

# Phytochemical Evaluation and Anti-bacterial Activity of *Brachystegia eurycoma* Harms. Ethanolic Seed Extract.

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## ABSTRACT

*Ethanolic seed extract of Brachystegia eurycoma was investigated in the laboratory for its phytochemical properties and anti-bacterial activities. Standard procedures were employed in this study. The results of quantitative and qualitative phytochemical analysis of the seeds revealed the presence of tannins (0.32±0.87), alkaloids (0.87±0.24), flavonoids (1.18±1.28), cardiac glycosides (1.67±0.01) and saponins (1.46±1.02). Anthraquinones and phlobatannins were not detected in the seeds extract. The anti-bacterial potency of the extract was tested against some pathogenic bacteria which include Bacillus subtilis, Pseudomonas aeruginosa, Shigella spp. and Escherichia coli. All the organisms tested were sensitive to the undiluted crude extract and 150mg/ml extract concentration. Three of the organisms: Shigella spp., Bacillus subtilis and E. coli were sensitive to the extract at the concentration of 100mg/ml while Pseudomonas aeruginosa was resistant. Again, all the tested organisms were resistant to the extract at the concentration of 50mg/ml. The anti-bacterial activity of the seed extract compared favourably with the reference drug (ciprofloxacin) used as a standard. This research work proved the ethnobotanical claims on the anti-bacterial potency of the seed in herbal therapy is therefore supported.*

## Keywords:

*Brachystegia eurycoma*; Phytochemicals; Anti-bacterial; Organisms; Extract; Seeds

## INTRODUCTION

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Jigna and Sumitra, 2007) and antibiotics resistance has become a global concern thus, there is a need to look for substances from other sources with proven antibacterial activities. Though several investigations on anti-bacterial properties of Nigerian herbs, trees and shrubs have been conducted (Sofowora, 1993; Adekaku *et al.* 2001, Abdelrahim *et al.* 2002; Aguna *et al.* 2003, Alenika *et al.* 2004; Idowu *et al.* 2005 and Odoemena *et al.* 2005, 2007). Information on the phytochemistry and bioassay of majority of the plants are still scarce. The universal role of plants in the treatment of diseases is exemplified by their employment in all major systems of medicine irrespective of the underlying philosophical premises (Trease and Evans, 2002). Since the origin of human race, man relied amongst the surrounding formed by plants, he found things he needed in these natural surroundings, over years, man came across many such plants which were either directly fulfilled one of the important needs, having discovered by the utility of these natural resources his life came to heavily depend on plants (Ashok and Pande, 2007).

The use of herbal remedy as anti-bacterial regimen in Nigerian traditional homes is gaining prominence and is on the increase due to the resistant bacteria strains. Natural anti-biotics have been a part of

African traditional medicine since time immemorial, and its continual efficacy suggests that micro-organisms such as bacteria have not been able to develop effective resistance against them (Idu, 2007). Studies on African Medicinal plants for antibacterial activities rank highest among all biological test carried-out in plants because of the increase resistance. It is interesting to note that most plants extract have been reported to possess inhibitory growth and lethal activities on some pathogenic microorganisms in-vitro (Aghna *et al.* 2003, Dogon *et al.* 2006).

*Brachystegia eurycoma* is a tropical tree commonly known as Okwen, “Ukpantoton or Odukpa” in Ibibio, “Achi” in Igbo and “Akalodo” in Yoruba, South-eastern and south-southern Nigeria (Iwu, 1980, Etukudo, 2003, Sofowora, 2006). *B. eurycoma* belongs to the family Caesalpiniaceae (Hutchinson and Dalziel, 1980). Okwen is a dicotyledons plant classified as a legume. It flowers between April and May and its fruiting period is between September and January (Keay *et al.*, 1986). The fruits are very conspicuous and persistently woody. The tree is vaguely buttressed with low-branching, large and flat crown. The bark is yellowish grey to grey in colour and often shallowly scaly with lenticels and horizontal folds. The bark exudes gums and translucent-whitish resin, as well as a good source of fibre (Burkill, 2002). *B. eurycoma* seeds are edible as they are used as a soup thickener just like the melon seeds. The seed produces oil; the leaves are excellent browsing material for sheep, cattle and goats (Etukudo, 2003). It is one of the legumes which have not been fully utilized to alleviate the problem of protein-energy malnutrition common in developing countries. The seed is a good source of dietary fibre, it has high carbohydrate content which is quite significant to health (Okwu and Jasiah, 2006).

*Brachystegia eurycoma* has the lowering effects on blood glucose level and blood cholesterol due to its fibre content (Daniel *et al.*, 2000). Diet supplementation for diabetes, oxidative and anticancer properties was reported (Pederson *et al.*, 1980). The plant has the ability to lower the risk of heart

diseases and provides anti-inflammatory activity (Okwu, 2004). The stem bark of *B. eurycoma* has diuretic effect and is one of the polyherbal drugs in the treatment of some complicated gynecological cases such as fibroids (Adikwu, 2007 and Nwosu, 1998).

The practice of herbal medicine in Modernised form is now gaining momentum in Nigeria with many health officials appreciating the potency of some of the indigenous plants. These have been an upsurge in the interest in herbal remedies in several part of Nigeria with many of the herbs being incorporated into orthodox medicine practice. *Brachystegia eurycoma* has been widely known as soup thickener and also been claimed to have anti-bacterial effects by local medicine practitioners, hence this study is expected to provide concrete information on its usage as an anti-bacterial regimen and also to verify the claims of its herbal efficacy.

## MATERIALS AND METHODS

### Source and Collections of Seeds

The mature dry seeds of *Brachystegia eurycoma* were obtained from its plantation in Mbak Etoi in Uyo Local Government Area, Akwa Ibom State in November, 2009. The plant seeds were authenticated and identified by Dr. (Mrs.) U. A. Esseit, a plant taxonomist in Department of Botany and Ecology Studies, University of Uyo, Uyo.

### Seed Treatment/Extraction

The seeds were roasted in oven at 80°C for 10-15 minutes, and then soaked immediately for 1 hour in cold distilled water to remove the seed coat. The cotyledons were soaked overnight in distilled water. The water was drained off and the cotyledons were sun-dried and ground into fine coarse powder using electric blender. Three hundred and fifty-two (352g) grams of the powdered samples were macerated in 1000ml of 70% ethanol for 72 hours. The liquid filtrate obtained was concentrated in-vacuo at 40°C. The yield was 78.6g (Sofowora, 2006). The extract was stored in a refrigerator at -40°C until used for this experiment.

### Phytochemical Screening

The experiment was carried-out in Botany and Ecological Studies, Microbiology, Pharmacognosy and Natural Medicine Departments, University of Uyo, Uyo. The phytochemical test involved the simple chemical test to detect the secondary metabolites using standard method of Trease and Evans (2009) and Sofowora (2006).

### Anti-bacterial Analysis

Stock cultures of *Shigella* spp., *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* were obtained and confirmed at University of Uyo Health Centre, Uyo. The cultures were checked for its purity and maintained on nutrient infusion agar and stored at 4<sup>0</sup>C (Inyang and Adegobe, 2008).

### Anti-bacterial Sensitivity Test

The anti-bacterial activity test of the seed extract was carried-out using the agar-disc (6mm in diameter) diffusion method (Prescott *et al.*, 2008). The labeled plates were seeded with the test organisms. The crude ethanol extract at different concentration (150mg/ml, 100mg/ml and 50mg/ml). Six (6mm) filter paper discs were impregnated with various concentrations of the extract; anti-biotic (ciprofloxacin) was used as a reference drug which served as control. The plates were then incubated at 37<sup>0</sup>C for 24hours. Thereafter, the diameter of the zone

of inhibition were measured in millimeters (mm). The zone was determined by the area of growth around the sensitivity disc and subtracting from the diameter of the disc (Inyang and Adegobe, 2008).

### RESULTS

The results of quantitative and qualitative phytochemical analysis of the ethanolic seed extract of *Brachystegia eurycoma* revealed the presence of alkaloids (0.87±0.24), saponins (1.46±1.02), flavanoids (1.18±1.28), cardiac glycosides (1.67±0.01). Anthraquinones and phlobatanins were not detected in the seed extract (Table 1).

The anti-bacterial activity of the seed extract on four (4) bacterial species (*Escherichia coli*, *Shigella* spp., *Pseudomonas aeruginosa* and *Bacillus subtilis*) in this study showed inhibitory and non-inhibitory growth. All the bacterial species were totally non-sensitive to the concentration of 50mg/ml. *Pseudomonas aeruginosa* was non-sensitive at the concentration of 100mg/ml. The four tested organisms showed inhibitory effects on concentration of 150mg/ml. *Shigella* spp., *Bacillus subtilis* and *Escherichia coli* were sensitive to the extract at 100mg/ml concentration. Similarly, all the crude undiluted extract compared favorably with the reference drug. Each of the four tested organisms showed various zones of inhibitions when treated with reference drug (ciprofloxacin) as standard control (Table 2).

**Table 1: Quantitative/Qualitative phytochemical analysis of *Brachystegia eurycoma* seeds**

S/N	Test	Qualitative Analysis	Quantitative Analysis
1	Alkaloids	+++	0.87±0.24
2	Saponins	+++	1.46±1.20
3	Tannis	++	0.32±0.81
4	Phlobatannins	ND	ND
5	Flavonoids	+++	1.18±1.28
6	Anthraquinones	ND	ND
7	Cardiac glycosides	+++	1.67±0.01

Legend: ++ = Moderate, +++ = Abundant, ND = Not Detected.

**Table 2: Result of Anti-bacterial of Pour bacterial species on ethanolic seed extract of *B. eurycoma*.**

Test Organisms	Concentration/Zone of Inhibition				
	150mg/ml	100mg/ml	50mg/ml	Undiluted Stock Extract	CP
<i>Bacillus subtilis</i>	20mm	18mm	NZI	22mm	25mm
<i>Escherichia coli</i>	20mm	15mm	NZI	25mm	30mm
<i>Shigella spp.</i>	15mm	10mm	NZI	23mm	25mm
<i>Pseudomonas arruginosa</i>	13mm	NZI	NZI	15mm	18mm

**Legend:** CP = Ciprofloxacin, NZI = No Zone of Inhibition

#### DISCUSSION

The pronounced presence of alkaloids, saponins, flavonoids, tannins and cardiac glycosides in the seed extract of *Brachystegia eurycoma* clearly indicates the phyto-therapeutic potentials of this plant. The qualitative and quantitative phytochemical evaluation of bioactive chemical compounds of the seed extract previously reported by Uhegbu *et al.* (2009) is in-line with the findings of this study.

The in-vitro anti-bacterial activity of the ethanolic seed extract against pathogenic organisms showed a remarkable inhibitory effect. The presence of alkaloids in the seed extract may be connected with the anti-bacterial efficacy, this is in agreement with the work of Odoemena *et al.* (2008). Flavonoids occurring in the seed extract in abundant have biological properties and could be used as anti-bacterial agent, this is ascertained with the report of Xu and Lee (2001) who studied the anti-bacterial activity of *Argemonve mexicana*. Tannins cause astringency and also interfere with digestion and utilization of foods (Odoemena, 2010; Okon and Umoh, 2013), it was also reported by Adesina *et al.* (2001) and Ajibesin (2005) to exhibit anti-bacterial activities. Thus, the seed of *Brachystegia eurycoma* could be used as a regimen for bacterial infections.

The susceptibility of the test organisms to the seed extract at higher concentration of undiluted crude extract is found to be dose dependent. The high sensitivity activity by the extract on the bacterial isolates may be either due to the presence of alkaloids, flavonoids, tannins, saponins and glycosides. Although previous report on *Rauvolfia serpentine* implicated alkaloids as being responsible for the anti-bacterial activity of the root (Ahmed *et al.*, 2002). The variation of the degree of anti-bacterial activities of the seed extract in this study is presumed to be due to different levels of active compounds presents in the seed of *B. eurycoma*. Some workers have also attributed the anti-bacterial effects of plant extracts to the presence of these secondary metabolites (Nweze *et al.* 2004 and Ogueke *et al.*, 2006).

#### CONCLUSION

The findings in this study have shown the effectiveness of the extract of *Brachystegia eurycoma* on bacteria, thereby justifying its culinary and therapeutic purposes. The ethnobotanical claims on the anti-bacterial potency of the seed in herbal therapy are supported by this study. These findings could also be of commercial interest to both pharmaceutical industries and the Nigeria Raw

Materials Research Institute in the production of new novel drugs.

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