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Antibacterial activity of tannins and flavonoids of *Parkia biglobosa* stem bark

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Abstract
In this study, three different solvents of different polarities were used to extract flavonoids and tannins from *parkia biglobosa* stem bark which were further subjected to antibacterial activity using four strains of bacteria. The antibacterial studies showed inhibition zones exhibited by the extract against the tested bacteria ranged between 27.5-57.5 mm (*E. coli*), 30-62.5 mm (*B. subtilis*) 22.5-50mm (*S. aureus*) and 25-60mm (*S. typhi*.) as compared to 21-45mm for the control Ciprofloxacin. Flavonoid fractions of all the three different polarity extracts showed higher zones of inhibition; in some cases, higher values than the control while others are competing with it. These results were taken to indicate that crude flavonoid fractions at 200mg/ml had the highest antibacterial activity and therefore the extracts can be used as antimicrobial agents. Also, these antibacterial activities may serve to explain and support the continued use of the *P. biglobosa* stem bark for treating cuts, wounds and other disease conditions by traditional medicine men.

Keywords: tannins, flavonoids, *Parkia biglobosa*, antibacterial activity

Introduction
Phenolics compounds are extremely researched because of their significant properties of biological, pharmacological, and especially their anti-inflammatory, antioxidant, and anti mutagenic and anti carcinogenic activities. (K. Muniyandi *et al.* (2019) [6]. Therapeutic effects shown by medicinal plants is as a result of the high contents of these phenolic compounds they produce, while some plants are not medicinal because of their relative low content of these compounds (Ghasemzadel, 2011). Flavonoids, phenolic acids and tannins are essential secondary metabolites that are found in plants which exhibits natural antioxidants properties and other therapeutic effects in remedying different human ailments that includes anti-inflammatory and healing potentials. The antioxidant properties of these metabolites includes; inhibition of free radicals, ROS, RNS, and other biological conditions that results from oxidative stress. (Ghasemzadel, 2011; Mamta, 2012). These free radicals are responsible for causing various types of ailments which includes, cardiovascular disease, inflammatory, gastric, and other ailments. The use of some flavonoids and tannins in the treatment of inflammations, wound-healings because of their potentials in therapeutic use as anti-inflammation, anti-fungal, antioxidants and healing properties (Pinto, *et al.* (2019) [1].
This paper therefore provides scientific basis of the use of stembark of *parkia biglobosa* and the roles that phenolic acids plays in the treatments of ailments that are related to wound healing, anti-inflammatory and antioxidants activity that has been in continuous use by traditional people of Yobe State.

Materials and Methods
Apparatus
Ultrasonicator (Model/AS3120) an ultra-sonic water bath, power sonic 420, with 40KHZ frequency and maximum power of 700w, internal dimension (d): 500 x 300 x 150mm were purchased from Auto science Ltd USA, analytical weighing balance (Model/PA 214) U.S.A. Analytical weighing balance (Model/PA214) was from Chaus Corporation, Pine Brook, USA; Blender (Model: HR 2815), China, Drying cabinet (Model: FSM 140), Japan, Genlab (Water bath) from Thermal Engineers (Model: WBH22/FL), United Kingdom, Unico Spectrophotometer (Model No.:UV2150), USA, Beam weighing balance, and ultrasonic cleaner (AS3120) purchased from Autoscience Instrument Co. Limited., Nephelometer and Vortex mixer Bibby Scientific Limited Stone, Staffordshire,(Model: ST15 0SA), UK. Whatman No.1 filter paper was purchased from Whatman international Ltd, Maidstone, England,

Chemicals and Reagents
Ethyl acetate, n-Hexane and Methanol were purchased from BDH Chemicals, Pools, England; Iodine Crystals, Potassium iodide, mercuric chloride and bismuth iodide were from Hopkins and Williams Co Ltd, England 2, 2-diphenyl-1-picrylhyrazyl (DPPH) was purchased from Sigma-Aldrich Co. St Louis, Germany. Ascorbic acid was obtained from Griffin and George, England. All other chemicals used were of highest analytical grade and purchased from BDH Chemical Ltd, Pools, England. Sabouraud Dextrose Agar (SDA) HCM049, Microbial Sci. & Tech. CO., LTD. Blood Agar Base CM0055, Oxoid Ltd., Basingstoke, Hampshire, England, herein referred to as Nutrient Agar (NA).

Sample collection
The fresh *Parkia biglobosa* stem bark samples were collected behind Federal Government Girls' College (FGGC) Potiskum, Yobe state, Nigeria. The plant was identified by the combined efforts of the traditional healers and botanists attached to the Ministry of Environment, Yobe State, Damaturu, Nigeria.

Extracts preparations

The sample was washed and chopped into small bits and dried under shade in the laboratory at ambient temperature. The dried sample was then pulverized in a mortar and pestle into coarse particles and the drying was continued until a constant weight was obtained. A total of 400g of the coarse powder was then successively extracted with solvents of different polarities namely n-hexane, ethyl acetate and methanol as described previously using Ultrasonicator (Samaram, Mirhosseini, Tan, & Ghazali, 2013) [19]. After the ultrasonication the volume of the extracts were reduced and finally dried in a drying cabinet. Each extract was weighed and labeled appropriately and stored until required for analysis.

Phytochemical Screening

Screening tests for the presence of secondary metabolites were performed using standard methods
(Ezekwe, *et al*., 2013; Sabri, *et al.*, 2012; and Tiwari, *et al*., 2011) [3, 8, 10]

DPPH Radical Scavenging Activity

The free radical scavenging activity of the plant extracts were measured against DPPH at 517nm wavelength using Ascorbic acid as the standard antioxidant as described by other workers (Gupta *et al.*, 2003; Hatano *et al.*,1988) [4, 5]

Fractionation of Flavonoid and Tannins

Tannins

Determination of tannins was carried out by gravimetric method by transferring 8ml of the prepared tincture to 100ml volumetric flask. To this, 60ml of distilled water was added followed by 8ml of 12.5% lead acetate to precipitate resins, tannins and pigments. The mixture was shaken well and the volume completed to the 100ml mark with distilled water. This was mixed well and filtered through a filter paper. The residue was dried and weighed to obtain the quantity of the crude tannins. (El-Olemy, Al-Muhtadi, & Afifi 1994) [2].

Flavonoids

A 5ml portion of the preparation (Extract), accurately measured, was transferred to a small flask and then hydrolysed by heating on a water bath with 10ml of 10% H_2SO_4 for 30 minutes.

The original volume was then reduced to one half and the mixture was cooled on ice for 15min, where Flavonoid aglycones were precipitated out and cold solution was filtered. The solid on the filter paper was the flavonoid aglycones mixture, while the remaining flavonoids (glycones) remain in solution. (El-Olemy, Al-Muhtadi, & Afifi 1994) [2].

Microorganisms

The strains of bacteria used in this study (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhimurium*) were clinical isolates obtained from the School of Medical Laboratory Technology and Department of Dermatophilosis, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The strains were maintained and tested on Nutrient Agar (bacteria) with ciprofloxacin as control. The antibacterial activity screening was carried out using agar well diffusion and dilution technique as described elsewhere (Perez *et al.*, 1990) [7].

Results and Discussion

Antioxidant and Phytochemical screening

The results of antioxidant and preliminary phytochemical screening was reported by Umar *et al.* 2020[11] in press

Antibacterial activity

The antibacterial activity was carried out on the crude extracts of flavonoids and tannins of the three different solvent extracts of n-hexane, ethyl acetate and methanol of Parkia biglobosa stem bark on two Gram positive (G +ve) (Bacillus subtilis & Staphylococcus aureus) and Gram negative (G -ve) (Escherichia coli & Salmonella typhimurium) bacteria. The results of the zone of inhibition (ZOI) are presented in Table 1 and summarized in Fig 1(a-c) for crude tannins and Table 2 and summarized in Fig 2 (a-c) for crude flavonoids.

Table 1: The results of antibacterial activity of crude tannin extract of *Parkia biglobosa*

Samples	Bacteria	Zones of Inhibition (mm) of Concentration (mg/ml)							
		200	100	50	25	12.5	6.25	3.125	ciprofloxacin
A1	B.S	30	NCZ	NCZ	NI	NI	NI	NI	31
	E.C	20	8	NCZ	NI	NI	NI	NI	21
	S.A	22.5	6	NCZ	NI	NI	NI	NI	22
	S.T	25	NCZ	NI	NI	NI	NI	NI	21
B1	B.S	NI	NI	NI	NI	NI	NI	NI	31
	E.C	27.5	7	NCZ	NI	NI	NI	NI	36
	S.A	22.5	7	NCZ	NI	NI	NI	NI	40
	S.T	30	8	NCZ	NI	NI	NI	NI	30
C1	B.S	32.5	7	NCZ	NI	NI	NI	NI	30
	E.C	27.5	8	NCZ	NI	NI	NI	NI	36
	S.A	27.5	8	NCZ	NI	NI	NI	NI	45
	S.T	30	8	NCZ	NI	NI	NI	NI	41

Key: A1= n-Hexane crude Tannin extract
B1= Ethyl acetate crude Tannin extract
C1= Methanol crude Tannin extract
NCZ= No clear zone NI = No inhibition
B.S= *Bacillus subtilis* (G+ve)
E.C= *Escherichia coli* (G -ve)
S.A= *Staphylococcus aureus* (G+ve)
S.T= *Salmonella typhimurium* (G -ve)

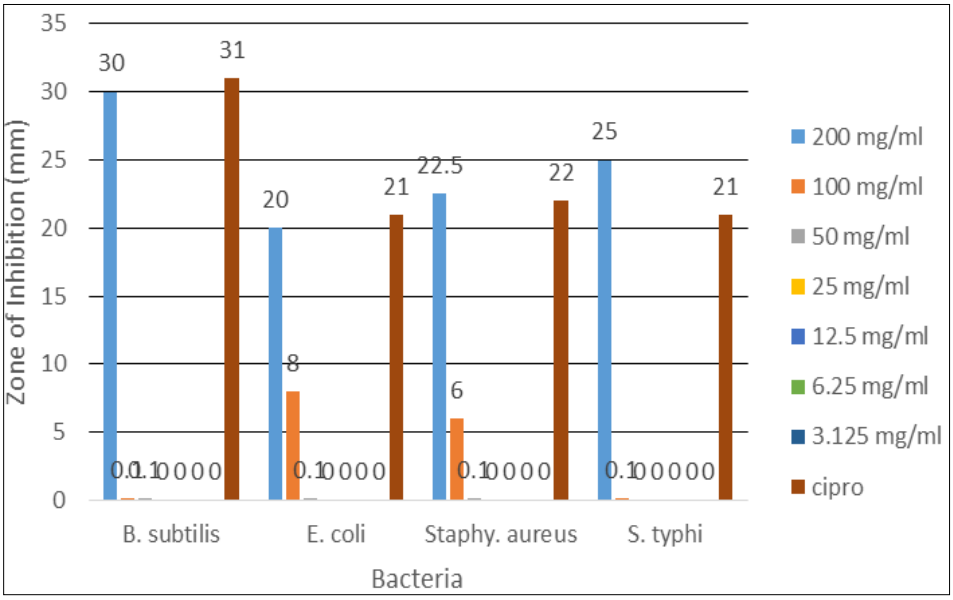

Fig 1a: The Zones of Inhibition Produced by N-hexane crude Tannin Extracts of parkia biglobosa on Gram negative and Gram positive bacteria.

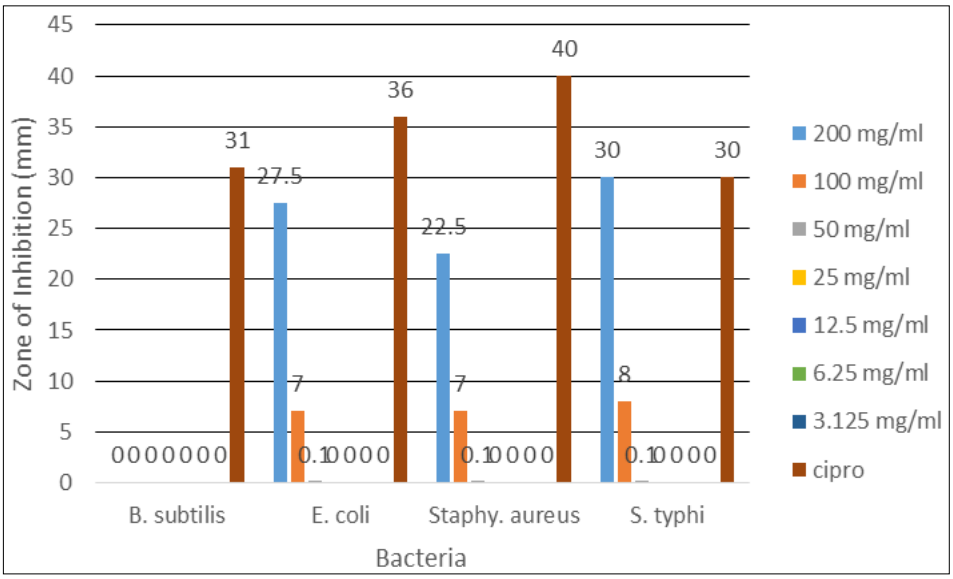

Fig 1b: The zones of inhibition produced by ethyl acetate crude tannin extracts of parkia biglobosa on gram negative and gram positive bacteria.

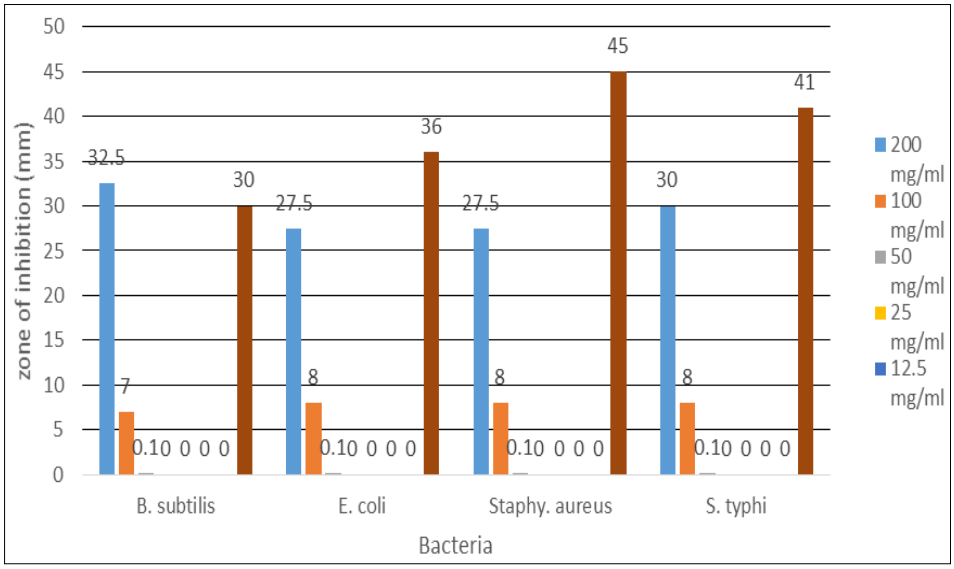

Fig 1c: The zones of inhibition produced by methanol crude tannin extracts of parkia biglobosa on gram negative and gram positive bacteria.

Table 2: The results of antibacterial activity of crude flavonoid extract of *Parkia biglobosa*

Samples	Bacteria	Zones of Inhibition (mm) of Concentration (mg/ml)							
		200	100	50	25	12.5	6.25	3.125	ciprofloxacin
A1	B.S	40	NCZ	NCZ	NI	NI	NI	NI	22
	E.C	30	8	NCZ	NI	NI	NI	NI	23
	S.A	30	10	NCZ	NI	NI	NI	NI	24
	S.T	42.5	NCZ	NI	NI	NI	NI	NI	33
B1	B.S	45	9	NI	NI	NI	NI	NI	30
	E.C	40	8	NCZ	NI	NI	NI	NI	38
	S.A	35	6	NCZ	NI	NI	NI	NI	45
	S.T	47.5	8	NCZ	NI	NI	NI	NI	40
C1	B.S	62.5	13	9	NCZ	NCZ	NI	NI	22
	E.C	57.5	12	8	NCZ	NI	NI	NI	20
	S.A	50	11	NCZ	NI	NI	NI	NI	23
	S.T	60	10	8	NCZ	NI	NI	NI	24

Key: A1= N-hexane crude flavonoid extract
B1=Ethyl acetate crude flavonoid extract
C1= Methanol crude flavonoid extract
NCZ= No clear zone
NI = No inhibition
B.S= *Bacillus subtilis* (G+ve)
E.C= *Escherichia coli* (G -ve)
S.A= *Staphylococcus aureus* (G+ve)
S.T= *Salmonella typhimurium* (G -ve)

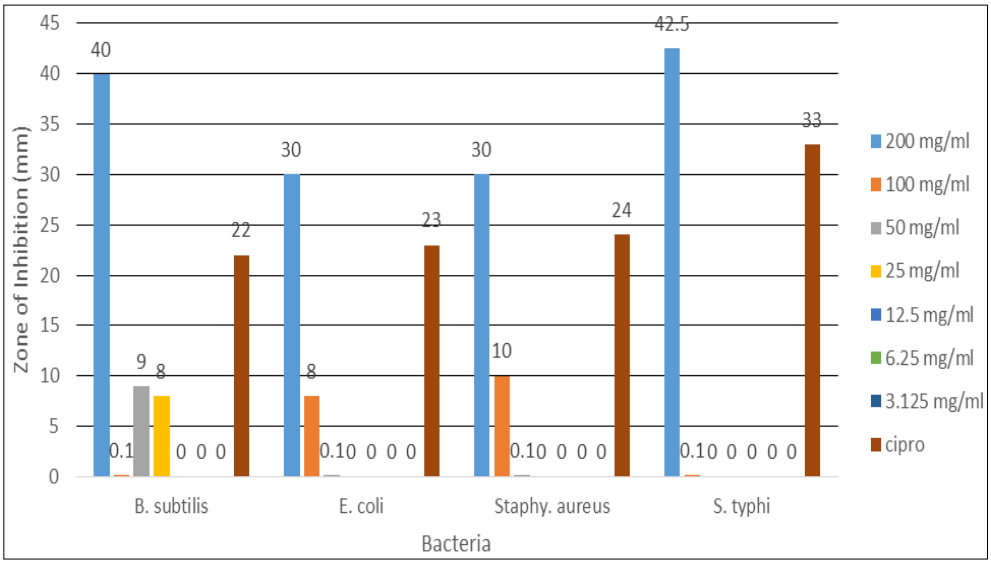

Fig 2a: The Zones of inhibition produced by N-hexane crude flavonoid extracts of parkia biglobosa on gram negative and gram positive bacteria.

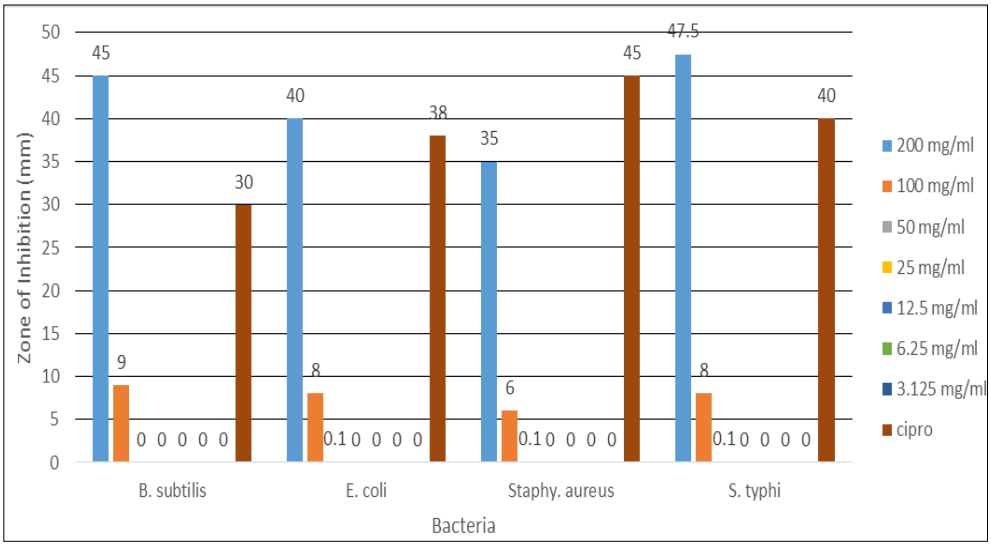

Fig 2b: The zones of inhibition produced by ethyl acetate crude flavonoid extracts of parkia biglobosa on gram negative and gram positive bacteria.

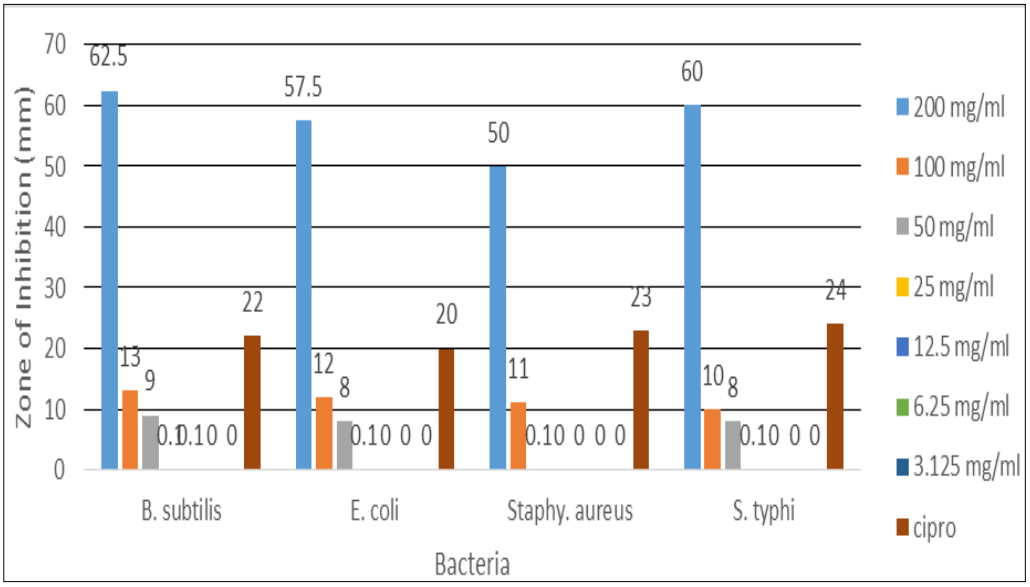

Fig 2c: The zones of inhibition produced by methanol crude flavonoid extracts of parkia biglobosa on gram negative and gram positive bacteria.

Crude tannins

The crude extracts of tannins showed comparable inhibitory activities with the control ciprofloxacin only at the concentration of 200 mg/ml. At this concentration the G+ve bacteria showed higher zones of inhibition (ZOI = 22.5-32.5mm) as compared to the Gram negative (ZOI = 20-30mm).

Crude flavonoids

The crude extracts of flavonoids showed comparable and sometimes even higher inhibitory activities with the control ciprofloxacin only at the concentration of 200 mg/ml. At this concentration the G+ve bacteria showed higher zones of inhibition (ZOI = 30- 62.5mm) as compared to the Gram negative (ZOI = 30-60mm).

These findings suggest that *Parkia biglobosa* extracts possessed strong antibacterial activities against both Gram positive and Gram negative bacteria, and hence the stem bark of this plant can be used as antibacterial agent in wound healing and other similar ailments.

Conclusion

In summary, flavonoid and tannins were extracted using three different solvents. The antibacterial study revealed the highest activity with the flavonoid of the EtAc extracts which is more active in inhibiting the bacterial growth of both the gram positive and negative bacteria as compared to the other solvents extracts, then followed by the tannins. Based on the results, the findings of the research suggest that these crude extract can be used as antibacterial drugs and, hence its continuous use by traditional medicine men for wound healing and anti-inflammatory effects.

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