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RESEARCH PAPER

THE PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EFFECTS OF STEM BARK EXTRACTS OF *BRACHYSTEGIA EURYCOMA* HARMS

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ABSTRACT

Plant based antimicrobials represent a vast potential of untapped sources of medicines. Antimicrobial sensitivity patterns change over time due to resistance developed by microorganisms, underpinning the great need for search of novel antimicrobial drugs. Phytochemical and antibacterial effects of crude aqueous (hot and cold) and alcohol extracts of stem bark of *Brachystegia eurycoma* was investigated using standard methods. The preliminary phytochemical screening revealed the presence of saponins, alkaloids, steroids and tannins as major components. Also, of the three different extracts tested against four pathogenic bacteria (*Sphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris*), only the cold aqueous extract showed a mild zone of inhibition (3mm) against *Escherichia coli*, with an MIC of 12.5mg/ml and MBC of 25mg/ml. This suggests that cold aqueous extract of *B. Eurycoma* has antibacterial activity, which might account for its inclusion in traditional herbal preparations in the treatment of wounds and infections.

Key words: *Brachystegia eurycoma*, phytochemical, stem bark, antibacterial, extracts

INTRODUCTION

Plants are the source of some very useful drugs (Parfitt, 1978). It is estimated that plant materials have provided the models for about 50% of orthodox drugs (Sofowora, 1993). Several investigations have been conducted on medicinal properties of herbs, trees and shrubs; good examples include the isolation of cardiac glycosides (digoxin) from *Digitalis purpurea*, quinine from cinchona bark, reserpine from *Rauolfia spp (serpentina and vomitoria)*, physostigmine or eserine from *Physostigma venenosum* (the Calabar bean), anticancer taxols from *Taxus spp*, the spindle poisons (vincristine and congeners from *Catharanthus roseus* -Vinca or Madagascar Rose periwinkle), which are used in the management of leukaemia and Hodgkin's disease (Reis and Lipp, 1982). By the early nineties, screening work on African medicinal plants has advanced with publications arising from the following research areas: antimicrobial (16%), molluscicidal (11%), antimalarial (7%), plant toxicology (7%), antitumour-related studies (4%) and others (54%) (Sofowora, 1993). Indeed, many studies have revealed the antimicrobial potency of a variety of plants and plant products like *Allium sativum* (Block, 1985); *Acapyphaterta* (Akinyanji *et al*., 1986); Nutmeg -*Myristica frangrans*, Kola nut- *Kola nitida* (Njoje, 1997) and Japanese green tea (Thomas, 2001). Similarly, *Aloe vera* has antimicrobial effects that is useful in the treatment of burns and also aids the healing

process (Fajimi *et al.,* 2004), while Dogon-yaro *(Azadiractha indica)* has analgesic and antibacterial properties (Obadoni and Ochuko, 2001).

Available literature have shown that various parts of the tree of *Brachystegia eurycoma* known as Achi (in Igbo), Akalado or Eku (in Yoruba), Akpakpa or Taura (in Hausa), Apaupan (in Ijaw), Okweri (in Edo), Okung (in Efik) and Okwen, "Ukpantoton or Odukpa (in Ibibio) are used as food additives and as medicine. Among the Igbos of South East, Nigeria, the seed is used as thickening agent for soup and as a flavoring agent (Enwere, 1998). The seed is a good source of nutrients and rich in carbohydrate and fiber. It is known to control body temperature, softens stool, and protects against colon and rectal cancer (Ndukwu, 2009). Its blood sugar and cholesterol lowering effects and the ability to lower the risk of heart diseases has been reported (Okwu, 2004). The stem bark is reported to have diuretic effects and anti-inflammatory activities, which makes it useful in some gynaecological conditions such as premenstrual syndrome and uterine fibroids (Adikwu and Nwosu, 1998).

Further investigations have also shown that *Brachystegia eurycoma* has antifungal activities and in combination with snail mucin and honey in native treatment of wound and can prevent scar formation, as well as promote the regeneration of hair follicles (Adikwu and Enebeke, 2007). The red liquid gum of the stem bark is used as binding substance in pharmaceutical industry (Ikegwu *et al.,* 2010). Extracts of *B. eurycoma* inhibited the growth and celluloytic activity of *Bacillus subtilis* (Beguin, 1990). Adekunle (2000) reported that water extracts of *B. eurycoma* has antifungal activities against various species of fungi; but the ground powder quickly became contaminated and yielded *Aspergillus* species. Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are teratogenic, mutagenic and carcinogenic (hepatic carcinoma), and have been associated with growth retardation, underweight and modification of immune function in West African children (Gong *et al., 2002*).

Johnny *et al.* (2014) tested the potency of ethanolic extracts of *B. eurycoma* seed against some pathogenic bacteria such as *Bacillus subtillis, Pseudomonas aeruginosa, Shigella spp, Escherichia coli* and found that all the organisms tested were sensitive to the undiluted crude extract and compared favorably with ciprofloxacin. Okenwa and Echeme (2013) showed that naphthalene pentenoic acid from ethanol extract of the stem bark of *B. eurycoma* has marked antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus fecalis.* In addition, *Brachystegia eurycoma* contain B vitamins (thiamin, riboflavin, and niacin), ascorbic acid as well as minerals such as calcium, potassium, sodium, phosphorus, magnesium, zinc, iron, and copper- a confirmation of its nutritional values (Okwu, 2004; Bolanle *et al.,* 2014).

In addition, the phytochemical analysis of seeds and stem bark of *B. eurucoma* revealed tannins, terpenes, flavonoids, saponins, phenols, cardiac glycosides and antioxidants (Igwe and Okwu, 2013a,b; Okwu and Okoro , 2006; Johnny *et al.,* 2014). Anthraquinones and phlobatannins were not detected in the seed extracts (Johnny *et al*., 2014). The presence of the antioxidant phytochemicals may underlie the basis for the use of the plant in the treatment/management of tissue inflammation, arthritis, wounds, cancer, artherosclerosis and other cardiovascular disorders (Igwe and Okwu, 2013).

Regardless of the fact that fungi were the original sources of very potent antibacterial drugs, plant based antimicrobials represent a vast potential of untapped sources of medicines and antimicrobial sensitivity patterns change over time, as micro organisms constantly develop resistance to existing antimicrobial drugs. This worrisome trend makes the continuous search for new and more efficacious antimicrobial agents imperative (Hesing, 2001). The last decade witnessed an increase in the investigation of plants as a source of drugs for treatment of disease for plant, animals and man (Aiyelagabe, 2000). Overcoming antimicrobial resistance is therefore a major challenge in this millennium (WHO, 2002). This study therefore, was designed to analyze the phytochemical contents of stem bark of *Brachystegia eurycoma* and determine the antibacterial activities of its various crude extracts (aqueous – hot/cold and alcohol) on selected bacterial pathogens (*Staphylococcus aureus, Escherichia coli, Pseudomonas eurugenosa, Proteus vulgaris)*

MATERIALS AND METHODS

Collection and preparation of *Brachystegia eurycoma*: Fresh stem bark of *Braschystegia eurycoma* was obtained from Umuaku Isuochi, Umunneochi Local Government Area, Abia State, South East, Nigeria, and identified by the Taxonomist at the Herbarium in the Department of Botatny, Ambrose Alli University, Ekpoma. Voucher specimen number AAUBH00115. The peeled bark of *B. Eurycoma* was sun-dried and weighed daily for three (3) weeks, until a constant weight was obtained. Inside a fume cupboard, the chopped pieces of the dried bark were then pulverized to powder using clean sterile ceramic mortar and pestle, which had been sterilized by flaming. The *B. eurycoma*

powder was sieved (0.23µm) to remove larger particles and then stored in airtight glass containers protected from direct sunlight until required for use.

Experimental procedure: The extraction, phytochemical analysis and antibacterial sensitivity were carried out at CDR Laboratory, an accredited/ registered medical laboratory (MLSCN RF 918) at Ekpoma, South-South, Nigeria using standard procedures; the antibacterial sensitivity tests used was agar diffusion according to the methods of Schwalbe *et al*. (2007). Sterile distilled water was used as negative control while ciprofloxacin and tetracycline served as positive controls.

Crude extraction: One gram (1g) each of *B. eurycoma* powder was placed into three different sterile universal bottles (labelled A, B, C), each with 10ml of cold distilled water, hot water (80^oC), and 70% ethanol respectively and shaken every 30minutes for three hours in each case. The samples were allowed to stand for twenty four (24) hours, after which they were filtered using Whatman's No 1 filter paper. The filtrates were appropriately labeled and allowed to evaporate to dryness in the Gallenkemp oven drier at 40^oC. The labeled extracts were stored in the refrigerator at 4^0C until used for this experiment.

Phytochemical analysis: Qualitative phytochemical test involved the simple chemical test to detect the secondary metabolites using standard method of Trease and Evans (2009). For each of the cold water, hot water, and ethanol extracts powder of *B. Eurycoma* qualitative phytochemical screening was determined for the presence of: tannins, saponins, cardiac glycosides, steroids, alkaloids and flavonoids, One gram (1g) of the powder was subjected to qualitative phytochemical tests for Alkaloid **(**Mayer reagent); Tannins **(**$FeCl_3$**)**; Saponins (chloroform and H_2SO_4**);** Cardiac glycosides **(**glacial acetic acid + $FeCl_3$ + H_2SO_4.**);** Steroid **(**chloroform + acetic anhydride + Conc. H_2SO_4**)**; and Flavonoid **(**5ml of Ammonia solution + H_2O). Total phenolic content was estimated spectrophotometrically using Folin Ciocalteu reagent, as described by Spanos and Wrolstad (1990), with slight modification using Gallic acid as a standard.

Antibacterial Sensitivity Test: For the antibacterial sensitivity tests, agar diffusion and broth dilution methods were used according to Schwalbe *et al*., 2007. 5.6g of nutrient agar was weighed and diluted with 200ml of sterile distilled water, mixed and sterilized at 21^oC for 15minutes, allowed to cool and then poured into different sterile Petri dishes to solidify. The Nutrient agar plates were dried and each was inoculated with the bacterial isolates (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Proteus vulgaris*), isolated from urine samples of patients with urinary tract infections, using standard procedure. Different concentrations of the various extracts (cold water, hot water, alcohol) were absorbed on standard filter paper discs and applied on the inoculated isolates along with control discs (sterile distilled water, ciprofloxacin and tetracycline). All the cultures were incubated for 24hours at 37^o C. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) respectively of the cold water extract of *B. eurycoma* were determined according to the method of Schwalbe *et al*., 2007.

RESULTS

The qualitative screening results showed the presence of moderate quantities (++) of tannins, saponins, phenols and flavonoids, and low concentrations (+) of alkaloid, cardiac glycosides and steroids (Table 1).

Table 1: Phytochemistry of stem bark of *B. eurycoma*

Tannins	Saponin	Flavonoid	Cardiac Glycoside	Alkaloid	Steroids	Phenol
++	++	++	+	+	+	++

Of all the preparations of *B. eurycoma*, only the cold aqueous extract showed significant zone of inhibition for *E. coli.* Hot aqueous and alcohol extracts showed no zone of inhibition. All the bacterial organisms tested (*Staphylococcus aureus, Escherichia coli, Pseudomonas eurugenosa, and Proteus vulgaris*) were inhibited by ciprofloxacin, while *Staphylococcus aureus* and *Escherichia coli* were inhibited by tetracycline. Cold aqueous extract and tetracycline showed the same zone of inhibition to *Escherichia coli*. The negative control containing sterile distilled water showed no zone of inhibition (Tables 2 and 3)

Specifically, table 2 below shows the sensitivity of the organisms and diameters of zones of inhibition in millimeters (mm) by the various extracts of *Brachystegia eurycoma*, and the controls- sterile distilled water (placebo) and the antibiotics - ciprofloxacin and tetracycline. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of cold aqueous extract of *B. eurycoma* on *Escherichia coli* as shown in table 3, was 12.5mg/ml and was 25mg/ml respectively.

Table 2: Sensitivity and diameters of zones of inhibition

Test Organism	Extracts of *Brachystegia eurycoma* (100mg/ml)			Ciprofloxacin (1mg/ml)	Tetracycline(3 mg/ml)
	Hot Water	Cold water	Alcohol		
Staphylococus aureus	0	0	0	16	12
Escherichia coli	0	3	0	12	2
Pseudomonas. aeruginosa	0	0	0	18	12
Proteus vulgaris	0	0	0	5	3

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Extract conc. (mg/ml)	100	50	25	12.5	6.25	3.125
MIC Inhibition	+	+	+	+	-	
MBC Inhibition	+	+	+	-	-	-

Key: + Growth; - No growth

DISCUSSION:

Phytochemical screening helps to assess the chemical constituents of plant extracts. It may also be used to search for bioactive agents that could be used in the synthesis and formulation of drugs (Yakubu *et al*., 2005). Phytochemical screening of the stem bark of *Braschystegia eurycoma* revealed the presence of tannins, saponins, flavonoids and phenols, as the major phytochemical components (Table 1). Alkaloids, steroids and cardiac glycosides content were moderate. These findings are similar to that of Igwe and Okwu (2013).

On the other hand, studies have shown that flavonoids in many plant parts have multiple biological activities, including antioxidant (Steffan, *et al.*, 2005, Cushnie and Lamb, 2005**),** vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial (Ao *et al.*, 2008**)**, anti-allergic, antiviral, estrogenic and immune system stimulating effects **(**Cowan, 1999**)**. Thus, the detection of moderate quantity of flavonoids in the stem bark of *B. eurycoma* confers both nutritional and medicinal value on the plant. Similarly, the presence of saponin in the stem bark of *B. eruycoma* may also account for the results of this study as saponin possess antibacterial (Rebeiro *et al*., 1995) and antifungal properties (Escalate *et al*., 2002, Joyce *et al*., 2007).

Other secondary metabolites in *B. eurycoma* included alkaloids, tannins and phenols. A review of the literature shows that alkaloids are plant bases that exhibit certain physiological properties when used in herbal medicine. Most of them have anti-malarial, antifungal and antimicrobial activities (Scalbert, 1991). Tannins in plants are known to improve healing of ulcers and burns, and also known for their antioxidant and antimicrobial properties. It possesses astringent properties and is thought to act as inhibitors of oxidative phosphorylation and electron transport (by depletion of iron); thus depriving bacteria of iron (Scalbert, 1991). In addition, *B. eurycoma* contains phenol and this phytochemical is known to possess anti-inflammatory and antimicrobial properties (Joyce *et al*., 2007)**.**

Indeed, there is an ever increasing need for newer antibacterial agents on account of development of bacterial resistance to the existing ones. *B. eurycoma* is a plant used in trado-medical practice in the treatment of sexually transmitted diseases, purportedly for its antibacterial effects. The observed inhibition of *Esch. coli* in this study has justified its use in the trado-medical circle, as the cold aqueous extracts showed a 3mm zone of inhibition against *Escherichia coli* in a disc agar plate (Table 2); slightly more than the 2mm zone shown by tetracycline, but far less than the 12mm zone of inhibition shown by ciprofloxacin - both used as positive controls. It can be said that with the MIC of 12.5mg/ml and MBC of 25mg/ml and 3mm zone of inhibition, the cold water extract of *B. eurycoma* could be more effective than tetracycline, but less so for ciprofloxacin; bearing in mind the pharmacokinetic influences and its potential toxicities, which was not part of the design of this particular study. This finding is similar to that of Okenwa and Echeme (2013), who showed that 4-(4-phenyl-,4-dihydronaphthalen-1yl) Pentenoic Acid from the Stem Bark of *Brachystegia eurycoma* has antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus fecalis*; further justifying the use of the extracts of the plant in traditional medical practice for the treatment of gonorrhea and other infections, including healing of wounds. However, the hot aqueous and alcohol extracts of *B. eurycoma* did not show any zone of inhibition (Table 2). The phytochemicals could have been retained in the cold aqueous extract and could be heat sensitive and hence destroyed in the hot water extract. It could also be that some of the active principle of the plant did not dissolve in the solvents used in the present study (Ellof, 1998).

Concerning the inhibition against *Escherichia coli*, the antibacterial effects of *Brachystegia eurycoma* could be attributed to the presence of chemical substances such as resins, flavonoids, taninins and phenols which are known to inhibit the growth of bacteria (Obadoni and Ochuko, 2001) and act by inhibiting DNA synthesis, cytoplasmic membrane function as well as inhibition of energy metabolism in bacteria (Cowan, 1999**,** Cushnie, 2005). Furthermore, the organisms that did not show zones of inhibition (*Pseudomonas aeruginosa, Proteus vulgaris and Staphylococcus aureus*) and were adjudged resistant are known to be resistant to multiple antibiotics by various mechanisms; even though they were sensitive to the standard antibiotics in this work. One or more of these mechanisms may be in operation in the present study for the hot aqueous and alcohol extracts of *Brachystegia eurycoma. E. coli*, the organism sensitive to the cold aqueous extract may not have acquired the resistant factor(s).

In conclusion, the cold aqueous extract of the bark of *Brachystegia eurycoma* had antibacterial activity against *Escherichia coli* and may be used against infections caused by this organism if further toxicological and pharmacokinetic studies confirm it to be safe. The findings have also justified its use in trado-medical practice in the treatment of infections and for the healing of wounds. It is our recommendation therefore, that further studies using more bacterial organisms be carried out to ascertain the antibacterial spectrum of cold aqueous extract of *Brachystegia eurycoma*.

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AUTHOR'S CONTRIBUTIONS

All the authors played significant roles towards the successful completion of this study, including the revision of the manuscript. No conflict of interest is declared.