EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 3
90-94
Year: 2019

FOLIAR EPIDERMAL MICROSCOPY, PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL ANALYSIS OF THE LEAVES AND STEM BARK OF *ARTHOCARPUS HETEROPHYLLUS* LAM. (FAMILY: MORACEAE) GROWN IN NIGERIA

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Article Received on 01/01/2019 **Article Revised on 22/01/2019** **Article Accepted on 12/02/2019**

ABSTRACT
The Foliar Epidermal Microscopy, Preliminary Phytochemical screening and Anti-microbial screening of the leaves and stem bark of *Arthocarpus heterophyllus* Lam. were carried out using standard methods. The leaf epidermal microscopy revealed numerous anomocytic stomata type on the lower epidermis while the upper layer had no stomata. The cell walls on the upper surface were wavier than on the lower surface and those of the lower surface had knobs. Trichomes were not observed on lower surface but had trichome base on the upper surface. The preliminary phytochemical screening on the leaf and stem bark revealed the presence of flavonoids, saponins, alkaloids, steroids, terpenes and anthraquinones and absence of tannins and phlobatanins in the leaves but present in small quantity in the stem bark. Antimicrobial screening carried out on 2.0mg/ml of the 70% methanol extract of the leaves and stem bark by cold maceration at room temperature (25 -30^{o}C) for 24hrs were screened against standard strains of *Salmonella paratyphi* (ATCC 69150), *Candida albicans* (ATCC 22015) and clinical isolates of *Pseudomonas aeroginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsiella pneumoniae* from NIPRD clinic using agar dilution method. At the concentration of 2.0mg/ml, the stem bark extract exhibited anti-microbial activity against *Salmonella typhi* (ATCC 69150), *Pseudomonas aureginosa, Candida albicans* (ATCC 22015) and *Escherichia coli* while the leaf extract had no activity against all the test micro-organisms. Amoxicillin, which was used as standard, inhibited the growth of all the test micro-organisms at the same concentration. The results from the study provide finger print for the identification and anti-microbial activities of the 70% methanol extracts of the leaves and stem bark of *Arthocarpus heterophyllus*.

KEYWORDS: Microscopy, Phytochemical screening, Anti-microbial, Micro-organisms and Stomata.

INTRODUCTION
The genus *Arthocarpus* (Moraceae) consists of approximately 50 species and is widely distributed in Nigeria. Members of the genus have been used medicinally to treat various diseases (Heyne, 1987; Perry, 1980). Different compounds isolated from some species of *Arthocarpus* have been shown to exhibit interesting biological properties (Nomura *et al*., 1998). Achmad*et al* 1996; Hakim *et al*., 1999; Makmur *et al*., 2000; Hakim *et al*., 2000; Syah *et al*., 2001; Suhartata *et al*., 2001 and Ersam *et al*., 2002 have studied the phenolic constituents of several species of the Indonesian *Artocarpus* and isolated a number of flavonoids with prenyl groups, several of which have been shown to exhibit cytotoxic activities. Euis *et al*., 2002 reported the isolation of prenylated stilbene, artoindonesianin N and prenylated arylbenzofuran artoindonesianin O, together with a known stilbene, oxyresveratrol from the dichloromethane extract of the tree bark of *Arthocarpus gomeziaanus*. In this paper, we report the foliar epidermal microscopy, preliminary phytochemical screening and anti-microbial analyses of the leaves and stem bark of *Arthocarpus heterophyllus* from Nigeria.

MATERIALS AND METHODS
Foliar epidermal microscopy
The dried leaf was used for the microscopy and the method used by Ugbabe and Ayodele (2008) was adopted. About 5mm-1cm squared leaf fragments were obtained from the standard median portion of the leaf

and macerated in concentrated nitric acid in petri-dish for a period of 24 hrs. The appearance of bubbles on the surface of the leaf fragment indicated their suitability for separation. The fragments were transferred into water in a petri-dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling each epidermis back at itself. The leaf epidermises were cleaned with the Carmel hair brush. These were rinsed in distilled water and later transferred into 50% ethanol to harden. They were then stained in Safranin O for 5 minutes and excess stain washed off in water. They were then mounted on glycerin on a slide with the edge of the cover slips ringed with nail vanish to prevent dehydration. The slides were labeled appropriately and examined under the light microscope while photographs were taken using NICON AFX-DX microscope with NICON FX-35DX camera attached at magnifications of x100 and x400.

Phytochemical screening

Plant preparation: The leaves and stem bark of *Arthocarpus heterophyllus* were collected from Imo State on 6th February, 2014. A herbarium specimen (NIPRD/H/6630) was deposited at NIPRD herbarium. The plant leaves and stem bark were dried at room temperature (27 ± 1°C) and then powdered using a mortar and pestle. The powdered samples were used for the phytochemical screening. Screening of the filtrate for possible phytochemicals was carried out as described by Tiwari *et al*., 2011, Harborne, 1998, Trease and Evans, 2002 and Sofowora, 2008.

Anti-microbial screening

Preparation of extracts: The powdered leaf and stem bark were macerated separately using 70% methanol at room temperature (28 ± 2°C) for 24hrs. (Sofowora, 2008). The extracts were filtered and the filtrates concentrated *in vacuo* using a rotary evaporator at 45°C. The crude extracts were kept in sealed containers until required.

Preparation of the test organisms

A wire loop-full (wire loop flamed red hot and cooled) of each test micro-organisms were taken aseptically from their respective slants and sub-cultured into Mac-Cartney bottles containing 5ml of freshly prepared nutrient broth and placed in the incubator for 24 hours at 37°C. The 24 hours culture was sub-cultured using a wire loop-full into freshly prepared nutrient broth and incubated at 37°C for 3 hours (Containing approximately $1.25 \times 10^6 - 1.25 \times 10^7$ colony forming units). This is equivalent to half McFarland standard.

Screening against micro-organisms

The extracts were screened for anti-microbial activity against *Salmonella typhi* (ATCC 69150) and *Candida albicans* (ATCC 22015) and clinical isolates of *Pseudomonas aeroginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsiella pneumoniae* using agar dilution method (Mitscher *et al*, 1972). 0.5 ml of dimethylsulphoxide (DMSO) was used to dissolve the crude extracts and made up to 1ml with sterile water. 1 ml each of the crude extracts (containing 32mg) was introduced into 15ml of molten nutrient agar placed in water at 45°C. These were mixed properly and poured into sterile petri-dishes to give final concentration of 2.0mg/ml. The dishes which were prepared in duplicates and then allowed to gel and thereafter, the test micro-organisms were inoculated by surface streaking onto the nutrient agar using a wire loop. Control dishes were also set containing only agar and test organisms (organism viability control), dishes containing agar and DMSO and plates containing agar and sterile water, also served as controls. The standard amoxicillin, at a final concentration of 2.0mg/ml was treated the same way as the crude extracts. The petri-dishes were incubated over night at 37°C (20 - 24hrs.) after which they were observed for microbial growth inhibition. All procedures were done aseptically in the biosafety cabinet to avoid the introduction of unwanted micro-organisms from the environment.

RESULTS

Foliar Epidermal Microscopy

The leaf epidermal microscopy revealed anomocytic stomata type on the lower epidermis while the upper surface had no stomata. The cell walls on the upper surface are wavier than on the lower surface. Trichome bases were observed on the upper leaf surface of the plant while knobs were observed on the cell walls of the lower surface (Plate 1).

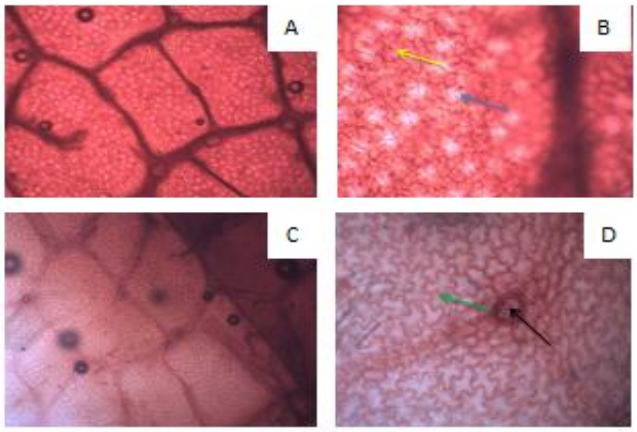

Plate 1: Foliar Epidermal surfaces of *Arthocarpus heterophyllus*
(A) Lower Surface x100: Numerous stomata
(B) Lower Surface x400 : Anomocytic stomata (blue arrow), wavy cell walls with knobs (yellow arrow)
(C) Upper Surface x 100: No stomata and trichome base (black arrow)
(D) Upper Surface x400: No stomata, wavy cell walls (green arrow) and trichome base (black arrow)

Phytochemical screening

The phytochemical screening revealed the presence of flavonoids, saponin, alkaloids, steroids, terpenes and anthraquinones and absence of tannin and phlobatanins in the leaves while all the tested phytochemicals were present at different concentrations in the stem bark (Table 1).

Table 1: Phytochemical screening of the leaf and stem bark of *Arthocarpus heterophyllus*.

Constituent	Leaf Methanol extract (70%)	Stem bark Methanol extract (70%)
Saponin	+++	+++
Tannins	-	+
Phlobatannin	-	+
Flavonoids	+++	+++
Terpenes	+	++
Steroids	++	++
Alkaloids	++	+
Antraquinone	++	++

Key: - = absence; + = presence

The anti-microbial screening using these micro-organisms *Staphilococcus aureus, Salmonella paratyphii, Bacillus subtilis, Pseudomonas aeroginosa, Candida albicans, Klebsiella pneumonia and Escherichia coli* revealed these activities (Table 2).

Table 2: Anti-microbial Screening of the Leaf and Stem bark of *Arthocarpus heterophyllus*.

Parameters	Sa	St	Bs	Ps	Ca	Kp	Ec
Stem bark extract at 2.0mg/ml concentration	-	+	-	+	+	-	+
Leaf extract at 2.0mg/ml concentration	-	-	-	-	-	-	-
Agar (Control)	-	-	-	-	-	-	-
DMSO (Control)	-	-	-	-	-	-	-
Acetone (Control)	-	-	-	-	-	-	-
Amoxicillin at 2.0mg/ml concentration (Std)	+	+	+	+	+	+	+

Key:
Sa = *Staphilococcus aureus*
Sp = *Salmonella paratyphii*
Bs = *Bacillus subtilis*
Ps = *Pseudomonas aeroginosa*
Ca = *Candida albicans*
Kp = *Klebsiella pneumoniae*

Ec = *Escherichia coli*
(+) = Activity
(-) = No activity
(Std) = Standard

DISCUSSION

The epidermal microscopic study revealed the presence of stomata on the lower surface and not on the upper surface (i.e. hypostomatic), showing that gaseous exchange takes place only on the lower surface of the leaf (Plate 1). Knobs were also present on the cell walls of the lower surface. Trichome bases were found on the upper surface of the leaf. Trichomes are single or multi-cellular outgrowths of the epidermis of a plant that form hairs over the plant's surface; these epidermal hairs are in many types of plant specialized in defense against attack by insects and mites. Trichomes are also special part of the plant that produce, hold and secrete essential oils. Trichomes protect plants from pests because the pests do not like the taste or smell of the trichomes. The type, presence and absence and location of trichomes are important diagnostic characters in plant identification and plant taxonomy (Davis and Heywood, 1963). In forensic examination, plants such as *Cannabis sativa* can be identified by microscopic examination of the trichome (United Nations office on Drug and Crime, 2009). Although, trichomes are rarely found preserved in fossils, trichome bases are regularly found and in some cases, their cellular structure is important for identification. Trichomes also serve as models for cell differentiation as well as pattern formation in plants (Hulskamp *et al*., 1999). Plants may use trichomes in order to deter herbivore attacks via physical and/or chemical means, e.g. in specialized, stinging of *Urtica* (Nettle) species that deliver inflammatory chemicals such as histamine. Studies on trichomes have been focused towards crop protection, which is the result of deterring herbivores (Brooks *et al*., 2016). In *Urtica*, the stinging trichomes induce a painful sensation which is a defense mechanism via secretion of metabolites. This involves a rapid release of toxin (such as histamine) upon contact and penetration via the globular tips of said trichomes (Fu, *et al*., 2003).

From the phytochemical screening results (Table 1), the tannins and phlobatannins being present in the stem bark extract may be responsible for the anti-microbial activity observed (Table 2) because they were absent in the leaf extract. Tannins have been reported to disrupt the cytoplasmic membrane, interrupt the PMF (proton motive force), active transport, coagulation of cell substances and electron flow (Burt S., 2004). Tannins can exist in two forms, either as condensed tannins or as hydrolysable tannins. Higher levels of tannins especially condensed tannins and polyphenols when present in plant extracts might be responsible for antibacterial mode of action and anticandidal activity (Sampaio T, *et al*, 2017). Phlobatannins along with other chemical classes has been widely reported in the implication of causing inhibitory action against a wide range of micro-organisms when present in plants (Arekemase M. *et al*, 2011). The anti-microbial results (Table 2) also showed that the nutrient agar used was not contaminated and the micro-organisms were viable. The solvents that were used to dissolve the extracts did not inhibit the growth of all the test micro-organisms. These results clearly indicate that the anti-microbial activities exhibited by the leaves and stem bark of *Arthocarpus heterophyllus* are as observed.

CONCLUSION

The cellular structure of the leaf epidermis is important for identification and standardization of *Arthocarpus heterophyllus* in crude drugs. Flavonoids, saponins, alkaloids, steroids, terpenes, anthraquinones, tannins and phlobatannins were present in the stem bark while tannins and phlobatanins were absent in the leaves of *Arthocarpus heterophyllus.* The leaves did not inhibit the growth of all the 7 test organisms while the stem bark inhibited the growth of 4 of the test organisms used in this study. Tannins and phlobatannins present in the stem bark of *Arthocarpus heterophyllus* may be responsible for the anti-microbial activity observed. From the results of this study, antibiotic agents could be developed from the stem bark of *Arthocarpus heterophyllus* to combat these pathogens.

ACKNOWLEDGEMENT

The authors acknowledge the management of NIPRD, for providing enabling environment for the study. We appreciate the Technologists in the department of Medicinal Plant Research and Traditional Medicine, NIPRD, for their various assistances during the course of the study.

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