ChemSearch Journal 10(1): 58 – 63, June, 2019
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 08/04/2019 Accepted: 26/06/2019

In vitro Antimicrobial and Radical Scavenging Activities of *Adenanthera pavonina* Stem Bark Fractions

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ABSTRACT

The present investigation deals with the *in vitro* antimicrobial and radical scavenging activities of four different fractions obtained from the stem bark of *Adenanthera pavonina*. Phytochemical screening of the fractions revealed the presence of alkaloids, flavonoids, glycosides, phenol, protein, saponins, steroids, tannins, and terpenoids. Evaluation of the extracts against three different bacterial strains (*Escherichia coli, Salmonella typhimurium and Staphylococcus aureus*) showed that the ethyl acetate fraction (F4) possess the best antimicrobial activity with good zones of inhibition. While antifungal activity against (*Aspagillus flavus, Candida albican and Mucor*) further indicate F4 has the best activity. Lastly, the radical scavenging activity revealed that the F1 (ethanol extract) and F4 (ethyl acetate fraction) demonstrated promising antiradical power on diphenylpicrylhydraxyl (DPPH) with an excellent percentage scavenging effect. At 1000 µg/mL and 10 µg/mL the DPPH activity of the ethyl acetate fraction was 97.9 and 71.3% respectively. While it was observed to be 94.9 and 69.6% for ethanol at 1000 and 10 µg/mL respectively. These values are found to be higher than that of the standard reference (ascorbic acid) which was 96.4 and 58.4 % at 1000 and 10 µg/mL respectively. This shows that the assay for DPPH free radical scavenging activity is concentration dependent. This strengthens part of the ethnomedicinal claims on the plant, *Adenanthera Pavonina* as a curative agent of different diseases of clinical concern.

Keywords: *Adenanthera pavonina*, Alkaloid, Antimicrobial, Ethyl acetate, Phytochemical, Radical

INTRODUCTION

Medicinal plants have been used in virtually all cultures as a source of medicine to treat health disorders and to prevent diseases including epidemics (Rojas, *et al.*, 2003). The knowledge of their healing properties has been transmitted over the centuries within and among human communities. The active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used for various purposes, such as treatment of infectious diseases (Refaz, *et al*., 2017). Hence assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries (Sigh, 2015).

Free radicals are atoms or group of atoms with unpaired electron. They are highly reactive due to their tendency to capture electrons from stable molecule to reach their electrochemical stability thereby causing a large number of diseases including cancer (Partha and Rahaman, 2015 and Kinnula and Crapo, 2004), Cardiovascular diseases (Singh and Jialal, 2006), neural disorders (Sas, *et al*., 2007), Alzheimer's disease (Smith, *et al*., 2000), mild cognitive impairment (Guidi, *et al.*, 2006), Parkinson's disease (Bolton, *et al*., 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna, *et al*., 1997), aging (Hyun, *et al*., 2006) and atherosclerosis (Upston, *et al*., 2003). When the increase in intracellular free radicals exceeds the antioxidant defense, the cell oxidative stress occurs, where by damage to biomolecules such as lipids, proteins and nucleic acid is induced. Protection against free radicals can be enhanced by ample intake of dietary antioxidants (Alam, *et al*., 2013).

Adenanthera pavonina belongs to the family Fabaceae. The scientific name is derived from a combination of two Greek words *aden*, "a gland," and *anthera*, "anther" (Khan and Kanum, 2007). It is a deciduous tree, about 6-15 m tall, erect and up to 45 cm in diameter depending on the location. It is commonly known by English names as coral wood, red sandal wood, red lucky tree, red bead tree, and food tree. It is also known by a common name, Ghirni (Babur/Bura) in the southern part of Borno state, Nigeria.

The aim of this work was to determine the phytochemical constituents, *in vitro* antimicrobial and radical scavenging activities of the different fractions obtained from the stem bark extract of *Adenanthera Pavonina L.*

MATERIALS AND METHODS

Chemicals

All chemicals used in the present work were purchased from Sigma Aldrich and were used without further purification.

Sample Collection and Identification

Fresh stem bark of the plant was collected at Kwaya Bura Village, Hawul Local Government Area, Borno State, Nigeria. The leaves of the plant together with the stem bark was identified and authenticated at the Department of Plant Biology, Bayero University, Kano, Nigeria. A with herbarium accession number of 0493 was given.

Sample Preparation

The stem-bark of *Adenanthera pavonina L.* was cut into smaller pieces and air dried under shade at ambient temperature for three (3) weeks. It was then pulverized mechanically (using mortar and pestle) to form a coarse powder. After pulverization the powdered sample material was stored in an air tight container in cool dry place away from light and was later subjected to ethanol extraction (Rohit, 2015).

Extraction

About 500 g was percolated with 1.5 Liter of absolute ethanol with shaking at regular intervals for one week. After which the extract was separated from the debris by filtration. The filtrate was then concentrated using a rotary evaporator (R110) at 40^{o}C to afford ethanol crude extract (F1). The extract was kept in a cool dry place away from any form of contaminant (Shovon, *et al.*, 2016).

The crude ethanol extract (F1) was further macerated with n-hexane, chloroform and ethyl acetate to yield fractions F2, F3 and F4 respectively.

Phytochemical Screening

The qualitative phytochemical tests of alkaloids, flavonoids, glycosides, phenols proteins, saponins, steroids, terpenoids, and tannins for all the four fractions (F1, F2, F3 and F4) were carried out according to standard protocols (Gurav *et al*., 2014 and Harborne 1998).

Test Organisms

Standard bacterial isolates both gram negative (*Escherichia coli* and *Salmonella typhimurium*) and gram positive (*Staphylococcus aureus*) as well as fungal isolates (*Aspagillus flavus, Candia albican and Mucor specie*) obtained from the Department of Microbiology, Bayero University, Kano, Nigeria, were used.

Preparation of Concentrates

The stock solutions were prepared by dissolving 60 mg of each fraction in 1ml of DMSO to produce 60 mg/ml. From the stock solution, 0.5 ml was diluted with 0.5 ml DMSO to produce 30 mg/ml and 0.5 ml of 30 mg/ml solution was also diluted with 0.5 ml DMSO to give 15 mg/ml.

Antibacterial Screening

Antibacterial activity of the four fractions was carried out using agar well diffusion method (Azoro, 2002 and Chung, *et al.*, 1990). Nutrient agar (NA) and Potatoes dextrose agar (PDA) plates were swabbed (sterile cotton swab) with eight-hours-old broth culture of bacteria and fungi respectively. Using the sterile cork borer three wells of 6 mm each were made into each petri-plate. Different concentrations made from F1, F2, F3 and F4 were used to evaluate their dose dependent activity. They were dissolved and diluted with dimethylsulphoxide (DMSO) and impregnated into the well with the help of a sterile micropipette. Gentamycin, 125 mg/ml was used as standard and the plates were incubated at 37^{o}C for 24 hours. After incubation, the diameter of the zones of inhibition around each well was measured and the values were noted for the eventual antibacterial activity (Albin, *et al.*, 2015).

Antifungal Screening

Antifungal activity of the four fractions was carried out using agar well diffusion method, the same method for the antibacterial activity as described by Azoro, 2002 and Chung, *et al.*, 1990.

Free Radical Scavenging Activity of the Extracts

The free radical scavenging activity of the extracts (F1 and F4) was determined according to the method described by (Mensor, *et al*., 2001). A solution of 30 µg/mL DPPH was used for the assay. It is prepared by dissolving 3 mg of DPPH in 100 ml of methanol in amber bottle covered with aluminium foil paper and kept below 25^{o}C. The Stock solutions were prepared by dissolving 0.02 g (20000 µg) of each in 2 mL of methanol to give 20,000 µg per 2 mL. From the stock solution, concentrations of 1000 µg/mL was prepared by measuring 0.2 mL of the stock solution and then diluted with 1.8 mL of methanol. The concentration of 500 µg/mL was prepared from 1000 µg/mL by measuring 0.5 mL and then diluted with 0.5 mL of methanol. Using this method concentration of 250, 100, 50 and 25 µg/mL were also prepared. The concentration of 10 µg/mL was prepared by measuring 0.4 ml of 25 µg/mL and dilute with 0.6 mL of methanol. A blank standard solutions of each sample (100 µgL) was impregnated into a 96-well micro-plate using a sterile micro pipette in triplicate based on concentration gradient. After impregnation, absorbance of each blank sample solution was calibrated at 518 nm using a micro-plate reader. Standard solution of DPPH (40 µL) was added to the blank solution and allowed to react in complete darkness for 30 minutes at room temperature. The absorbance of the mixture (sample + DPPH) was also measured at same

wavelength using same machine and was converted to percentage inhibition according to equation (1).

$$\%\text{Inhibition} = 100 - \frac{(\text{ABS}_{\text{sample}} - \text{ABS}_{\text{blank})}}{\text{ABS}_{\text{control}}} \times \frac{100}{1} \quad (1)$$

Ascorbic acid was used as positive control and its concentration was prepared in the same manner as that of the sample solutions and were also performed in triplicate too.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of stem bark of *Adenanthera pavonina* uncovered the presence of alkaloids, phenols, flavonoids, sterols, tannins, glycosides among others in different extract fractions. Phytochemical constituents are presented in Table 1.

Table 1: Phytochemical Ingredients of Stem bark fractions of *A. pavonina*

Phytochemicals	F1	F2	F3	F4
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Flavoniods	+	+	-	+
Phenols	+	-	-	+
Protein	+	+	+	-
Saponins	+	-	-	+
Steroid	+	+	+	+
Tannins	+	+	-	+
Triterpenoids	+	+	+	+

(+): present and (-): absent

F1= Crude ethanol fraction, **F2**= n-Hexane fraction, **F3**= Chloroform fraction and **F4**= Ethyl acetate fraction

Previous studies by Hussain, *et al*. (2011), on the ethanol and aqueous extracts of stem bark of *Adenanthera pavonina* uncovered the presence of alkaloid, phenol, flavonoid, sterol, tannin, and glycoside. Evidently, the finding also presents promising antimicrobial and antiradical power of the plant, which is in agreement with the studies conducted by Mujahid, *et al*. (2015) and Hooper *et al.* (2011).

Antimicrobial activity

The antibacterial activity for ethanol crude extracts (F1) and the three other fractions (F2, F3 and F4) displayed promising activity on gram negative bacteria isolates (*Escherichia Coli and Salmonella typhimurium*), gram positive bacterium (*Staphylococcus aureus*) as depicted in Fig. 1.

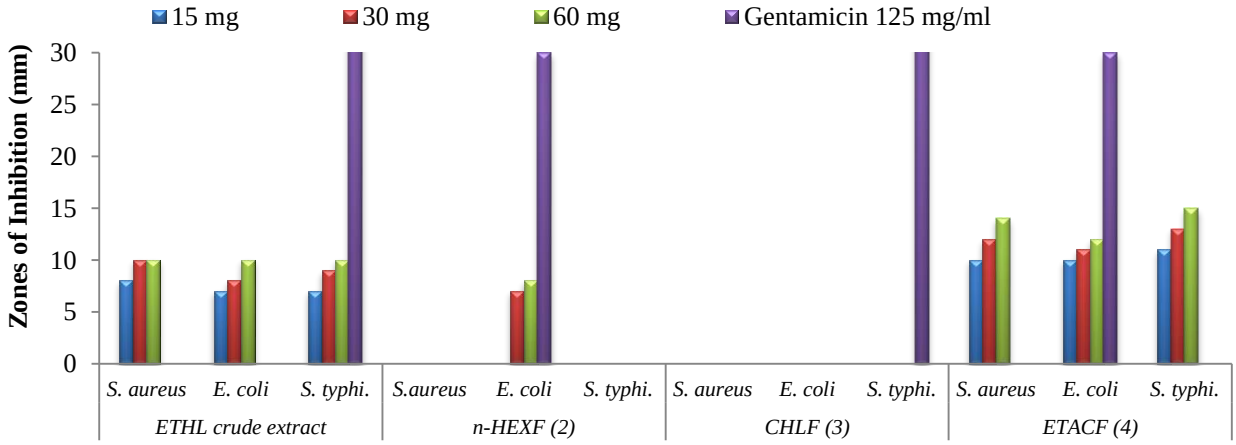

Fig. 1: Antibacterial activity of *Adenanthera pavonina* fractions

The antifungal activity for ethanol crude extracts (F1) and the three other fractions (F2, F3 and F4) displayed promising activity on the fungal isolates (*Aspagillus flavus, Candia albican and Mucor specie*) as depicted in Fig. 2.

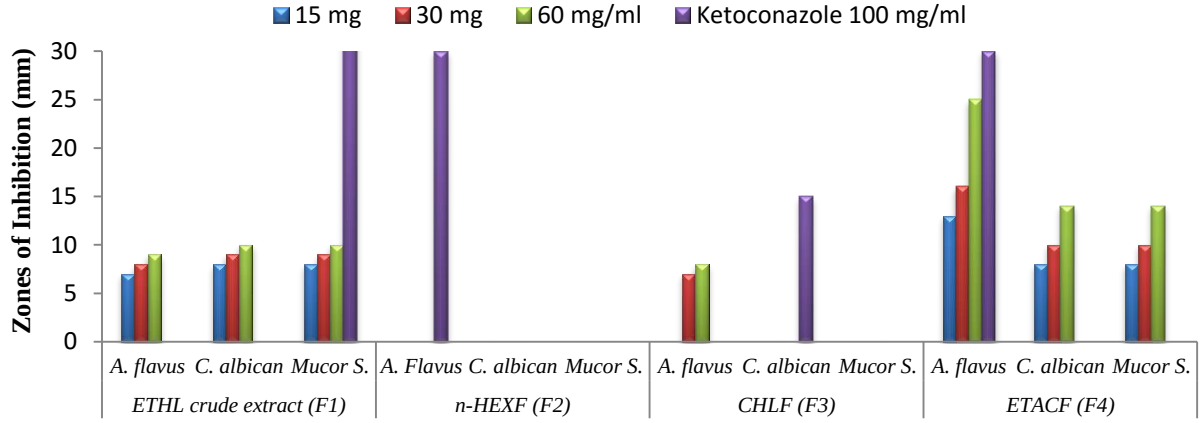

Fig. 2: Antifungal activity of *Adenanthera pavonina* fractions

The ethyl acetate fraction (F4) was observed to be the fraction having the highest antimicrobial effect as can be seen in Figs. 1 and 2.

Previous work on the different extract of *Adenanthera pavonina L.* on the Gram positive and negative bacterial strains showed good activity on *Pseudomonas aeruginosa, Bacillus subtilis, Enterbacter aerogenes, Staphylococcus epidermidis,* and *Salmonella typhimurium* (Parejo, 2003). The overall result showed that *E. aerogenes* was the most sensitive strain and *S. typhimurium* was the most resistance strain. In comparable with the results obtained from the extracts and commercially available standard antibiotics, the inhibitory effects of extracts are even higher than that of standard antibiotics used (Hussain *et al*., 2011).

The antibacterial activity of the *Adenanthera pavonina L.* stem bark extracts collected in petroleum ether, acetone, chloroform and methanol against the 15 bacteria showed apparent zones of inhibition of 21 mm for chloroform extract and 18 mm for methanol extract against *S. typhimurium*, while the same organism developed resistance to the rest of the extracts. Highest zone of inhibition were found to be 17 mm and 16 mm against *S. aureus* and *E. coli* respectively, for acetone extract, while both bacteria showed resistance to the remaining extracts (Abdul *et al.*, 2015).

The antimicrobial activity of the extracts (F1, F2, F3 and F4) showed that, ethyl acetate fraction (F4) was the most promising extract on both gram positive and negative bacterial isolates, as well as fungal isolates used in the study.

In comparison with the studies conducted by Hussain *et al*. (2011) and Albin *et al*. (2015), the antibacterial activity of the stem bark extract (F1 and F4) of *A. pavonina* evidently appears promising and the plant bark has a potential to be regarded as a possible source for new antimicrobial agent against bacteria and fungi of clinical and veterinary concern.

Radical Scavenging Activity

The antiradical potential of the two extracts (F1 and F4) on DPPH were found to be promising with percentage scavenging effects of 97.9 and 71.3 % for ethyl acetate, 94.9 and 69.6 % for ethanol at 1000 and 10 µg/mL respectively. These values are higher than those of ascorbic acid (96.4 and 58.4 %) used as reference standard at same concentration. These findings entails that, assay for free radical scavenging activity is concentration dependent. As can be seen from the Fig. 3.

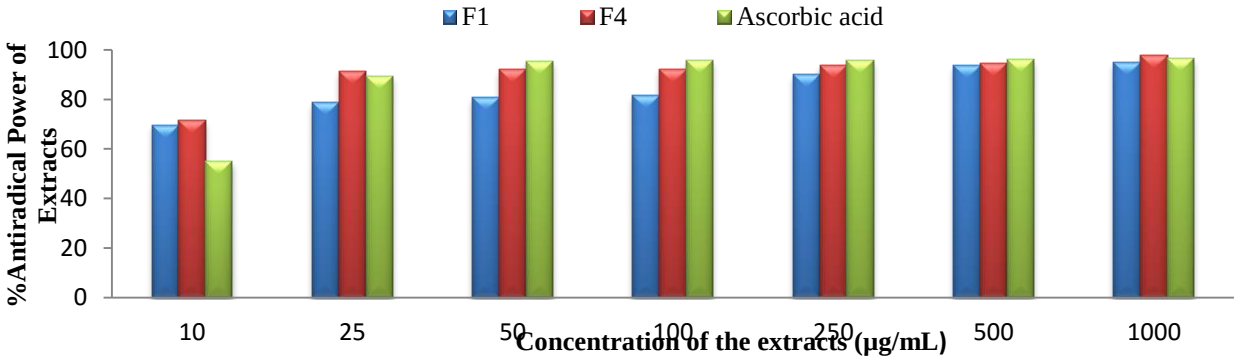

Fig. 3: Percentage radical scavenging activity of Ethanol and ethyl acetate extracts on DPPH

Many synthetic chemicals are toxic and their risk to health has increased the demand for natural antioxidant (Liu *et al.*, 2011). With the antiradical potential of the stem bark of *A. pavonina*, the plant stem bark has the potential to be used as a natural source that can help in the prevention of oxidative stress that occurs in the living body.

The results of the bioactivity and the antiradical studies justify some aspects of the ethno medicinal claims on the plant and could therefore be regarded as a preliminary scientific validation for the use of the plant for antibacterial, antifungal and antioxidant purposes to promote proper conservation and sustainable use.

CONCLUSION

The findings on the present study furnished supportive information on the phytochemical contents, antimicrobial activity and antiradical power of compounds imbedded in *Adenanthera pavonina L.*, which validates the primitive use of the plant as a curative medicine for many diseases.

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