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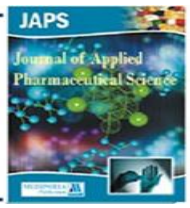

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Bioactive Compounds of the Stem Bark of *Parkia Biglobosa*

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ABSTRACT

Phytochemistry, elemental analysis and Infra red spectroscopy were used in combination to confirm the bioactive compounds encountered in the aqueous fraction of the extract of the stem bark of *Parkia biglobosa*. Bioautography results obtained confirmed the activity of these compounds singly and in combination against *Staphylococcus*. *aureus* and *Pseudomonas aeruginosa* -all organisms of medical and pharmaceutical importance. All results obtained brings us closer to the real identity of these compounds. Work is going on in our laboratory for the structure elucidation of the compounds.

Keywords: Phytochemistry, Bioactive, *Parkia biglobosa*, Pharmaceutical, Elucidation, Compounds.

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INTRODUCTION

Plants are known to have the ability to produce and store a wide range of chemical substances. Most of these substances are secondary metabolites which are organic compounds that are not directly involved in the normal growth and development of plants. In most cases, these compounds serve to defend the plant against attacks from microorganisms and other predators. A lot of them are also responsible for plant flavours (e.g. the terpenoid capsaicin from chili peppers) while others give plants their characteristic odour. Yet, others are responsible for the kind of pigments seen in plants (Cowan, 1999). The antimicrobial properties of plants are traceable to these substances which can be divided into several groups. The major group of plant chemicals widely reported to exhibit antibacterial activity is the phenolic group. They consist basically of a single substituted phenolic ring and the sites and number of substituting hydroxyl groups is believed to explain their toxicity to microorganisms since it has been observed that increased hydroxylation leads to higher toxicity to microorganisms (Cowan 1999). The origin of *Parkia biglobosa* is traced to the West African sub-region where it was first encountered by the Scottish surgeon, Mungo Park as he explored the Niger basin between 1795 –1799. He went ahead to describe this tree in his writing "Travels in the interior districts of Africa. *Parkia biglobosa* is a multipurpose fodder tree that belongs to the family MIMOSACEAE. Also called the "African Locust Bean Tree", it is crown large and spreads wide with low branches. The leaves are alternate, dark green, bipinnate and about 8 – 30mm x 1.5 – 8mm in size with about 13-60 pairs of leaflets of distinct venation on a long rachis (Agroforestree Database, 2008). *Parkia biglobosa* has found so much medicinal use especially in West Africa. It is used against bronchitis, pneumonia and diarrhea. A decoction of the stem bark is used as a mouthwash (Ajaiyeoba, 2002).

The bark is also used with lemon for wounds and ulcers. In cote d' Ivoire and Nigeria, a bark infusion is used as a tonic for diarrhea and as an enema (Duker-Eshun *et al*, 2001). The leaves are also used for burns and toothache as well as for sore eyes in Gambia (Banwo *et al* 2004). Recently, the attention of researchers has been drawn to the great potentials in *Parkia biglobosa* as a source of an antibacterial agent. Ajaiyeoba, (2002); Banwo *et* al, (2004); Millogo-Kone *et al*, (2006) and El Mahmood, (2006) have all reported the presence of plant secondary metabolites which are known to exhibit antibacterial activity against a wide range of organisms. This paper presents the report of the efforts made to identify the compounds in the stem bark of *Parkia biglobosa* responsible for their bioactivity.

METHODOLOGY

Plant collection and authentication

The plant material was collected from Samaru– Zaria in Kaduna state Nigeria. It was authenticated in the herbarium section of the Biological Science Department of the Ahmadu Bello University Zaria, Nigeria and a voucher specimen number 2846 was deposited there for further reference.

Preparation and extraction of Plant Samples

The plant material was air dried for five days on the laboratory bench and then ground into powder in a mortar. 250g was then extracted to exhaustion with Methanol, using a Soxhlet apparatus. Afterwards, the solvents were removed and the extracts obtained were stored in the desiccator until needed.

Fractionation of Extract

The extract was fractionated using Petroleum ether, Chloroform and water. 20g of the dried extract was ground in a mortar and dissolved in 200mls of water before shaking vigorously in a separating flask. The mixture obtained was filtered using a filter paper to remove debris. 200mls of petroleum ether was then added to the mixture, shaken vigorously and allowed to settle. The petroleum ether layer (on top) was removed and concentrated while a further 200mls of chloroform was added to the aqueous layer and also vigorously shaken and allowed to settle. The aqueous and the chloroform layers were further separated and while the chloroform portion was concentrated to dryness by allowing to stand on the laboratory bench until all the solvent evaporated, the aqueous layer(WS) was concentrated to dryness using (mild) heat. The resulting fractions were appropriately stored in a desiccator until needed.

Bioautographic studies

This was done using the method described by Udobi, et al (2010).

Phytochemical Analysis

The extract was subjected to Phytochemical analysis using standard methods as described by Trease and Evans (1989) and Harborne (1973).

Preparative TLC

45g of silica gel were mixed with 90mls of deionized water, stoppered and properly shaken to ensure homogeneity of the slurry which was then poured over a set of glass plates- 10x20cm and 20x20cm on a TLC spreader using an applicator to achieve a thickness of 0.5mm.The plates were air dried and further activated in an oven at about 100-115^{0}C for between 1-4 hours. They were then allowed to cool to room temperature before use. The fraction (WS) was dissolved in water to make a concentration of about 10-20mg/ml and then applied as a thin line across the plate using a micropippet about 1.5cm above the bottom edge of the plate.

Compound development and detection:

N-butanol, acetic acid and water in the ratio 6:1:2 was used as the preferred solvent system and was mixed in such volumes as to achieve 135mls in the tank and the silica coated plates were allowed to develop in it after the solvent had been allowed to saturate for about 20 minutes. The plates were removed from the tank when the solvent front was a few millimeters from the top of the plate and air dried. Each plate was developed by allowing it to stand for about 24 hours.

Desorption and Recovery of Compounds:

The straight line bands which developed were scrapped off using a clean razor blade along with the sorbent into a conical flask. Ethanol was added and the suspension obtained was left to stand for 30 minutes to facilitate leaching of the compound into the solvent and then filtered. This process was repeated two to three times to ensure maximum recovery. The filtrate was left in an open crucible for ethanol to evaporate and the compound scrapped off and stored in a desiccator (Simon and Gray, 1998)

Functional group determination (Infrared Spectroscopy)

The functional groups present in the fraction was determined using Infra red spectroscopy. Characteristic peaks of different functional groups in the IR spectra will help in the prediction of the molecular structure of the compounds in the fraction. A little quantity of the sample (in solid form) was finely ground in a small mortar with a few drops of liquid hydrocarbon (nujol). The mull was then pressed between two flat plates of sodium chloride and fed into an infra-red spectrophotometer. The spectrum for each sample so treated was produced by the instrument within one minute.

RESULTS

The four bioactive components identified could not be eluted from the adsorbent (silica gel) used for the separation. This must have been due to the high attraction between them (both are highly polar). NMR spectra of what was considered the eluate (Figs 1 and 2) confirmed this as no characteristic peaks were observed. A reversed phase TLC of what was believed to be the eluted compounds after the separations confirmed also that the compounds did not elute from the adsorbent. Results of the bioautographic studies revealed that all the 4 compounds separated

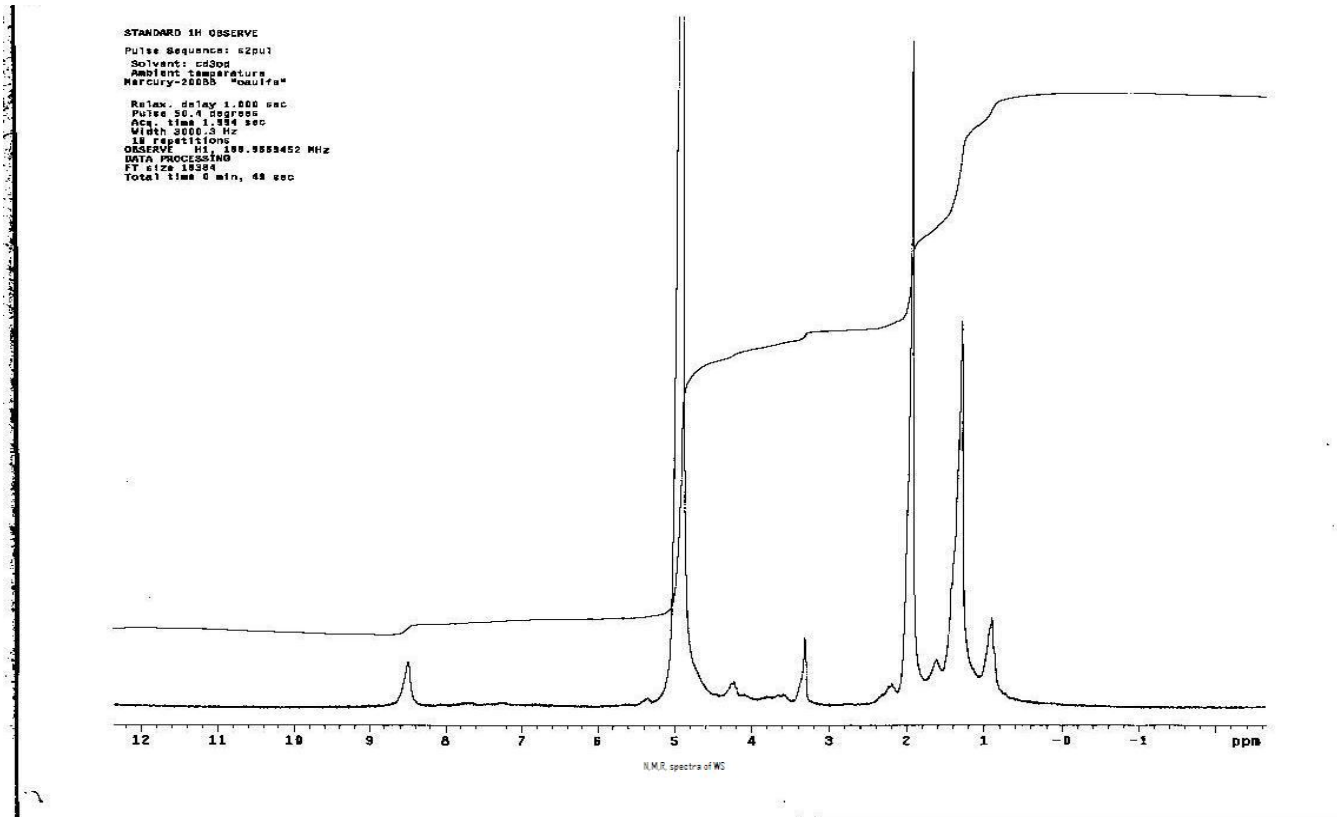

Fig. 1: NMR Spectra for WS (Hydrogen).

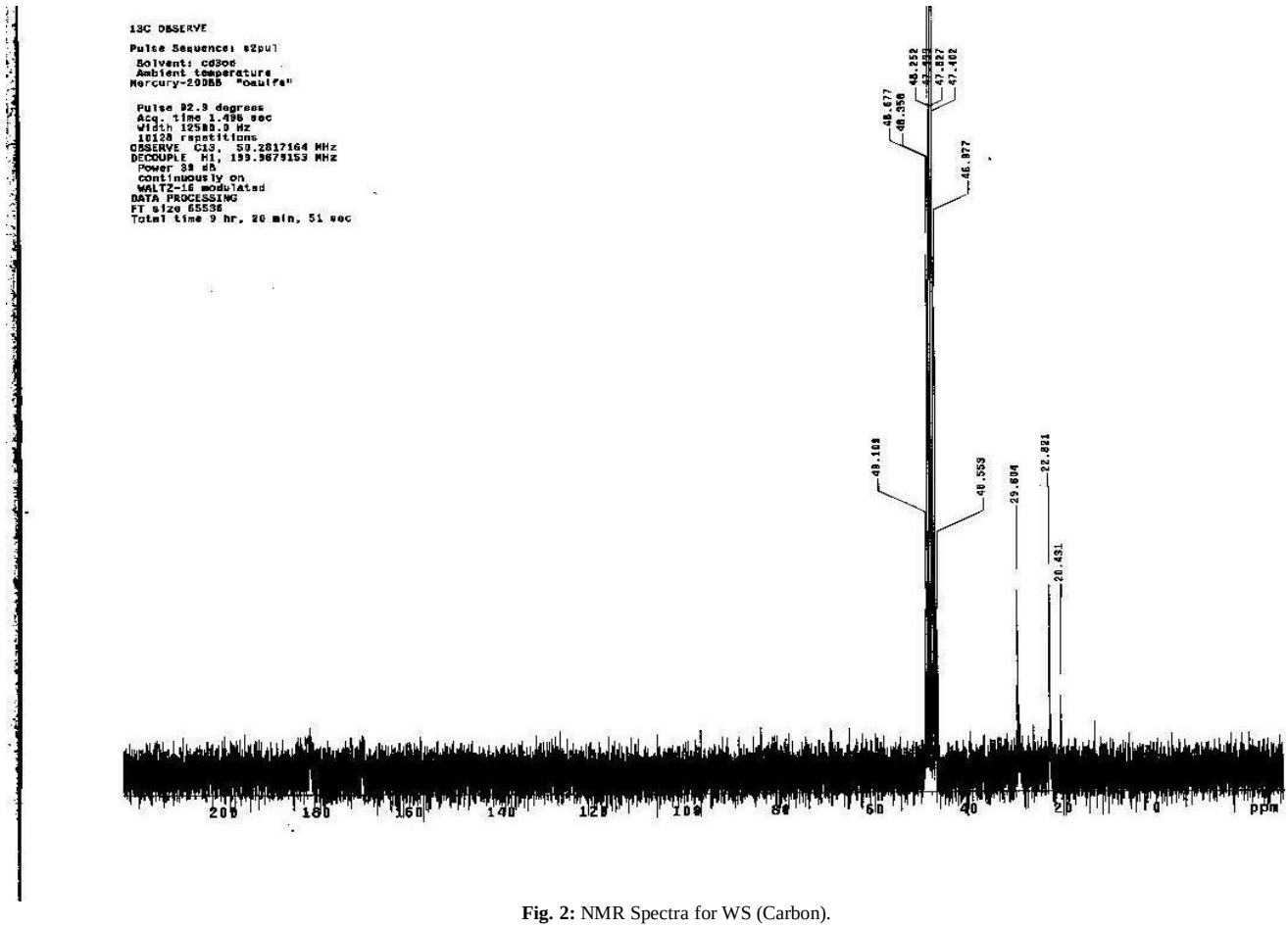

Fig. 2: NMR Spectra for WS (Carbon).

by TLC, had good activity against *Staphylococcus aureus* and very mild effect on *Pseudomonas aeruginosa* while there was no activity against *Escherichia coli*. It also showed that the zone of inhibition observed around the extract was much more than that around the separated compounds.

Table. 1: Results of Phytochemical screening.

Metabolites	WS
Saponin	+
Carbohydrates	+
Alkaloid	-
Tannins	+
Flavonoids	+
Anthraquinones	-
Cardiac glycosides	+
Resins	-

Preparative TLC

The preparative TLC results using n-butanol, acetic acid and water at a ratio of 6: 1: 2 as solvent system revealed 4 bands of different R_f values depicting 4 different compounds 1-4.

Table. 2: R_f values of the bioactive compounds of WS.

Bioactive compounds	1	2	3	4
R_F Values	0.92	0.72	0.62	0.56

Infra Red Spectroscopy

The spectral patterns obtained showed that there were peaks at 1377.22 and 1461.13 which suggest a nitrogen containing compound. The peak at 1461.13 particularly suggest the presence of an aromatic nucleus. Although the elemental analysis indicated the presence of aliphatic aldehyde or ketones (Table 3). The absorption peak at 1619 suggest an oxygen containing compound. This is even more supported by the adsorption within 2853-2955 which may be for an oxygen containing chromophore –C-H. The presence of absorption peaks within3386-3489^{m-1} for the fraction suggests OH-stretching frequency for alcohol or phenol. The signal at 1615.4 suggests the presence of a chelated keto group normally found in flavonoids. It also confirms the 5-OH substitution in the ring.

Table. 3: Elemental analysis of the aqueous fractions of the methanolic extract of stem bark (WS) of *Parkia biglobosa.*

Test	Observation	Inference
Sample in H_2O	Soluble	Polar Compounds Present Lower molecular weight acidic compounds
Sample in Diethyl-ether Fraction (aq. solution) with 1 – 2 drops of neutral ferric chloride solution	Not Soluble Wide range of colouration	Present phenol Present
0.2g of fraction (in 5m Hcl) and 2,4 dinitro phenylydrazine (Brady's Reagent	Yellow- Red ppt Clear solution was obtained	aldelydes or Ketones present
Fraction + 2M ammoniacal silver Nitrate solution. Warm in water bath	(silver mirror absent)	Aliphatic aldehyde Present
Fraction + 2M Hydrochlonic acid. Cool to 5^oC in ice. Add 3 or 4 drops of aqueous sodium nitrate	There was an efferescence. Nitrogen evolved and clear solution was obtained	Amines, Amino acids or Amide Present

DISCUSSION

The need for the real identity and structure of the compounds confirmed to be responsible for biological activities against certain organisms cannot be overemphasized. This is because ultimately, the goal of the researcher is that the compounds so identified will form the basis for a new agent which will be used for the control of the said organisms. The compounds in the fraction studied were successfully separated using the thin layer chromatography with the intention of making valid statements about each of them. The number of spots observed during the separation by the TLC depicted the compounds that are present in the extract. The preparative TLC plates using butane l(6): acetic acid(1): water(2) as solvent system however showed four bands of different R_f values (Table 2). The combined effects of the compounds may have brought about the original activity of the fraction noted during the antimicrobial activity, since such combinations are known to produce a synergistic effect (Esimone *et al* 1999) and they have proved to be efficient (Fritz, 1986, Klastersky *et al*, 1986). This is confirmed by the bioautography result which shows the fraction inhibiting the organisms better than the separated compounds. Plants are known to produce compounds that can be effective antimicrobials if they find their way into the cells of pathogens especially across the double membrane barrier of Gram negative bacteria (Sibanda and Okoh, 2007).

The characteristic peaks observed in the IR spectra are suggestive of members of the phenolic group e.g the peak at 1615.4 suggests the presence of a chelated keto group normally found in flavonoids while the peaks observed at 3386-3489^{cm-1} suggest an OH stretching frequency for phenols. The elemental analysis results (Table 3) also confirms the presence in the fraction of acidic, polar compounds of low molecular weight which points to phenols. The secondary metabolites (Tannins, Saponins and flavonoids) which phytochemical analysis results confirmed to be responsible for the activity of the fractions, all belong to the phenol group of compounds. Antimicrobial activity tests had earlier shown that the activity of the fractions is brought about by secondary metabolites which are soluble in water and are therefore taken into the aqueous fraction during the fractionation of the whole extract. (Udobi, et al 2009). Before now, other workers have reported the presence of the same metabolites in *Parkia biglobosa* parts (Ajaiyeoba, 2002; Millogo kone, et al 2006; Banwo, et al 2004; El-Mahmood, et al 2007. Present efforts made to isolate these compounds were not successful because it was not possible to elute the identified compounds from silica gel. This can be explained by the fact that these compounds are as highly polar as the silica gel and they will be tightly bound to one another since like will attract like.

The elemental analysis along with the infra red spectroscopy further confirmed the phytochemistry result that the bioactive compounds are phenolic and actually brings us closer to the true identification of the compounds. Further work is going on in our laboratories for the isolation, further identification and structural elucidation of these compounds.

REFERENCES

Agroforestree Database. International Centre for Research in Agroforestry (ICRAF).

Ajaiyeoba, Edith O. Phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. Afr. J. Biomed. Res. 2002; 5 (3) 125 – 129.

Banwo, G.O. Abdullahi, I and Duguryil, M. The antimicrobial activity of the stem bark of *Parkia clappertoniana* keay family Leguminosae against selected microorganisms. Nig. J. Pharm. Res. 2004; 3(1): 16-22.

Cowan, M.M. Plant products as antimicrobial agents. Clin. Microbial. Rev. 1999; 12(4) 564 – 582.

Duker-Eshun, G. D. Beni, C. T. Asonaming, W. A. and Akwamoah, R. A. Chemical investigation of the stem bark of *Parkia clappertoniana* Keay. Journal of Ghana Science Assoc. 2001; 3:2.

El-Mahmood, A.M and Ameh J. M. In-vitro Antibacterial activity of Parkia biglobosa(Jacq) root bark extract against some micro-organisms associated with urinary tract infections. Afr. J. Biotech. 2007; 6 (11): 1272-1275.

Esimone, C.O., M.U. Adikwu and J.M.Okonta. Preliminary Antimicrobial Screening of the ethanolic extract of the Lichen *subfloridans*(L). J. Pharm. Res. And Dev. 1999; .3(2).

Fritz, W. R. Efficacy of *inipenem*/*cilastin* in patients with severe bacterial infections. Journal of Antimicrobial chemotherapy, 1986; 18; 141 – 144.

Harborne, J.B. Phytochemical Methods. Chapman and Hall, London. (1984) 166-226.

Klastersky J, Van der AP. Cephalosponins, Vancomycin, Aminoglycosides and other drugs especially in combination for thetreatment of methicilin – resistant staphylococcal infection. J.Antimicrobial Chemother. 1986; 17: 19-24.

Millogo-Kone, H., Guisson, I. P. Nacoulna, O.; Traore, A. S. Study of the antibacterial activity of the stem bark and leaf extracts of *Parkia biglobosa* (Jacq) Benth on *Staphylococcus aureus*. Afr. J. Trad. Comp. and alternative medicines. 2006; 3(2) 74- 78.

Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance; plant extracts as potential sources of antimicrobial andresistance modifying agents Afr. J. Biotechnol. 2007; 6(25): 2886-2896.

Simmon, G and Gray, A.I. Methods in Biotechnology 4: Natural Products Isolation.R. J. P. Cannel (ed). Humana Press. Inc. Totowa N. J. (1998).

Trease G. E., & Evans W. C. *Pharmacognosy*. 15th ed. Brailliar Tridel can, Macmillan Publishers. (1989).

Udobi, C. E. and J. A. Onaolapo. Phytochemical analysis and antibacterial evaluation of the leaf, stem and root of the African Locust Bean (*Parkia biglobosa*). J. Med. Pl. Res. 2009; 3(5): 338 344.

Udobi, Chinweizu Ejikeme, Onaolapo, Ademola Josiah, & Abdulsalam, I. A. Bioautographic determination of the antistaphylococcal components of the stem bark of Parkia biglobosa (Jacq) benth (mimosaceae). *Journ. Pharmacog. Phytother*, 2010; 2(8), 108-112.