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PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF EXTRACT AND FRACTIONS OF *PACHYPODANTHIUM staudtii* (*ANNONACEAE)* ENGL & DIELS

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ABSTRACT

The rapid spread of microbial infections and effort by scientists to identify novel antimicrobial agents from natural products has necessitated further search for antimicrobial agents from medicinal plants of *Annonaceae* family. This study was, therefore, designed to evaluate the phytochemical constituents and antimicrobial activities of *Pachypodanthium staudtii* stem bark used by traditional medicine practitioners to treat infectious disease. The phytochemical test was conducted by standard protocols while the antimicrobial activity was evaluated by agar dilution method. The phytochemical screening revealed the presence of alkaloids, flavonoids, fat and oil, terpenoids, and cardiac glycoside in the bulk extract; terpenoids, fat and oil in the *n*-hexane fraction (PSHF); alkaloids, flavonoids and terpenoids in the ethyl acetate fraction (PSEAF) and alkaloids and cardiac glycoside the in the methanol fraction (PSMF). The minimum inhibitory concentrations (MICs) of extracts and fraction range from 12.5 mg/ml - 100 mg/ml against some selected bacterial strains (ciprofloxacin 2.5 - 5.0 mg/ml) and 12.5 mg/ml - 50 mg/ml against some fungal strains (ketoconazole 1.25 mg/ml) respectively. Interestingly, the overall result of the MICs indicated that the extract and fractions showed good antimicrobial activity comparable to the standard drugs. Our findings justified the folkloric uses of *P. staudtii* stem bark in treating infectious diseases caused by pathogenic microorganisms and therefore, could be a possible a source of new and effective antimicrobial remedy. Further studies are required to isolating and characterize the bioactive constituents for identification of antimicrobial lead compound(s).

KEYWORDS: Antimicrobial activity, minimum inhibitory concentrations, *Pachypodanthium staudtii Phytochemistry*

INTRODUCTION

Medicinal plants are important source of novel chemical structures leading to drugs in all major infectious diseases. These assume a more scientific and wider dimension as the emphasis on ethno-medicine is on the increase especially in the developing countries. The World Health Organization (WHO) reported that over 80 % of world population depends on traditional medicines for their primary healthcare needs and 20 % of the synthetic drugs are based on plants and their derivatives [1]. In the developing countries the use of herbal medicine is attracting attention as a result of resistance posed by synthetic drugs. These synthetic drugs are expensive with numerous side effects. The support for the use of medicinal plants by the WHO is quite encouraging due to their unlimited therapeutic benefits[2]. So there is need to search for new and more potent antimicrobial agents from herbs to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic

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microorganisms [3]. Medicinal plants have curative properties due to presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants[4]. These plant metabolites according to their composition are grouped as alkaloids, glycosides, essential oils, flavonoids, tannins, terpenoids, saponins etc. *Pachypodanthium staudtii* Engl & Diels (*Annonaceae)* is a large forest tree that grows up to 40 cm in height with horizontal branches and long narrow leaves. The stem is straight, cylindrical up to 60 cm in diameter with rough brownish-grey bark[5]. It is widely distributed throughout the West and Central African region[6]. The bark decoction is taken to treat cold, cough and other complaints of respiratory tracts and as purgative, anthelmintics and aphrodisiac[6]. It is also used as mouth wash against toothache and to wash hair to get rid of lice. A paste of pounded bark is applied externally to treat smallpox and measles. The bark is also used to treat tumor, edema, leprosy and gonorrhea[5]. Pulped bark with kola nut is taken against gastrointestinal disorder. This study was initiated with the aim of evaluating the phytochemical constituents and antimicrobial activity of *P. staudtii* stem bark.

MATERIALS & METHODS

Collection of Plant Materials

The stem bark of *P. staudtii* was collected from Uyo, Akwa-Ibom state, Nigeria in October 2013 by Abia Williams Okon of Department of Pharmacognosy University of Uyo. It was identified and authenticated by Mr. Alfred Ozioko of the International Centre for Ethno-medicine and Drug Development (InterCEDD) Nsukka, Enugu State, where a voucher specimen was deposited (specimen number: InterCEDD/1584)

Extraction and Fractionation of plant material

The pulverized stem bark (2 kg) was macerated with methylene chloride:methanol (1:1) for 48 h. The mixture was filtered, and the filtrate concentrated in vacuum to get the crude methylene: methanol extract. A 200 g of the crude extract was fixed on silica gel (60-200 mesh) and subjected to column chromatography using n-hexane, ethyl acetate, and methanol based on increasing polarity of the eluting solvents. Three fractions were obtained and each concentrated in vacuum to afford PSHF (*P. staudtii* hexane fraction), PSEAF (*P. staudtii* ethyl acetate fraction) and PSMF (*P. staudtii* methanol fraction) respectively.

Phytochemical Screening

The extract and fractions were tested for the presence or absence of major secondary metabolites such as alkaloids, flavonoids, tannins, cardiac glycosides, terpenoids, steroids, saponins. These were screened by standard methods described by Soforowa[7], Trease and Evans[8].

Antimicrobial Activity Determination

Twenty-four hours cultures of six human pathogenic bacteria made up of both Gram positive (*S. aureus*, and *B. subtilis*), Gram negative (*P. aeruginosa*, *E. coli* and *S. dysentirae)* bacteria and fungi *(Candida albicans* and *Aspergillus nigger)* were used for the *in-vitro* antimicrobial assay. All microorganisms were obtained from the laboratory stock of the Department of Pharmaceutics, Faculty of Pharmaceutical Sciences University of Nigeria, Nsukka.

Agar dilution method

The minimum inhibitory concentrations (MICs) of the extract and fractions were evaluated by agar dilution method. Thirty-eight g/l of Muller Hilton agar was prepared following manufacturers description by dissolving the required quantity of the powder in distilled water, homogenized by heating until the contents completely goes into solution. The Mueller Hilton agar prepared was distributed in 19 ml portions using Mac Cartney bottle capped with the stopper. It was sterilized in an autoclave at 121^{o}C for 15 minutes. A 200 mg of the extract and fractions were weighed and transferred into sterile test tube, 2 ml of dimethylsulphoxide (DMSO) was added for complete dissolution of the extract and fractions thereby producing a stock solution of 100 mg/ml. From the stock solution, two-fold serial dilutions were carried out to produce 100, 50, 25 and 12.5 mg/ml respectively. The following concentrations were used for standard drugs 10, 5, 2.5 and 1.25 mg/ml for ciprofloxacin and 5, 2.5, 1.25 and 0.625 mg/ml for ketoconazole. The antimicrobial agents from the prepared concentrations were incorporated into the Muller Hilton agar at concentration of 12.5 – 100 mg/ml while the control drugs were 1.25 – 10 mg/ml for ciprofloxacin and 0.625 – 5 mg/ml for ketoconazole respectively. The antimicrobial agents and the Muller Hilton agar were mixed thoroughly and poured into corresponding plates. The plates were allowed to solidify and dried in oven for 30 minutes

at 50°C. The plates were labeled appropriately and each of the test organisms was inoculated on their respective plate previously labeled accordingly before incubation at 37°C for 24 h (bacteria) and 25°C for 48 h (fungi). After the incubation period, the results were recorded by observing for any visible growth. The MICs were taken as the lowest concentration of the antimicrobial agents without visible growth.

RESULT AND DISCUSSION

The results obtained for the yield of crude extract was (465.72 g) whereas each of the fractions yielded PSHF (21.89 g), PSEAF (127.36 g), and PSMF (7.04 g) respectively. Among the fractions obtained, the yield of PSEAF was high compared to others indicating that the major phytochemical constituents of the stem bark were composed of moderately polar compounds (Table 1). The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, fat and oil, cardiac glycosides and terpenoids in the extract whereas terpenoids, fat and oil were present in PSHF. Likewise, alkaloids, flavonoids and terpenoids were present in PSEAF while PSMF contained alkaloids and cardiac glycosides as summarized in (Table 2). The antimicrobial activity determination was evaluated by agar dilution method to determine the minimum inhibitory concentrations of the extract and fractions that inhibited the growth of the test organisms as presented in Figure 1. All the test microorganisms were susceptible to *P. staudtii* extract and fractions though to varying degree. The ethyl acetate fraction showed the highest level of inhibition against *P. aeruginosa, E. coli, B. subtilis, C. albicans and A. nigger.*

Table 1: The yield of crude extract and fractions

Sample	Quantity (g)	Yield (g)	% yield
PSCE	2000	465.72	23.29
PSHF	200	21.89	10.95
PSEAF	200	127.36	63.68
PSMF	200	7.04	3.52

Table 2: Qualitative Phytochemical constituents of Crude Extract and Fractions

Sample	Alkaloids	Flavonoids	Terpenoids	Cardiac glycoside	Fat and oil	Tannins	Saponins	Steroids
PSCE	+++	++	+++	++	++	-	-	-
PSHF	-	-	+++	-	+	-	-	-
PSEAF	+	++	++	-	-	-	-	-
PSMF	++	-	-	+	-	-	-	-

Key: +++ High, ++ Moderate, + Low, - Ni

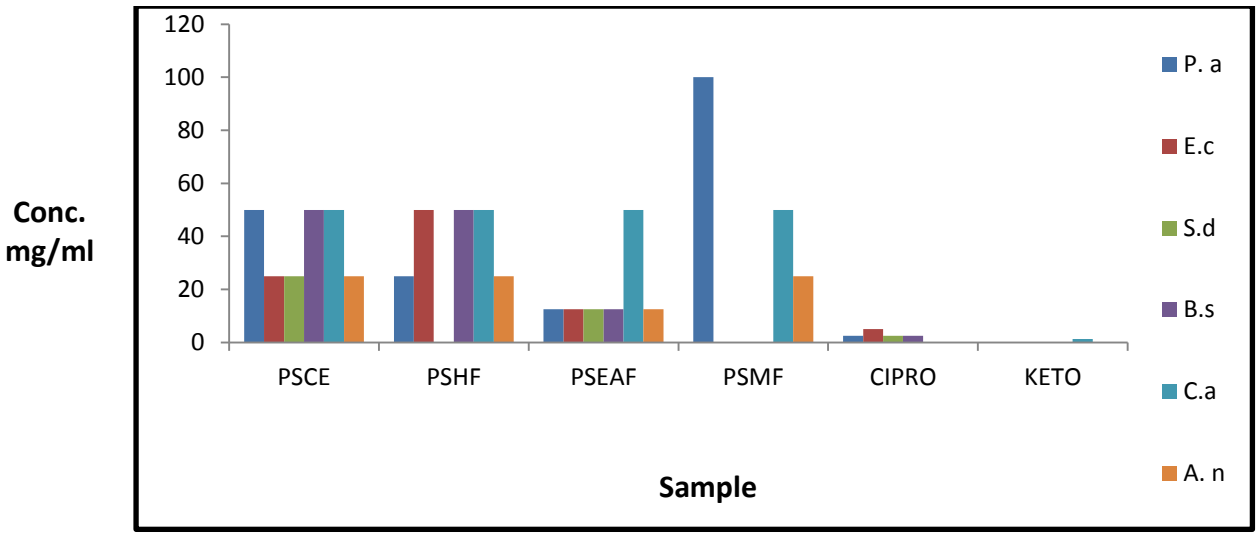

Fig 1: Minimum Inhibitory Concentrations Determination

The yields of the crude extract and some fractions were relatively small which could be as a result of factors like age of the plant and the polarity of the solvent used. The phytochemical screening of the crude extract and fractions of the plant showed that the stem barks were rich in some bioactive components as seen in (table 2). They are known to show medicinal as well as some physiological activities. These phytochemicals also have some strong antimicrobial significance against some enteric pathogens. The minimum inhibitory concentrations were determined as the lowest concentrations of the antimicrobial agents that showed no visible growth of the test organism on the plate (Fig. 1). The total clearance produced by the antimicrobial agent against the test organism is an indication of the potency of the secondary metabolites present in the plant. The PSCE showed MICs at 25 mg/ml for *E. coli, S. dysentriae, A. nigger* and at 50 mg/ml for *P. aeruginosa, C. albicans and B. subtilis* respectively.

The PSHF produced MICs at 25 mg/ml against *A. nigger, P. aeroginosa*, and at 50 mg/ml against *E. coli, B. subtilis and C. albicans*. The MICs produced by PSMF against *P. aeroginosa* was 100 mg/ml, *C. albicans* 50mg/ml, A. nigger 25 mg/ml while others were not inhibited at the tested concentrations. In the case of PSEAF, It showed MIC value at 12.5 mg/ml against *P. aeroginosa, S. dysentriae, B. subtilis, A. nigger* and 50 mg/ml for *C. albicans* and *E. coli*.The standard antimicrobial drugs produced MICs of 2.5 mg/ml against *P. aeroginosa, S. dysentriae, B. subtilis* and 10 mg/ml against *E. coli*. Ketoconazole produced MICs of 1.25 mg/ml against only *C. albicans*. This result indicated that the extract and fractions were more effective against the tested Gram-positive bacteria than against Gram negative bacteria. These findings corroborate some previous reports and could be explained by differences in cell wall architecture of these bacteria. Gram negative bacteria have outer membrane comprising of phospholipids and lipopolysaccharide which act as barrier to the entrance and reaction of most antimicrobial agents the through cell envelope [9, 10]. The results of the MICs indicated that the PSCE and fractions showed good antimicrobial activity compared to the standard drugs. It has been reported that compounds belonging to the class of alkaloids, flavonoids, and terpenoids from *P. staudtii* have shown activity against microorganisms [11]. This showed that the plant contains bioactive substances with proven antimicrobial activities which are extended through different modes of action. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found to be effective *in vitro* antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins of the bacterial cell walls [12]. The presence of terpenoids has been reported to be useful in herbal medicines; thymol, betulinic acid, ursolic acid and β-sitosterol isolated from plants are terpenoids with antimicrobial activity [13 - 18]. The mechanisms of action of terpenes are not fully understood but it is speculated to involve membrane disruption by the lipophilic compound [19]. Thus, the bark of *P. staudtii* is rich in bioactive secondary metabolites with medicinal properties and this supports its traditional use in treating infectious and non-infectious diseases.

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CONCLUSION

This evaluation provided evidences for the use of *P. staudtii* stem bark in treating infectious diseases caused by pathogenic microorganisms. Henceforth, the plant could be a possible source of new and effective antimicrobial compounds. The isolation and elucidation of the bioactive constituents from the plant will further be carried out to identify and develop lead antimicrobial agents.

CONFLICT OF INTEREST

Authors declared no conflict of interests in the conduct and reporting of this study

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