing the nervous system in case of mental illness. The presence of tannins could also show that it is an effective astringent, that is, helps in wound healing and as anti-parasitic. The presence of terpenes suggest its possible use as an anti-tumor and anti-viral agent as some terpenes are known to be cytotoxic to tumor cells, while the saponins in plants are believed to have antioxidant, anti cancer, anti-inflammatory and anti-viral agent (Ronan et al., 2009; Yoshida et al., 1990; Balas and Agarwal, 1980).

The large zone of inhibition exhibited by the plant extract against *Methicillin R. staph aureus*, *S. aureus*, *P. aeruginosa*, *E. coli* and *P. vulgaris* justified its use by the traditional medical practitioners in the treatment of sores, boils, open wounds, sexually transmitted diseases and in the treatment of dysentery (Table 2). *S. aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds (Braude et al., 1982). The MIC exhibited by the plant extract against *S. aureus* is of great significance in the health care delivery system, since it could be used as an alternative to antibiotics in the treatment of

Table 2. Zone of inhibition obtained from the microorganisms.

Test organism	n- hex (mm)	CHCl ₃ (mm)	EtoAc (mm)	n- BuOH (mm)	Aq (mm)
<i>Mithicilin R. Staph. aureus</i>	17	27	25	18	20
<i>Staphylococcus aureus</i>	18	25	24	36	21
<i>Streptococcus feacalis</i>	-	-	-	-	-
<i>Escherichia coli</i>	19	20	21	28	22
<i>Pseudomonas aeruginosa</i>	15	24	22	29	19
<i>Salmonella typhi</i>	-	-	-	-	-
<i>Proteus vulgaris</i>	12	25	22	25	18
<i>Candida albicans</i>	17	24	22	25	20
<i>Candida krusei</i>	-	-	-	-	-

n-hex, n-hexane; CHCl₃, chloroform; EtoAc, ethylacetate; n-BuOH, n-Butanol; Aq, aqueous.

Table 3. Minimum inhibitory concentration of the extracts against test micro organism.

Test-organism	n-Hexane (mg/ml)					CHCl ₃ (mg/ml)					EtoAc (mg/ml)					n-BuoH (mg/ml)					Aq (mg/ml)				
	60	30	15	7.5	3.25	60	30	15	7.5	3.25	60	30	15	7.5	3.25	60	30	15	7.5	3.25	60	30	15	7.5	3.25
<i>Mithicilin R. staph. aureus</i>	-	-	OX	+	++	-	-	-	OX	+	-	-	-	OX	+	-	-	OX	+	++	-	-	-	OX	+
<i>S. aureus</i>	-	-	OX	+	++	-	-	-	OX	+	-	-	-	OX	+	-	-	OX	+	++	-	OX	+	-	++
<i>S. feacalis</i>			Nil					Nil					Nil					Nil					Nil		
<i>P. aeruginosa</i>	-	-	OX	+	++	-	-	-	OX	+	-	-	-	OX	+	-	-	-	-	OX	-	-	-	OX	+
(-) <i>E. coli</i>	-	OX	+	++	+++	-	OX	+	++	+++	OX	-	+	++	+++	-	-	OX	+	++	-	OX	+	++	+++
<i>S. typhi</i>			Nil					Nil					Nil					Nil					Nil		
<i>P. vulgaris</i>	-	-	OX	+	++	-	-	-	OX	+	-	-	-	OX	+	-	-	OX	+	++	-	-	OX	+	++
<i>C. albicans</i>	-	-	OX	+	++	-	-	-	OX	+	OX	-	+	++	+++	-	-	OX	+	++	-	OX	+	++	+++
<i>C. krusei</i>			Nil					Nil					Nil					Nil					Nil		
<i>C. tropicalis</i>	-	-	OX	+	++	-	-	OX	+	++	-	-	OX	+	++	-	-	-	OX	+	-	OX	+	++	+++

n-hex, n-hexane; CHCl₃, chloroform; EtoAc, ethylacetate; n - BuOH, n-Butanol; Aq, aqueous; ox, MIC.

infections caused by this microbe, especially as it frequently develops resistance to known antibiotics (Singleton, 1999). Their use will also reduce the cost of obtaining health care delivery. The inability of the extract to inhibit *S. typhi* may be

due to a bacterial mechanism for detoxifying the active principle contained in the extract. Some bacteria are known to possess mechanisms by which they convert substances that inhibit their growth to non-toxic compounds (Singleton, 1999;

Srinivasan et al., 2001). Therefore, the observed antimicrobial properties of this plant *E. africanum* corroborate its use in folkloric medicine.

Traditionally, extract from this plant is used in sore and wound dressing, treatment of boils,

Table 4. Minimum bactericidal/fungicidal concentration of the extracts against test micro organisms.

Test-organism	n-Hexane (mg/ml)					CHCl ₃ (mg/ml)					EtoAc (mg/ml)					n-BuoH (mg/ml)					Aq (mg/ml)				
	60	30	15	7.5	3.25	60	30	15	7.5	3.25	60	30	15	7.5	3.25	60	30	15	7.5	3.25	60	30	15	7.5	3.25
<i>M. R. staph. aureus</i>	Ox	+	++	+++	++++	-	-	Ox	+	++	Ox	+	++	+++	++++	Ox	+	++	+++	+++	Ox	+	++	+++	++++
<i>S. aureus</i>	Ox	+	++	+++	++++	-	Ox	+			-	Ox	+	++	+++	Ox	+	++	+++	+++	-	Ox	+	++	+++
<i>S. faecalis</i>			NIL					Nil					Nil					Nil					Nil		
<i>P. aeruginosa</i>	Ox	+	++	+++	++++	Ox	+	++	+++	++++	Ox	+	++	+++	++++	-	Ox	+	++	+++	-	Ox	+	++	+++
(-) <i>E. coli</i>	Ox	+	++	+++	++++	Ox	+	++	+++	++++	Ox	+	++	+++	++++	Ox	+	++	+++	++++					
<i>S. typhi</i>			Nil					Nil					Nil					Nil					Nil		
<i>P. vulgaris</i>	Ox	+	++	+++	++++	-	Ox	+	++	+++	Ox	+	++	+++	++++	Ox	+	++	+++	++++	Ox	+	++	+++	++++
<i>C. albicans</i>	Ox	+	++	+++	++++	-	Ox	+	++	+++	Ox	+	++	+++	++++	Ox	+	++	+++	++++	Ox	+	++	+++	++++
<i>C. krusei</i>			Nil					Nil					Nil					Nil					Nil		
<i>C. tropicalis</i>	Ox	+	++	+++	++++	-	Ox	+	++	+++	Ox	+	++	+++	++++	Ox	+	++	+++	++++	Ox	+	++	+++	++++

n-hex, n-Hexane; CHCl₃, chloroform; EtoAc, ethylacetate; n - BuOH, n-Butanol; Aq, aqueous; Nil, absent; ox, MIC; -, no turbidity (no growth); +, turbid (light growth); ++, moderate turbidity, +++, high turbidity; +++++, extremely high turbidity.

sexually transmitted diseases, dysentery and also in the treatment of cancer.

Conclusion

The leaf extract of *E. africanum* provides a promising solution in the ethno medicine practice of disease control. This can hence be used to replace the synthetic antibiotics used in the treatment of related ailments as phytochemicals from natural resources are generally considered safer, available and affordable compared to the synthetic drugs in the treatments of infectious diseases.

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