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# Phytochemical Screening and Antibacterial Evaluation of the Leaves Extracts of *Olea hochstetteri* Bak. (Oleaceae)

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**Abstract:** Phytochemical and antibacterial properties of *Olea hochstetteri* crude aqueous and ethanol leaf extracts were evaluated. The extracts were subjected to qualitative chemical analysis for identification of various classes of active chemical compounds. Disc diffusion method was used to determine the antibacterial properties of the extracts on some gram positive and gram negative bacteria. The extracts showed the presence of carbohydrates, tannins, saponins, glycosides, flavonoids, terpenes and steroids. The extract inhibited the growth of *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Salmonella typhi* and to some extent *Klebsiella pneumonia* but had no effect on the growth of *Bacillus* sp., *Shigella* sp. and *Escherichia coli*. The study revealed some antibacterial properties of this extracts that supported the use of the leaves of this plant in folklore medicine.

**Key words:** Phytochemistry, antibacterial activity, aqueous/ethanol extracts, *Olea hochstetteri* Bak., folklore medicine, Nigeria

## INTRODUCTION

Medicines from plants have given hope to man in the quest for treating old and emerging diseases that has defied many orthodox drugs. Plant medicines are considered safer and better than synthetic drugs, since the ingredients in plants such as carbohydrates, fats, proteins, vitamins and minerals are also of body composition (Kilham, 1999).

Approximately, 119 pure chemical substances extracted from higher plants are used in medicine throughout the world (Farnsworth *et al*., 1985). According to the World Health Organization, about 80% of the world population relies on the use of traditional medicine which is predominantly based on plant materials (WHO, 1993). It is estimated that approximately one quarter of prescribed drugs contains plant extracts or active ingredients obtained from or molded on plant substances (Tripathi and Tripathi, 2003). Several of these drugs are in extensive clinical use (Sofowora, 1982; Roja and Rao, 2000). Of the estimated 300,000 plant species acclaimed worldwide, only about 5% have been investigated scientifically for medicinal properties (Rabo and Sanusi, 2001). The African continent alone has over 5000 different plant species, many of which have been found to be useful in traditional medicine for prophylaxis and cure of diseases (Iwu, 1993). *Olea hochstetteri* Bak. belong to the family Oleaceae (Hutchingson and Dalziel, 1958). It is a small tree with dark green leathery leaves and thick, smooth, greyish bark that is found in Nigeria and many parts of Africa (Keay *et al*., 1964). The plant has been listed by the Food and Agricultural Organisation (FAO) among the plants of ethnomedicinal importance used by the Mukogodo Maasi people of Kenya (Ngethe *et al*., 1998). It is also among the plants used by traditional healers of Morogoro region of Tanzania in the treatment of non-insulin dependent diabetes mellitus (Moshi and Mbwambo, 2002). In Nigeria, especially in the North-

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Eastern part of the country, the plant is widely used as a traditional remedy for the treatment of febrile illnesses, wound dressing and diseases of unknown etiologies. However, the claim for the traditional use of this plant has not been scientifically validated.

The objective of this study was to determine the phytochemical constituents and evaluate the antibacterial properties of the crude and ethanol leaf extracts of *O. hochstetteri* Bak.

## MATERIALS AND METHODS

**Plant collection and identification:** Fresh leaves of *O. hochstetteri* Bak. were collected in September 2008 from Mafa town in Borno State of Nigeria. They were identified and authenticated by a taxonomist at the Department of Biological Sciences, University of Maiduguri, Nigeria. A voucher specimen (Species Vet. 206A) was deposited at the University's herbarium for reference.

**Preparation of aqueous extract:** The collected fresh leaves were air-dried, crushed and pulverized into fine powder and stored in a glass container at 4°C. About 250 g of the powdered sample was exhaustively extracted with distilled water using reflux method (Trease and Evans, 1989). The crude aqueous extract was then concentrated *in vacuo* until a brown colored extract weighing 163 g w/w was obtained. It was labelled and refrigerated at 4°C.

**Fractionation of the aqueous extract:** The crude aqueous extract obtained was suspended in cold distilled water and then filtered using Whatman No. 1 filter paper. The filtrate was thereafter subjected to fractionation using ethanol. The methods of Cho *et al.* (2003) and Motohashi *et al.* (2004) were used for the fractionation of the aqueous extract.

**Phytochemical analysis:** The crude aqueous and ethanol extracts of *O. hochstetteri* Bak. were subjected to qualitative chemical screening for identification of various classes of active chemical constituents. The phytochemical analysis was done according to standard methods (Trease and Evans, 1997).

**Preparation of bacterial cultures:** Laboratory isolates of pure cultures of some gram positive (*Staphylococcus aureus*, *Bacillus*) and gram negative (*Salmonella typhi*, *Pseudomonas aeroginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Shigella*) organisms obtained from the Department of Veterinary Medicine Laboratory, University of Maiduguri, Nigeria were used. The bacterial cultures were prepared according to standard methods as described by Geidam *et al.* (2007).

**Preparation of stock solutions of extracts:** Different stock solutions of the aqueous and ethanol extracts at the concentrations of 100, 200, 400 and 800 mg $mL^{-1}$ were prepared as described by Geidam *et al.* (2007). Standard antibacterial agent (Gentamycin) at a concentration of 250 mg $mL^{-1}$ was also used on all the bacterial cultures and its zone of inhibition compared with those of the extracts.

**Antibacterial sensitivity testing:** Discs containing different concentrations of dissolved extracts (100, 200, 400 and 800 mg $mL^{-1}$) were prepared with sterilized filter papers (Whatman No. 1; 6 mm in diameter) soaked in beakers containing extracts solutions at the concentrations of 100, 200, 400 and 800 mg $mL^{-1}$, respectively. The discs were dried at 50°C. The disc diffusion method as described by the National Committee of Clinical Laboratory Standards (1993) was used to determine the antimicrobial activity of the extracts and gentamycin as the positive control. Plates without the extracts or antibiotic disc were set up as negative control. Zone of inhibition above 6 mm diameter in each isolate was used as a measure of susceptibility to the extracts and this was compared to that of the standard antibiotic.

**Determination of Minimal Inhibitory Concentration (MIC):** The MIC of aqueous leaves extract of *O. hochstetteri* Bak. was determined using the method of Greenwood (1989) as described by Geidam *et al.* (2007). Serial dilution of the extract was done to obtain solution of different concentrations (50, 25, 12.5 and 6.25 mg $mL^{-1}$) which were used to determine the MIC.

The MIC was recorded as the least concentration of the extract that completely inhibited the growth of the test organisms. The contents of the tubes were further sub-cultured for 24 h to determine bactericidal or bacteriostatic activity. Bactericidal effect was demonstrated when no growth occurred on the sub-culture medium after MIC determination.

## RESULTS AND DISCUSSION

The phytochemical screening of aqueous and ethanol leaves extracts of *Olea hochstetteri* Bak. revealed that the two extracts contain similar chemical constituents. Carbohydrates, tannins, saponins, glycosides, terpenes and flavonoids were shown in the extracts (Table 1). There was notable absence of alkaloids and anthraquinones in the extracts. Inhibition of bacterial

growth with ethanol extract was observed on *S. aureus*, a gram positive organism and some gram negative organisms including *Salmonella typhi*, *Pseudomonas aeroginosa* and *Klebsiella pneumoniae*. *Klebsiella pneumoniae* was only inhibited by 200, 400 and 800 mg $mL^{-1}$ of ethanol extract. Similar result was obtained with aqueous extract of the plant (Table 2). The inhibition of bacteria by the ethanol and aqueous extracts appear to be concentration and organism dependent. *Salmonella typhi* was inhibited more by the extracts when compared to the other organisms used in this study. The extracts did not inhibit the growth of *Bacillus*, *Shigella* and *Escherichia coli*. Gentamycin (Standard antibacterial agent) inhibited the growth of all the organisms used in this study. The zones of inhibition produced by gentamycin on the organisms were far greater than those produced by the different extract concentrations.

The MIC of the aqueous leaves extract of *O. hochstetteri* Bak. for the three selected bacterial organisms tested is presented in Table 3. *Staphylococcus aureus* was found to be most sensitive to the extract since their growth was inhibited at a relatively lower concentration (25 mg $mL^{-1}$) than the other organisms. *Pseudomonas aeroginosa* and *Salmonella typhi* were both inhibited at concentrations of the leaves extract above 25 mg $mL^{-1}$. There was no growth of the tested bacteria following sub-culture of the contents of the tubes above the MIC. The phytochemical investigation of leaf extracts of *Olea hochstetteri* Bak. revealed the presence of useful chemical constituents such as tannins, saponins, flavonoids and glycosides. According to Villasenor *et al.* (1998) and Cho *et al.* (2003) medicinal plants usually contain many types of chemical compounds and that their biological activity are not attributable to a single compound. Tannins are diverse organic compounds with various compositions that have pronounced physiological astringent properties that hasten the healing of wounds and reduce diarrhoea (Tyler *et al.*, 1988; Yu *et al.*, 2000). It also suppresses bacterial cell proliferation by blocking key enzymes of microbial metabolism. Saponins lower the surface tension and possess emulsifying activities. They alter the

Table 1: Qualitative phytochemistry of aqueous and ethanol leaves extract of *Olea hochstetteri* Bak.

| | | Results | |
|---|---|---|---|
| Phytochemical constituents | Type of test | Aqueous extract | Ethanol extract |
| Carbohydrate | Molisch's | + | + |
| | Barfoed's | - | - |
| | Free reducing sugar | + | + |
| | Combined reducing sugar | - | - |
| | Ketones | + | + |
| | Pentoses | + | + |
| Tannins | Ferric chloride | + | + |
| | Formaldehyde | + | + |
| | Chlorogenic | - | - |
| Anthraquinones | Free anthraquinones | - | - |
| | Combined anthraquinones | - | - |
| Saponins | Frothing | + | + |
| Glycosides | General test | + | + |
| Terpenes and steroids | Lieberman – Buchard's | + | + |
| | Salkwoski's | - | - |
| Flavonoids | Lead acetate | + | + |
| | Sodium chloride | - | - |
| | Ferric chloride | + | + |
| | Pew | + | + |
| Alkaloids | Dragendorff's | - | - |
| | Mayer's | - | - |

(-) Not detected; (+) Detected

Table 2: Antibacterial efficacy of ethanol and aqueous extracts of *Olea hochstetteri* leaves

| | | Antibacterial activity (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Extract/ antibiotic | Conc. (mg $mL^{-1}$) | *P. aeroginosa* | *S. aureus* | *K. pneumonia* | *S. typhi* | *Bacillus* sp. | *Shigella* sp. | *E. coli* |
| Ethanol | 800 | 10 | 12.5 | 11 | 16 | R | R | R |
| | 400 | 9 | 10.0 | 9 | 15 | R | R | R |
| | 200 | 8 | 9.0 | 8 | 11 | R | R | R |
| | 100 | 7 | 8.0 | R | 10 | R | R | R |
| Aqueous | 800 | 13 | 11.0 | 10 | 17 | R | R | R |
| | 400 | 10 | 10.0 | 8 | 16 | R | R | R |
| | 200 | 9 | 10.0 | 7 | 12 | R | R | R |
| | 100 | 8 | 9.0 | R | 11 | R | R | R |
| Gentamycin | 250 | 23 | 13.0 | 14 | 20 | 16 | 19 | 20 |

R: indicates bacterial resistance

Table 3: Minimum Inhibitory Concentration (MIC) of leaf extracts of *Olea hochstetteri* Bak. against some bacterial organisms

| | Concentration of aqueous extract (mg mL$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Organisms | 100 | 50 | 25 | 12.5 | 6.25 |
| *P. aeroginosa* | - | - | + | + | + |
| *S. aureus* | - | - | - | + | + |
| *S. typhi* | - | - | + | + | + |

permeability of the cell wall and hence exert a general toxicity on all organized tissues. They are also known to have some antibacterial activity. Birk and Petri (1980) observed that saponins combine with cell membrane sterole to produce changes in cell morphology leading to lysis.

Flavonoids have been reported by many researchers to have potential beneficial effects on health. Several studies have shown biological and pharmacological properties of flavonoids such as their antibacterial activity (Narayana *et al*., 2001), anti-oxidant (Su *et al*., 2000), anti-tumor (Castillo *et al*., 1989) and antidiarrhoel (Rao *et al*., 2005) effects. Glycosides are known to exert pronounced physiological effects as well as antiseptic properties (Robinson, 1967; Frantisek, 1991). The present study shows that the aqueous and ethanol leaves extracts of *Olea hochstetteri* Bak. have inhibitory activity against some bacterial (*Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae*) organisms. The inhibition of the growth of these organisms *in vitro* by the extracts may be due to the presence of some active constituents in the extracts. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The activity of the extracts against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeroginosa* is very interesting since it is a known fact that these organisms are difficult to treat in clinical settings in the developing countries including Nigeria.

## CONCLUSION

In this study, some antibacterial properties of the extracts that justify the use of the plant in traditional medicine . However, further studies need to be carried out.

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