The Pharmaceutical and Chemical Journal, 2018, 5(1):174-181

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Preliminary Phytochemical and Antibacterial Screening of Crude Methanolic Extracts of Some Plants against Tested Bacterial Isolates

A. Abdulhamid*[1], Y.U. Dabai[2], Amar Mohamed Ismail[3]

[1]Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, PMB 1144, Aliero, Kebbi State, Nigeria
[2]Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, University of AL-Neelain, Kartoum, Sudan

Abstract Infectious diseases caused by bacteria, fungi, parasites and viruses are still a major threat to public health. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. This has lead to the search of new antimicrobial agents mainly among plant extracts. As part of our ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants, *Acacia nilotica* (Linn.), *Sclerocarya birrea* (A. Rich, Hochst.) and *Parkia biglobosa* (Jacq) were screened for their preliminary phytochemical and antibacterial activity. The preliminary phytochemical screening of the extracts was carried out using standard methods while the antibacterial activity was done using disc diffusion method. The results for the phytochemical secreening showed the presence of most of the phytochemicals tested. The results for the antibacterial activity of the crude methanolic extracts of the plants showed varying degree of antibacterial activity against the bacterial isolates. The extracts at a concentration of 100 mg/mL were found to inhibit the growth of most of the test bacterial isolates comprising of both Gram-positive and Gram-negative organisms. These findings support previous reports on the antimicrobial activity of these plants. However, Acacia nilotica leaves extract shows relatively high (18.00 ± 2.00 for *P. aeruginosa* and 22.67 ± 0.58 for *K. Pneumonia*.) zone of inhibition than all the other plants part used. The result of the present study signifies the potential of *Acacia nilotica* leaf as a source of therapeutic agents, which may provide leads in the ongoing search for antimicrobial agents from plants.

Keywords *Acacia nilotica*, *Sclerocarya birrea Parkia biglobosa*, phytochemicals, zone of inhibition

Introduction

Nigeria is well known for its rich ethno botanical wealth, particularly regarding medicinal plants which are traditionally used in the treatment of ailments and could be a good source for discovery of new, safe and biodegradable drugs. High population growth rate (2.8% per annum) and poverty coupled with dwindling economic reserves in the country make Nigerians resort to more affordable sources for their immediate health needs. As the population increases, demand for traditional medicine will increase. [1, 2]

Plants are rich in various active compounds including antimicrobial agents [3]. The recent discovery of novel drugs such as artemisinin, atropine, digitoxin, digoxin, emetine, pilocarpine, quabain, quinidine, quinine, reserpine,

vinblastine, vincristine, etc., from medicinal plants implies that vast potential still exist for the production of numerous more novel drugs. Consequently, the area of ethno pharmacology of medicinal plants has attracted increasing attention in new drugs research and development [4, 5]. It is estimated that two-thirds of the world population depend on traditional medications due to the limited availability, the high prices of most pharmaceutical products and the various side effects that they cause [6]. This further justifies the search for alternative products from plants used in traditional medicine.

Acacia nilotica (L.) Willd. ex Del. also known as Gum Arabic tree, Babul, Egyptian thorn, or prickly Acacia is multipurpose nitrogen fixing tree legume. It is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions. It is economically used as a source of tannins, gums, timber, fuel and fodder. In Nigeria, the plant is traditionally used to treat infections such as diarrhoea, dysentery, oxidative stress, intestinal pains, ulcer, cold, haemorrhages, tuberculosis, congestion, coughs and fever [7, 8].

Sclerocarya birrae (A. Rich.) Hochst is a tree about 13 m high and up to 2.5 m girth. It is a Nigerian medicinal plant used to cure diseases and heal injuries. This plant has various effects on living system. In Nigeria and in some other African countries, the stem bark, roots and leaves of *Sclerocarya birrea* are used for an array of human ailments, including: malaria fever, diarrhea and dysentery, stomach ailments, headache, toothache and body pains etc [9]. Anticonvulsant effect of aqueous stem bark of Sclerocarya birrea extract in mice was reported [10].

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. *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 [11].

As part of our ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants: *Acacia nilotica* (Linn.), *Sclerocarya birrea* (A. Rich, Hochst.) and *Parkia biglobosa* (Jacq). The plants were screened for their preliminary phytochemical and antibacterial activity.

Materials and Methods

Materials

Solvents for extraction

The solvents used were: 90% Methanol (BDH).

Materials for antimicrobial test

Microbiological media (nutrient broth): Muller Hinton agar.

Test Organisms: *Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Psedomonas aeruginosa and Proteus spp.*

Petri Dishes, Sterile Pipette, 6 mm cork borer, Incubator, Autoclave, Dimethylsulphoxide (DMSO) 10%

Methods

Plant Sample Collection and Identification

Fresh disease-free parts of the three plants used were separately collected from Bodinga, Sokoto State, Nigeria and was identified and authenticated by a Botanist at the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The plants were identified as *Acacia nilotica* (Linn.), *Sclerocarya birrea* (A. Rich, Hochst.) *and Parkia biglobosa* (Jacq) with voucher number UDUH/ANS/0247, UDUH/ANS/0245 and UDUH/ANS/0246 respectively. The samples were shed-dried, ground and kept in air-tight containers till further use.

Preparation of Plant Extracts

The methanolic crude extract was prepared by soaking a sample (50g) of different powdered plant parts material in 90% methanol (300 ml) for 72 h. At the end of the extraction, each extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30^{o}C and stored in sterile sample containers at 4^{o}C until further use.

Phytochemical Screening

The extracts were screened for the presence of major phytochemicals using standard qualitative methods as

described previously [12, 13, 14]. The plant extracts were screened for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, phenols, cardiac glycosides and anthraquinones.

Sensitivity test of the crude extracts

Agar well diffusion method was employed to assay for the antibacterial activity [15]. The antibacterial activity of the crude methanolic extracts of the eight plant samples (A to H) were determined using stock concentration of 100 mg/mL. The standardised inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), three appropriately labelled wells were bored into each agar plate. A 0.2 mL of the appropriate extract concentrate was placed in each well and then allowed to diffuse into the agar. The plates were later incubated at 37°C for 24 h after which zone of inhibition (diameter) formed was determined as an indication of antibacterial activity. These effects were compared with that of the standard antibiotic amoxicillin at a concentration of 1 mg/ml.

Results

Table 1: Weight (g) recovered and percentage (%) yield of the respective plant parts crude methanolic extracts

Extract	**Weight (g)**	**Percentage yield (%)**
Acacia nilotica leaves	9.98	19.96
Acacia nilotica stem-bark	4.86	9.72
Acacia nilotica seed	4.92	11.18
Acacia nilotica seed-less pod	11.46	22.92
Parkia biglobosa leaves	1.18	3.58
Parkia biglobosa stem-bark	3.50	7.00
Sclerocarya birrea leaves	.86	7.72
Sclerocarya birrea stem-bark	4.70	9.40

Table 2: Phytochemical constituents present in the methanolic crude extracts of the plant parts used (A to H)

Phytochemical	**Result**							
	A	B	C	D	E	F	G	H
Flavonoid	ND	+	+	+	N.D	+	N.D	+
Tannins	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	N.D
Alkaloids	+	N.D	+	+	+	+	+	+
Cardiac	+	+	+	+	+	N.D	+	+
glycosides	+	+	+	+	N.D	+	+	+
Steroids	N.D	N.D	N.D	N.D	+	N.D	N.D	N.D
Balsams	+	+	N.D	+	N.D	+	N.D	+
Anthraquinones	+	N.D	+	+	+	+	+	+
Terpenoids								

Key:

+ : present; N.D : not detected.

A: Sclorocarya birrea Stem – bark extract

B: Sclorocarya birrea Leaves extract

C: Acacia nilotica seed-less pod extract

D: Acacia nilotica leaves extract

E: Parkia biglobosa leaves extract

F: Parkia biglobosa stem – bark extract

G: Acacia nilotica seed extract

H: Acacia nilotica stem – bark extract

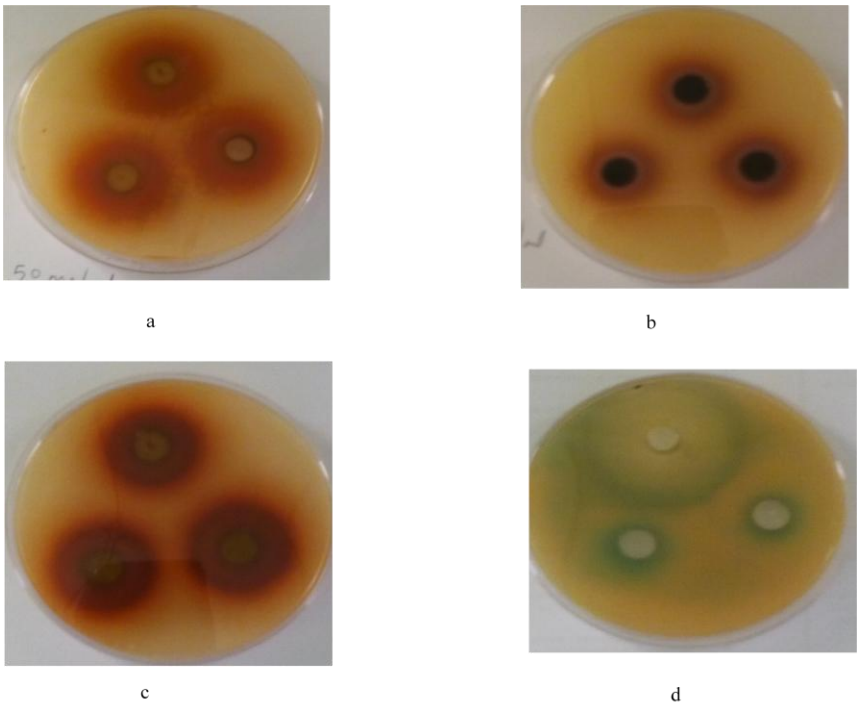

Figure 1: Zone of inhibition (mm) of (a) Acacia nilotica leaves extract against S. aureus (b) Parkia biglobosa stem-bark extract S. typhi (c) Acacia nilotica leaves extract against E. coli and (d) control (positive and negative) against E. coli.

Table 3: Antibacterial activity of methanolic crude extracts of *Acacia nilotica*

	Zone of inhibition (mm*)					
Bacterial isolates	**Leaves**	**Stem-bark**	**Seeds**	**Pods**	**Amox.**	**5% Meth.**
E. coli	22.00±2.00	14.33±1.53	11.33±2.31	18.33±2.08	28.67±1.15	0 ±0.00
K. pneumoniae	22.67±0.58	13.33±1.53	0 ±0.00	21.00±1.00	26.67±0.58	0 ±0.00
Proteus spp.	20.00±0.00	12.67±0.58	0 ±0.00	18.33±1.53	30.67±1.15	0 ±0.00
P. aeruginosa	18.00±2.00	12.33±0.58	14.00±1.00	14.67±1.53	25.37±1.53	0 ±0.00
S. aureus	20.67±1.15	13.67±1.53	11.00±1.00	20.33±0.58	23.00±1.00	0 ±0.00
S. typhi	19.33±1.15	12.00±1.00	10.33±1.15	14.00±1.00	28.00±2.00	0 ±0.00
S. pneumonia	22.33±2.08	14.00±1.00	0 ±0.00	22.00±1.00	21.67±1.53	0 ±0.00

*values are mean and standard deviation of three (3) replicates, 0 ±0.00 = No activity

Key: Amox. = Amoxicillin as positive control Meth (5%) = 5% methanol as negative control

Table 4: Antibacterial activity of methanolic crude extracts of *Parkia biglobosa*

	Zone of inhibition (mm*)			
Bacterial isolates	**Leaves**	**Stem-bark**	**Amox.**	**5% Meth.**
E. coli	14.00±2.00	11.33±1.53	28.67±1.15	0 ±0.00
K. pneumoniae	0 ±0.00	0 ±0.00	26.67±0.58	0 ±0.00
Proteus spp.	0 ±0.00	3.67±0.58	30.67±1.15	0 ±0.00
P. aeruginosa	10.67±0.58	12.67±1.53	25.37±1.53	0 ±0.00
S. aureus	17.33±2.52	11.33±0.58	23.00±1.00	0 ±0.00
S. typhi	13.61±3.51	14.33±0.58	28.00±2.00	0 ±0.00
S. pneumonia	0 ±0.00	12.33±1.53	21.67±1.53	0 ±0.00

*values are mean and standard deviation of three (3) replicates, 0 ±0.00 = No activity

Key: Amox. = Amoxicillin as positive control Meth (5%) = 5% methanol as negative control

Table 5: Antibacterial activity of methanolic crude extracts of *Sclerocarya birrea*

Bacterial isolates	Zone of inhibition (mm*)			
	Leaves	**Stem-bark**	**Amox.**	**5% Meth.**
E. coli	15.67±1.15	5.67±0.58	28.67±1.15	0 ±0.00
K. pneumoniae	12.33±2.08	0 ±0.00	26.67±0.58	0 ±0.00
Proteus spp.	14.33±1.53	0 ±0.00	30.67±1.15	0 ±0.00
P. aeruginosa	12.67±0.58	0 ±0.00	25.37±1.53	0 ±0.00
S. aureus	14.33±1.53	9.00±1.73	23.00±1.00	0 ±0.00
S. typhi	0 ±0.00	11.33±0.58	28.00±2.00	0 ±0.00
S. pneumonia	12.67±1.53	0 ±0.00	21.67±1.53	0 ±0.00

*values are mean and standard deviation of three (3) replicates, 0 ±0.00 = No activity

Key: Amox. = Amoxicillin as positive control Meth (5%) = 5% methanol as negative control

Discussion

The plant materials used in this study were initially extracted with methanol; the choice of methanol as a solvent of extraction was based on the earlier observation that an organic solvent, especially methanol, was a better solvent for consistent extraction of antimicrobial compounds from medicinal plants in comparison to other solvents such as water, hexane and ethanol [16, 17].

The result of the percentage yield of the crude methanolic extracts reported in Table 4.1 shows that the *Acacia nilotica* seed-less pod extract had the highest (22.92%) yield followed by *Acacia nilotica* leaves extract (19.96%), *Acacia nilotica* seed extract (11.18%), *Acacia nilotica* stem-bark extract (9.72%), *Sclerocarya birrea* stem-bark extract (9.40%), *Sclerocarya birria* leaves extract (7.72%), *Parkia biglobosa* stem-bark extract (7.00%), and lastly *Parkia biglobosa* leaves extract (3.58%). The amount of extract recovered and consequently the percentage yield depends largely on the fibre content of the plant/sample being extracted. High fibre contents gave a very low percentage yield. On the other hand, low fibre content gave a very high percentage yield [18].

The result of the phytochemical analysis of the eight (8) plant parts extracts is presented in Table 4.2. The result reveals the presence of flavonoids, tannins, saponins, glycosides, alkaloids, cardiac glycosides, anthraquinones and terpenoids in most of the extracts studied. Only steroids were not detected in all the eight (8) extracts expect in *Parkia biglobosa* leave extract. Several other studies have reported similar phytochemicals from these plants [19, 20, 21, 22, 23]; these support the data reported in this research. These compounds are known to be biologically active [24, 25] and thus may contribute to the observed antibacterial activities in these plants.

Phytochemicals exert antimicrobial activity through different mechanisms. For instance, flavonoids posses a wide range of biological activities which include antimicrobial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties [26]. The antibacterial activity of flavonoids had been shown to be a result of their ability to form complexes with bacterial cell walls' extracellular and soluble proteins [27]. Tannins act by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides [28, 29]. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery [28]; thus exhibiting antimicrobial activity. Thus these plants are traditionally used in treating diarrhoea and dysentery among communities in Northern Nigeria.

Saponins are known to produce inhibitory effects on inflammatory processes [30]. They were also reported to possess antibacterial property. Alkaloids are another kind of phytochemicals detected in most of the plant extracts tested. Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines [31].

Another important phytochemical detected in the plant extracts tested is cardiac glycosides. Cardiac glycosides are an important class of naturally occurring compounds whose actions help in the treatment of congestive health failure [32]. Steroid compounds detected only in the *Parkia biglobosa* leaf extract are of importance and interest due to their relationship with such compounds as the sex hormones [33]. Taken together all these facts support the

utilization of these plants (*Acacia nilotica, Parkia biglobosa* and *Sclerocarya birrea*) in various African countries such as Nigeria, Mali, Niger, Republic of Chad, Benin and Cote d'Ivoire in the preparation of local medications for the treatment of diseases.

The antibacterial activities of the eight (8) plant parts extract was investigated against the bacterial isolates of *E. coli, K. Pneumonia, Proteus spp., P. aeruginosa, S. aureus, S. typhi* and *S. pneumonia*. The result for the antibacterial activity of *Acacia nilotica* (leaf, stem-bark, seed, pod) extract is presented in table 3; while the antibacterial activity of *Parkia biglobosa* (leaf and stem-bark) extract is presented on table 4 and that of *Sclerocarya birrea* is presented in table 5. The extracts at a concentration of 100 mg/mL were found to inhibit the growth of most of the test bacterial isolates comprising of both Gram-positive and Gram-negative organisms. The zones of inhibition exhibited by the extracts ranged between 0.00 ± 0.00 mm and 22.67 ± 0.58 mm. The extracts tested displayed a varying degree of antibacterial activity against the bacterial isolates. These findings supports previous reports on the antimicrobial activity of these plants [19, 20, 21, 22, 34].

The bacteria isolates used in this study include pathogens such as *E. coli* known to cause urinary tract infections [35]; *S. typhi* known to cause typhoid fever and *K. pneumoniae* known to be the causative agent of pneumonia. All these pathogens were susceptible to most of the plant extracts used in this study, thus supporting the use of these plants in folklore remedies in the treatment of diseases caused by these pathogens. However, the antibacterial activity of *Acacia nilotica* against the bacterial isolates is relatively higher than all the other extracts used in this study (table 3). The zone of inhibition exhibited by the *Acacia nilotica* leaf extract ranged between 18.00 ± 2.00 for *P. aeruginosa* and 22.67 ± 0.58 for *K. Pneumonia*.

Conclusion

The result of the present study signifies the potential of *Acacia nilotica* leaf as a source of therapeutic agents, which may provide leads in the ongoing search for antibacterial agents from plants. Further, the activity exhibited by the extracts against tested bacteria species that are associated with various infectious diseases, may provide scientific justification for the ethnomedicinal uses of the plant.

Reference

1. Raphael, E. C. (2011). Traditional medicine in Nigeria: current status and the future. *Research Journal of Pharmacology*, *5*(6), 90-94.
2. El-Ghani, M. M. A. (2016). Traditional medicinal plants of Nigeria: an overview. *Agric. Biol. JN Am*, *7*, 220-247.
3. Sati, S. C., & Joshi, S. (2011). Aspects of antifungal potential of ethnobotanically known medicinal plants. *Research Journal of Medicinal Plants*, *5*, 377-391.
4. Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka, F., Kebede, T., Urga, K., Yersaw, K., Biza, T., Mariam, B. H., & Gipta, M. (2005). Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *Journal of ethnopharmacology*, *97*(3), 421-427.
5. Kilani-Jaziri, S., Bhouri, W., Skandrani, I., Limem, I., Chekir-Ghedira, L., & Ghedira, K. (2011). Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of Cyperus rotundus extracts. *South African Journal of Botany*, *77*(3), 767-776.
6. Tagboto, S., & Townson, S. (2001). Antiparasitic properties of medicinal plants and other naturally occurring products. *Advances in Parasitology*, 50, 199–295.
7. Aliyu, B. S. (2006). Common Ethinomedicinal Plants of the Semiarid Regions of West Africa; Triumph Publishing Company Limited; Nigeria, 2006, 8–26.
8. Saini, M. L., Saini, R., Roy, S., & Kumar, A. (2008). Comparative pharmacognostical and antimicrobial studies of Acacia species (Mimosaceae). *Journal of Medicinal Plants Research*, *2*(12), 378-386.
9. Masoko, P., Mmushi, T. J., Mogashoa, M. M., Mokgotho, M. P., Mampuru, L. J., & Howard, R. L. (2008). *In vitro* evaluation of the antifungal activity of *Sclerocarya birrea* extracts against pathogenic yeasts. *African Journal of Biotechnology*, *7*(20).

10. Ojewole, J. A., Mawoza, T., Chiwororo, W. D., & Owira, P. M. (2010). *Sclerocarya birrea* (A. Rich) Hochst.['Marula'](Anacardiaceae): a review of its phytochemistry, pharmacology and toxicology and its ethnomedicinal uses. *Phytotherapy Research*, *24*(5), 633-639.
11. Ajaiyeoba, E. O. (2002). Phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. *African Journal of Biomedical Research*, *5*(3), 125 – 129.
12. Harborne, J. B. (1988). *Phytochemistry*. London, Academic press, 92-135.
13. Sofowora, E. A. (1993). *Medicinal plants and traditional medicine in Africa*. 3rd ed. Spectrum book Ltd. Ibadan Nigeria, 23-25.
14. Trease, G. E., & Evans, W. O. (1989). *Trease and Evans Pharmacognosy*. 16th ed. New York: Sauders Elsevier Limited, 104-262.
15. Njoku, O. U., Boniface, J. A. E., Obitte, N. C., Odimegwu, D. C., & Ogbu, H. I. (2010). *Int. J. Appl. Res. Nat. Prod*., 2(4), 11-19.
16. Nebedum, J., Ajeigbe, K., Nwobodo, E., Uba, C., Adesanya, O., Fadare, O., & Ofusori, D. (2009). Comparative study of the ethanolic extracts of four Nigerian plants against some pathogenic microorganisms. *Research Journal of Medicinal Plant*, *3*(1), 23-28.
17. Rojas, J. J., Ochoa, V. J., Ocampo, S. A., & Muñoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC complementary and alternative medicine*, *6*(1), 232-239.
18. Ukwuani, A. N., & Igbokwu, M. O. (2015). *In vitro* antidiabetic effect of *Leptadenia hastata* leaves fractions. *Biosciences Research in Today's World*, *1*(1), 40-46.
19. Abioye, E. O., Akinpelu, D. A., Aiyegoro, O. A., Adegboye, M. F., Oni, M. O., & Okoh, A. I. (2013). Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of *Parkia biglobosa* (Jacq.). *Molecules*, *18*(7), 8485-8499.
20. Banso, A. (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *Journal of Medicinal Plants Research*, *3*(2), 082-085.
21. Kutama, A. S., Auyo, M., Umar, M., & Hadiza, M. (2013). Assessing the antibacterial activity of morula (*Sclerocarya birrea*) stem bark and leaf extracts against some selected bacterial isolates in Kano. *Nigeria World Journal of Agricultural Sciences*, *1*, 209-214.
22. Okoro, S. O., Kawo, A. H., & Arzai, A. H. (2014). Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts. *Bayero Journal of Pure and Applied Sciences*, *7*(1), 105-115.
23. Usman, H., Musa, Y. M., Ahmadu, A. A., & Tijjani, M. A. (2007). Phytochemical and antimicrobial effects of *Chrozophora senegalensis*. *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(4), 488-494.
24. Bruce, B., Spiller, G. A., Klevay, L. M., & Gallagher, S. K. (2000). A diet high in whole and unrefined foods favorably alters lipids, antioxidant defenses, and colon function. *Journal of the American College of Nutrition*, *19*(1), 61-67.
25. Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., Kretzschmar, G., & Panopoulos, N. (2007). Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnology journal*, *2*(10), 1214-1234.
26. Maikai, V. A., Maikai, B. V., & Kobo, P. I. (2009). Antimicrobial properties of stem bark extracts of *Ximenia americana*. *Journal of Agricultural Science*, *1*(2), 30-34.
27. Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, *30*(12), 3875-3883.
28. Dharmananda S. (2003). Gallnuts and the uses of tannins in Chinese medicine. Proceedings of institute for Traditional Medicine, Portland, USA.
29. Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus. Journal of antimicrobial chemotherapy*, *48*(4), 487-491.

30. Just, M. J., Recio, M. C., Giner, R. M., Cuéllar, M. J., Máñez, S., Bilia, A. R., & Ríos, J. L. (1998). Anti-inflammatory activity of unusual lupane saponins from *Bupleurum fruticescens*. *Planta medica*, *64*(05), 404-407.
31. Stærk, D., Christensen, J., Lemmich, E., Duus, J. Ø., Olsen, C. E., & Jaroszewski, J. W. (2000). Cytotoxic activity of some phenanthroindolizidine N-oxide alkaloids from Cynanchum v incetoxicum. *Journal of Natural Products*, *63*(11), 1584-1586.
32. Ikeda, Y., Fujii, Y., Nakaya, I., & Yamazaki, M. (1995). Quantitative HPLC analysis of cardiac glycosides in *Digitalis purpurea* leaves. *Journal of natural products*, *58*(6), 897-901.
33. Okwu, D. E. (2001). Evaluation of the chemical composition of medicinal plants belonging to Euphorbiaceae. *Pak Vet J*, *14*, 160-162.
34. Millogo-Kone, H., Guissou, I. P., Nacoulma, O., & Traore, A. S. (2008). Comparative study of leaf and stem bark extracts of *Parkia biglobosa* against enterobacteria. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(3), 238-243.
35. Dromigny, J. A., Nabeth, P., Juergens-Behr, A., & Perrier-Gros-Claude, J. D. (2005). Risk factors for antibiotic-resistant *Escherichia coli* isolated from community-acquired urinary tract infections in Dakar, Senegal. *Journal of Antimicrobial Chemotherapy*, *56*(1), 236-239.