Bima Journal of Science and Technology, Vol. 6 (3) Dec, 2022 ISSN: 2536-6041

DOI: 10.56892/bima.v6i03.49

ISOLATION AND CHARACTERIZATION OF BIOACTIVE CONSTITUENT OF N-BUTANOL SEED EXTRACT OF *Azadirachta indica*

[1]*MUSAH M., [1]MOHAMMED M., [1]MATHEW J. T and [1]UMAR M. T.

*Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria

Corresponding Author: mkwagana@gmail.com

ABSTRACT

Extraction of the seed powdered material of *Azadirachta indica* followed by an extensive column chromatography of the n-butanol portion on silica gel, purification over sephadex LH_{20} and subsequently HPTLC resulted in to the isolation of a pale yellowish solid coded as compound MND. The isolated bioactive constituent was found to have mp of 222-224^{o}C. The structure was elucidated using a combination of 500 MH_Z and 125MH_Z 1-D and 2-D NMR techniques (COSY, NOESY, HSQC, DEPT and HMBC). Thus, the isolate (MND) was determined as 4a, 7 – dihydroxy – 7 – methyl – 1 – (3, 4, 5 – trihydroxy – 6– hydro methyl – tetrahydropyran – 2 – yloxy) – 1 -, 4a, 5, 6, 7, 7a, hexahydro – cyclopenta [C] pyran – 4 – carboxylic acid methyl ester (Ipolamiide). The Antimicrobial properties of the compound MND and partition portion of the extracts were tested against *S. aureus*, *S. pyogenes*, *P. vulgari, P. aeruginosa, K. pneumoniae*, *E. coli*, *S. typhi, P. digitatum, C. albicans and P. nototum.* The antimicrobial sensitivity test indicated that various partition portion of the extracts inhibited the growth of *S. aureus* , *S. pyogenes P. aeruginosa, E. coli*, *S. typhi, K. pneumoniaer, P. digitatum, C. albicans and P. nototum* with 42mm, 38mm, 31mm, 29mm, 22mm, 30mm, 26mm, 27mm , 21 mm and 20 mm while the highest activity of the isolate (MND) was exhibited against *S. aureus, P. aeruginosa, E. coli and S. typhi* with 42 mm, 38 mm, 31mm and 39mm respectively.

Keywords: *Azadirachta indica,* Active constituent, Antimicrobial, Meliaceae, Spectral data

INTRODUCTION

Natural products research of plants with pharmaceutical value represents a key approach toward the development of new pharmaceutical products. The use of plants with therapeutic value for the treatment of diseases has been in practice for a long time and is documented (Twaij and Hasan, 2022). Humans, since primeval times have learnt to derive chemicals from plants and use them for therapeutic purposes (Jayawardene et al., 2021). The action of drugs in biological systems results from binding with receptors or enzymes. The biosynthetically altered active components of local plant extracts have enable them to bind successfully to human proteins in a process termed ‘evolutionary molecular modeling’. Bioactive plant products can bind to enzymes and receptors thereby obstructing or stimulating them (Tsado et al., 2018; Abubakar et al., 2015a). Natural products today as in times of old are having great influence on treatments for ailments and health status of people. Natural products have made significant contributions to the evolution of the pharmaceutical industry, as many drugs have been developed from natural products as precursors (Twaij and Hasan, 2022). World Health Organization acknowledged herbal remedies and have not frowned at developing countries who have incorporate the use of safe herbal medicine in their health system (Benarba and Pandiella, 2020). The patronage for natural products is further enhanced by their availability, affordability ‘perceived’ efficacy in treatment

DOI: 10.56892/bima.v6i03.49

of some ailments (Welz et al., 2018). Neem (*Azadirachta indica*) is one of those plants that have wide applications among locals especially in treatment of some ailments.

Azadirachta. indica is a fast-growing plant of the mahogany family (Meliaceae), it can reach can reach a height of 15–30 metres (49–98 feet). The leaves are evergreen toothed leaflets which drops amidst periods of drought. Several products from the plant, especially extracts have been used for medical purposes and in cosmetics. The plant's resilient nature allows it to grow well even in poor and rocky grounds. Neem is known to endure a wide variation in environmental conditions but does not grow well in waterlogged soils or freezing temperatures. The flowers are borne in clusters in the axils of the leaves with smooth yellow-green fruit (Petruzzello, 2022). Neem (*A. indica*) can be propagated from cutting suckers or from seed. It is grown for different purpose that include source of wood, provision of shade and reforestation; products obtained from *A. indica* have found applications in antiseptics, antipyretic and some in beauty products (Moin, et al., 2021; Adem et al., 2011).

Oil obtained from the seed have been used in the production detergent, soap and toothpaste. Other by-products have found application in the production of fertilizers. According to Imanuddin et al. (2020), *A. indica* contain about 300 secondary metabolites that are accountable for its properties. Research has shown over the years that neem seed is essentially non-toxic to vertebrate and it is the most potent growth regulator and feeding ever assayed (Wylie and Merrell, 2022). Neem seed is reported to be rich protolimonoids, fatty acid, cyclic tri- and tetrasulphides, alkyl sulphides and modified apoeuphol tetranortriterpenoids (Torres-Contreras, et al., 2022; Lin et al., 2021; Aarthy et al., 2018). In this research, a bioactive principle MND was isolated from the n-butanol seed extract of *A. indica* and the isolate was tested for its biological activities against the aforementioned microbes.

Plate I: *A. indica* tree

Plate II: *A. indica* seed

MATERIALS AND METHODS

Sample Collection and Treatment

Seeds of *Azadirachta indica* (Meliaceae) plant were obtained from a farm in Lapai town in Niger State, Nigeria. The plant was identified at the Department of Biological Sciences, Ahmadu Bello University (A.B.U.), Zaria, Nigeria. The seeds were decorticated and sliced into small pieces, then dried for 7 days

DOI: 10.56892/bima.v6i03.49

at room temperature after which they were ground into powder.

Experimental

Perkin- Elmer (Model 341 LC) spectrometer was used to measure optical rotations at room temperature. ^{1}H NMR and ^{13}C NMR experiments were performed using Bruker spectrometer at 500 MHz and 125 MHz for ^{1}H and ^{13}C NMR respectively. The NMR spectra obtained were referenced to CD_3OD solvent signals at δ3.30 ppm (^{1}H) and 49.00 for (^{13}C) using tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) obtained were in part per million (ppm) in relation to TMS (internal solvent standard). Thin layer chromatography (TLC) was done on plates which were precoated with RP-18 gel (merck) and silica gel F254. Spots on the TLC plates were made visible by careful spraying the plates with 10% sulphuric acid (H_2SO_4) then followed by heating the plate in the oven. Column chromatography was done on silica gel 60 (0.040 - 0.0653 mm) and column (40 – 63 µm, 310 mm and 15 mm i.d). HPTLC was carried on the concentrated pooled fraction with Fluka silica gel precoated glass plate (20 x 20 cm) having a layer thickness of 0.25 mm using Ethyl acetate: Methanol (7:3) as the solvent system. TLC visualization was done through UV absorption at 254 mm (Mohammed, 2022; Sofi and Nabi, 2018).

Antimicrobial Screening

Antimicrobial activities of the seed extract and isolated principle were determined using the microbes obtained from a Medical Microbiology Department of A.B.U. teaching hospital, Zaria, Nigeria. These microbes include, *Pseudomonas aeruginosa, Klebsiella pneumoniaer, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgari, Salmonella typhi, Penicillium digitatum, Bacillus subtilis, Aspergillus niger, Candida albicans, Penicillium nototum* and *Fusarium oxysorum*. The purity of the isolates was checked and maintained in slants of nutrient agar and saborand dextrose for bacteria and fungi respectively.

Extraction and Isolation

The powdered sample was macerated using the cold maceration technique using MeOH 100 % (3.0 dm^3) at 45°C for 48 hrs with intermittent shaking. The extract was concentrated at low pressure to dryness in order to obtain semi solid material. It was re-suspended in water (800 cm^3) and exhaustively partitioned consecutively with n-hexane, (3×500 cm^3), chloroform(3×600 cm^3), ethyl acetate (3×400 cm^3) and n-butanol (5×400 cm^3). The various partition portions of the extracts were concentrated by use of rotary evaporator to obtain n-hexane, (5.92 g), chloroform (3.15 g) ethyl acetate (4.25 g), n-butanol (5.20 g) and aqueous (7.22 g) residues respectively. The different fractions of the extract obtained were subjected to preliminary phytochemical screening according standard methods (Sofi and Nabi, 2018; Chóez-Guaranda et al. 2022).

The soluble portion of n-butanol was subjected to column chromatography on a silica gel (70-230 mesh). The column was eluted sequentially using gradient solvent system of n–hexane (100 %, 400 cm^3), Chloroform/EtOAC (2:8-1:9), EtOAC/MeOH (9:1-2:9) and MeOH 100 %. Fractions from other portions were pooled together on the basis of TLC analysis to obtain 83 fractions of 20 cm^3 aliquot (F_1 –F_{83}). Fraction (F_{8-68}= 161 mg) with three spots were subjected to repeated gel filtration using sephadex LH_{-20} and RP–18 column chromatography with 100 % MeOH (eluting solvent) to get amorphous mixture with two spots coded as FA(72 mg).

DOI: 10.56892/bima.v6i03.49

FA (72 mg) with two homogeneous spots was concentrated and further resubmitted for HPTLC analysis. The analysis was done using Fluka silica gel pre-coated glass plates 20×20 cm having thickness layer of 0.25mm. A thin line of about 1.5 cm was drawn with a pencil from the bottom of the plate. Pooled sample of FA (72 mg) was dissolved in MeOH to obtain concentration of 20 mg/cm^3. It was then uniformly applied along the thin line with the aid of capillary tube. The plate was then left to dry before it was developed with an appropriate solvent system. The plate which developed was dried under air in fume cupboard, pencil was used to mark the position of the band of interest then scraped off the back of the plate onto a foil. The size of the scraped sorbent was reduced using pestle and mortar, then transferred onto a sintered glass funnel and repeatedly washed with Acetone, followed by evaporation of the solution obtained to give a pale yellowish isolate coded as compound MND (40.3 mg, R_f0.6). Elution progress was monitored with TLC using pre-coated plate in different solvent systems; n-hexane:Ethylacetate (80:20), chloroform:ethylacetate (65:45) and EtOAC:MeOH (70:30). The chromatogram obtained was spread with 10 % H_2S0_4 and kept in an oven at of 105 ^oC for 5min then removed to ascertain the compound on the plate (Mohammed, 2022).

Chemical Test

Ferric Chloride Test

About 5.0 % iron(III)chloride in 0.5 N hydrochloric acid was spurted on the chromatogram, fluka-silica gel precoated glass plate of compound MND. This test was to check for the presence of phenolic compounds (Abubakar et al., 2015b).

Vanillin/Sulphuric Acid Test

4.0 g solution of vanillin was dissolved in 100 cm^3 of H_2SO_4. This solution was then spread using spray canister on the chromatogram pre-coated glass plate of compounds MND in a fume chamber. The plate was then heated in an oven at 110 ^oC for 5 – 10 minutes before it was removed from the oven to ascertain color formed (Mohammed et al., 2019).

Liebermann Buchard's Test.

1 cm^3 of CH_3COOH was added to 1 cm^3 of chloroform and cooled in a test tube to 0 ^oC. Then drops of conc. H_2SO_4 were added to the test tube which contain solution of compound MND (Mohammed, 2022).

Determination of Sugar in Compound MND

Compound MND (3.5 mg) was dissolved in 2.5 cm^3 of water; 2N solution of CH_2F_2-COOH (2.5 cm^3) was added and then refluxed on a water bath for 3 hours, after which the mixture was diluted with10 cm^3 of water then extracted with CH_2Cl_2 (2 x 5 10 cm^3). The extracts of CH_2Cl_2 were washed with water before evaporation to dryness *in vacuo*. The concentrated aqueous layer then was passed through Amberlite column (short) before evaporating to dryness in order to give sugar fraction (1.5 mg). These were analyzed with HPLC using CH_3CN/H_2O (85:15). Co-TLC of the sample was carried out and the sugar was analyzed with silica gel TLC in comparison to standard sugar using solvent system (Tsado et al. 2019).

Antimicrobial Assay

0.8g of the various partition portion of the extract were weighed separately and dissolved in 10.0 cm^3 of DMSO to get a concentration of 80.0 mg/cm^3. This concentration was the initial for the extract used to determine the antimicrobial activities of these extracts (Abu-Reidah and Taamalli, 2022). Mueller Hinton agar medium was prepared using method in the manufacturer's Instruction; sterilized at temperature of 121 ^oC for 15 min, the medium

DOI: 10.56892/bima.v6i03.49

sterilized was poured into sterile Petri dishes, the plates were then allowed to solidify on cooling. The extracts were screened using diffusion method. The medium was seeded with 0.1 cm^3 of standard inoculums of test microbes. The inoculums were evenly spread on the surface of the medium using sterile swab. After setting, the use of standard cork borer of a number 4 sterile cork borer of 6 mm in diameter were gotten, a well was cut at the middle of each inoculated plate medium. The medium inoculated was incubated at a temperature of 37 ^oC for 24 hrs. Each plate was observed for zone of inhibition of growth. Transparent ruler was used to measure the zone and the result recorded in millimeters (Adamu and Sajo, 2021).

The minimum inhibition concentration of the various partition portions of extracts were obtained with the use of broth dilution method. 10 cm^3 of prepared Mueller Hinton broth was dispensed into a test tube, then sterilized at 121 ^oC for 15 min and left to cool. Mc-farlands turbidity standard number 0.5 was prepared to give turbid solution. 10 cm^3 of prepared normal saline was dispensed into sterile test tubes and the test microbes inoculated and incubated at a temperature of 37 ^oC for 6 hrs. Test microbes in the normal saline were diluted until turbidity marched that of the Mc-Farland's scale using visual comparison; at this point the test microbe's concentration was about 1.5 x 10^8 cfu/ml (Tsado et al., 2018a). Two fold serial dilution of the extract in the sterile broth was done to obtain concentrations of 80 mg/cm^3, 40 mg/cm^3, 20 mg/cm^3, 10 mg/cm^3 and 5 mg/cm^3 respectively. 0.8 g of the extract was dissolved in 10 cm^3 each of the sterile broth to obtain the initial concentration. 0.1 cm^3 of standard inoculums of the test microbe was inoculated into different concentrations of the extract in the broth. Incubation was done at a temperature of 37 ^oC for 24 hrs, thereafter the test tubes were observed for growth (turbidity). The lowest concentration of the extract in the broth that did not show turbidity was noted as the minimum inhibition concentration (Ohikhena et al., 2017).

Minimum bactericidal and fungicidal concentrations were determined to check if the test microbes have been killed or only growth was inhibited. Prepared Mueller Hinton agar was poured into sterile Petri dishes, allowed to cool and solidify. Contents of the MIC in the serial dilutions were sub-cultured onto the prepared medium; incubation was made at temperature of 37 ^oC for 24 hrs, then each of the plates was observed carefully for colony growth. Plate having the lowest concentration of extract without colony growth can be considered the MBC for the bacteria and MFC for the fungi (Ohikhena et al., 2017).

The method of Mohammed (2022) was employed for the analysis of the isolated compound, MND. Organisms used were the same as those above. The medium of choice was Tryptic say Agar (Merck KGa A), it was prepared according to instructions of manufacturer. This was dispensed in sterile plates in 20 cm^3 aliquots after gelling and drying, the plates were seeded with the test microbes by streaking evenly in a cotton swab. The inoculums were allowed 5 min to dry, sterile filter paper disks (4 mm) earlier soaked with the isolated compound MND in (4µl/disk) placed and pressed down gently to ensure contact. The plates were inoculated at temperature of 37 ^oC for 24 hrs and ruler was used to measure the zones of inhibition.

RESULTS AND DISCUSSION

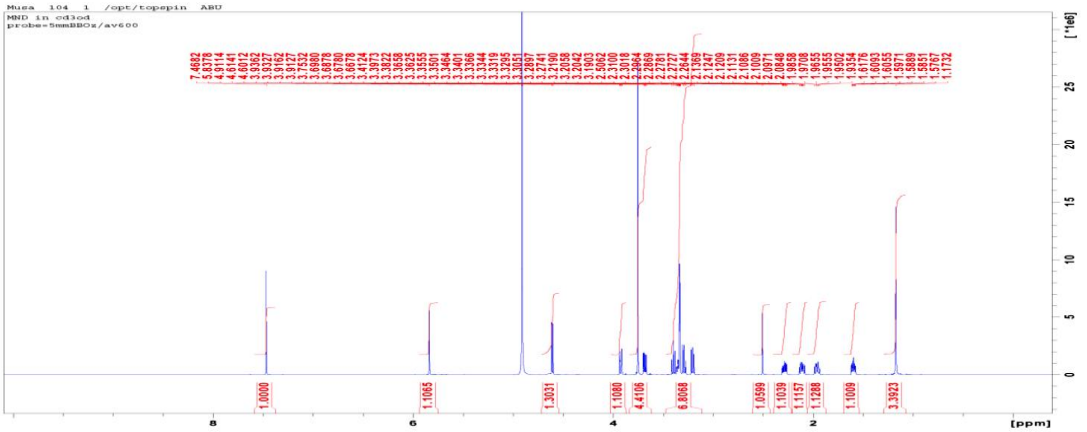
Figure 1 present TLC results of isolate MND in different solvent system. Extraction of the seed portion of *A. indica* followed by column chromatography of n-butanol portion of the extract on silica gel, then purification over sephadex LH–20 folloed by (HPTLC) lead to the isolation of compound MND. The isolate gave positive colour when tested with ferric chloride indicating the compound is phenolic (Mottaghipisheh and Iriti, 2020). It also gave

DOI: 10.56892/bima.v6i03.49

a red colour at the interphase indicating the presence of glycoside (Mottaghipisheh and Iriti, 2020).

Figure 1: MND in different solvent system

Compound MND was obtain as a pale yellowish powder (MeOH), having $[\propto]^{20}_{D}$+39.6(c1.1, MeOH). The ^{1}H NMR spectrum of MND (Figure 2 and table 1) displayed signal at δ_H 7.4ppm (IH, s) and δ_H 4.6ppm (IH d, 7.8Hz) corresponding to H – 3 and H – 1'. The signals at 1.2ppm (3H, s) and 3.6ppm (IH, dd, 6.0Hz) corresponds to H – 10 and H – 6' while the signal at δ_H 3.7ppm (3H, s) is attributed to OCH_3 (Brown, 2003). The signals at δ_H 3.2 – 3.6 above are all assigned to the sugar nucleus (Adem et al., 2011). The ^{1}H NMR spectra revealed the presence of single anomeric proton at δ_H (4.6 1H, d, 7.8Hz corresponding to H – 1'). The presence of signal at δ_H 7.4 ppm (1H, s) corresponding to H – 3, could be attributed to a methyl proton of the aglycone while signal at δ_H 3.7ppm (3H, s) are assigned to the methoxy group. The signal at δ_H1.20ppm which is shielded up field could be attributed to a tertiary methyl group (Mohammed, et al., 2015). The large J value of the anomeric proton at δ_H 4.0ppm (IH, d, 7.8Hz) of H – 1' and other resonance at δ_H 3.2ppm (3H, m) - δ_H 3.6ppm (1H, dd, 6.0) attributable to H – 2 – H – 6' is an indication of a β– glucosyl moiety (Francis, 2003). The ^{13}CNMR and DEPT experiment spectrum (Table 1) exhibited 17 carbon signals. The DEPT experiment showed four (4) quaternary carbon atoms, 3 oxymethelenes a single methine and one signal attributed to a methyl group. The signal observed at δc 51. 03 ppm could be attributed to a methoxy group (Guimarães, et al., 2012).



DOI: 10.56892/bima.v6i03.49

Figure 2: ^{1}H NMR spectrum of MND in CD_3OD

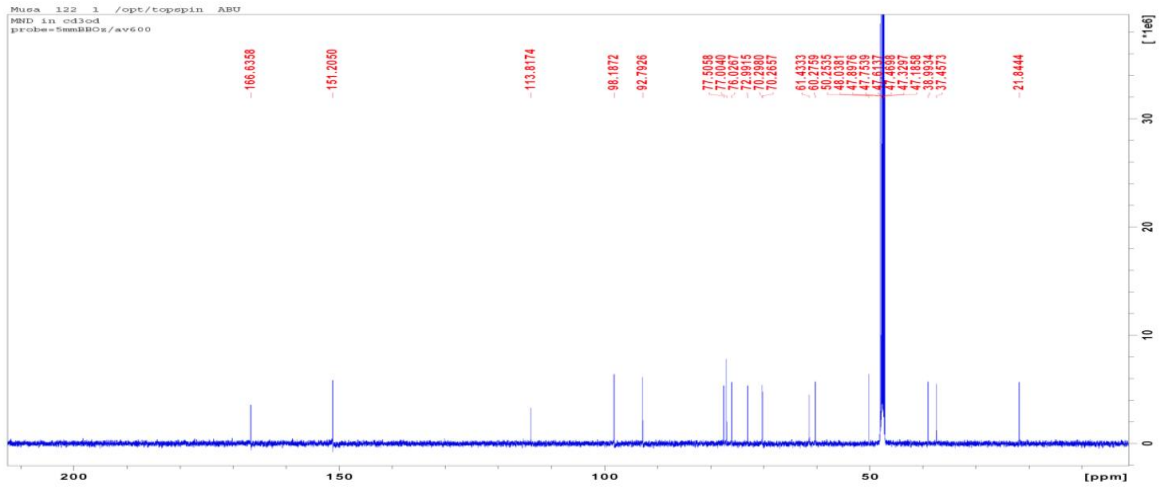


Figure 3: ^{13}C NMR spectrum of MND in CD_3OD

The HSQC spectra has further facilitated the assignment of the ^{13}CNMR signals at δc 93.0ppm (C–1), 151.2ppm (C–3), 38.0ppm (C–6), 39.0ppm (C–7), 60.3ppm (C–9), 22.0ppm (C–10) and 50.3ppm (OCH_3) with δ_H 5.8ppm (H–1), 7.4ppm (H–3), 1.9ppm (H–10) and 3.7ppm (OCH_3). These values are in complete conformity with the ipolamiide aglylone moiety (Oyvind, et al., 2006). The resonance observed at δc 98.2ppm (C–1') which correlate with δ_H 4.6ppm (H – 1') could be attributed to an anomeric carbon while signals at δc 73.0ppm (C–2'), 76.0ppm (C–3'), 70.3ppm (C–4'), 77.0ppm (C–5') and 61.4ppm (C–6') which correlates with δ_H 4.6ppm (H–1'), 3.2ppm (H–2'), 3.3ppm (H–3'), 3.4ppm (H–4'), 3.4ppm (H-5) and 3.6ppm (H–6'), could be attributed to a sugar moiety. The signals observed at δc 61.4ppm could be attributed to the oxymethylene carbon suggesting the presence of a glucopyranosyl moiety (Hostettmann, and Wolfende, 2004). The signal at δc 22.0ppm is assigned to the methyl group as confirmed by the HSQC data.

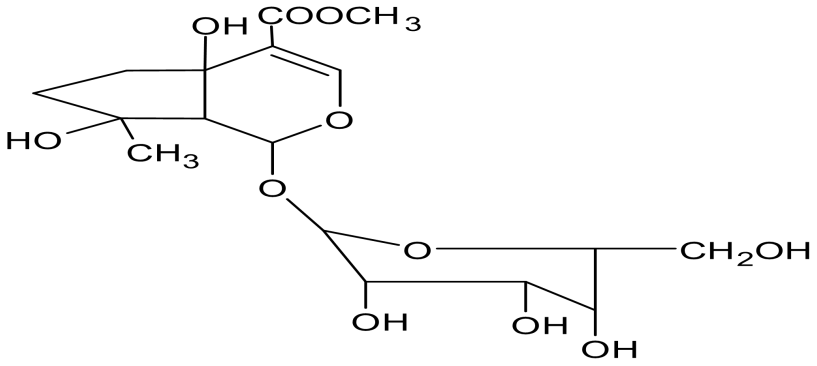


Figure 4: MND 4a, 7–dihydroxy–7–methyl–1–(3,4,5–trihydroxy–6–hydromethyl–tetrahydropyran–2–yloxy)–1-, 4a,5,6,7,7a, hexahydro–cyclopenta [C] pyran–4–carboxylic acid methyl ester (Ipolamiide)

DOI: 10.56892/bima.v6i03.49

Table 1: Summary of ^{13}C (125MHz) and ^{1}H (500 MHz) NMR spectra data of compound MND in CD_3OD, (δ ppm, J in Hz)

Position	δ_H (J in Hz)	δC	DEPT	COSY	HMBC (C-H)
1	5.8 (1H, d, 1.1 Hz)	93.0	CH	H-9	C -3, C -9, C -1'
3	7.4 (1H, s)	151.2	CH		C -1, C -5
4	-	114.0	C		
5	-	70.3	C		C -3, C -6, C -7
6	1.9 (1H, m)	38.0	CH_2		C -5, C -7, C -9
7	1.6 (1h, m), 2.05 (1H,m)	39.0	CH		C -9, C -1"
8	-	77.0	CH		C -7, C -9, C -1"
9	2.5 (1H, brs)	60.0	CH	H-10	C -4, C -5, C -1"
10	1.2 (3H, S)	22.0	CH_3		C -5, C -6
11	-	167.0	-		-
OCH_3	3.7 (3H, S)	50.3			-
1'	4.6 (1H, d, 7.8Hz)	98.2	CH	H-2[1]	C -1, C -2'
2'	3.2 (3H, m)	73.0	CH		C -3'
3'	3.3 (3H, m)	76.0	CH		C -2'
4'	3.4 (3H, m)	70.3	CH		C -3'
5'	3.4 (3H, m)	77.0	CH		C -6'
6'	3.6 (1H, dd, 6.0)	61.4	CH_2		C -1'

The COSY spectroscopic analysis has exhibited the correlation of δ_H 5.8ppm with δH 2.5 ppm and δ_H 4.6 ppm with 3.2 ppm (Table 1). The NOESY spectrum of MND has shown that δ_H 5.8 ppm (H – 1) is in the same environment with δ_H 1.2 ppm (H - 10) and δ_H 4.6 ppm (H – 1). This also shows the correlation of δ_H 4.6 ppm (H–1') with δ_H 3.6 ppm (H–6') (Table 1). The HMBC spectrum revealed the correlations between protons and the neighboring carbons up to 3 bonds. The connectivity between the various atoms and other units in the molecules was established, correlations were established between δc 93.0 ppm (C–1) with δc 151.2 ppm (C–3) δc 60.3 ppm (C–9) and δc 98.2 ppm (C–1'). So also correlations were established between δc 22.0 ppm (C–10) a methyl group of the aglycone with the δc 70.3 ppm (C-5) and δc 38.0 ppm (C–6). The correlation between δc 151.2 ppm (C–3) with δc 93.0 ppm (C–1) and δc 70.3ppm (C- 5) was established. The following correlations were also observed δc 60.0 ppm (C – 9) with δ_H 4.0 ppm (C– 4), δc 70.3 ppm (C–5) and δc 98.2 ppm (C–1') respectively (Zheng – Xiang et al., 2012).

Based on the aforementioned correlations from HMBC (Table,2) the aglycone was confirmed to be a cyclopentano pyran ring system (Zheng – Xiang et al., 2012).The connectivity between the sugar unit and the aglycone moiety was hence established, the anomeric proton δ_H, 4.6ppm (H–1') and the δc 93.0ppm (C–1) of cyclopentano pyran ring (agylcone) was ascertain. This firmly confirmed the structure of MND as Ipolamiide. However, on the basis of the spectral analysis (1D and 2D NMR) and comparison with ^{1}H – NMR and ^{13}CNMR of the reference data, Compound MND was unambiguously the same as (Ipolamiide), molecular formula of $C_{17}H_{27}O_{11}$ and $[M]^+$ 424 as the molecular weight. Thus, MND is named as 4a, 7–dihydroxy–7–methyl–1–(3,4,5–trihydroxy–6–hydromethyl–tetrahydropyran–2–yloxy)–1-, 4a, 5,6,7, 7a,

DOI: 10.56892/bima.v6i03.49

hexahydro–cyclopenta [C] pyran–4–carboxylic acid methyl ester.

Table 2 present results of the phytochemical screening of partitioned portion of the seed extract. The results of the various extracts indicated the presence of tannins, alkaloids, flavonoids, carotenoids and glycosides in the methanol and water fractions but were not detected in the hexane extract. The distribution of the studied phytochemicals in the solvents used was dependent upon their polarities and those of the extracting solvents (Ndamitso et al., 2013). These phytochemicals are ubiquitous in plants and are usually common in human diet. They are known to inhibit microbes which could be resistance to orthodox antibiotics. Flavonoids' free – radical scavenging property has given rise to multiple biological functions that include anti-bactericidal, anti-inflammatory, anti-carcinogenic, immune stimulatory, vasodialotory, anti-allergic and anti-viral functions (Agidew, 2022). Consequent, the presence of flavonoids in the ethyl acetate and n-butanol extract in this study has shown the therapeutic efficacy of the extract and has given credence to the folkloric use of the plant for treatment of ailments especially inhibitory effect on *S. aureus*, *K. pneumoniaer, S. typhi, E. coli* and *P. aeruginosa.*

Table 2: The results of phytochemical screening of the partition portions of the seed extract of the plant *Azadirachta indica*

Constituents	**Test**	**Observation**	**Portions of Extracts**					
			Hex	M_E	Cl	E_{ta}	n-But	Aq
Carbohydrate General Test	Molisch	Red colouring	-	+	-	-	-	+
Sugar Test	Aniline	Red colour	-	-	-	-	-	+
Sugar (Monosaccharide)	Barfoed's	Red ppt	-	+	-	-	-	+
Red Sugar	Fehling's	Red ppt	-	-	-	-	-	+
Tannins	Lead Ethanoate	White ppt.	-	+	-	-	+	+
	Iron (III) Chloride	Blue – Black	-	+	-	-	+	+
	Ethanoic acid	White ppt	-	+	-	-	-	-
	Methanol's	Red ppt	-	+	-	-	+	+
Saponins	Frothing	Persist frothing	-	-	-	-	-	-
	Liberman B.	Blue or green	-	-	-	-	-	-
Phlobatannins	Hydrochloric Acid	Red ppt	-	+	-	-	+	-
Carotenoids	Carr price's	Blue to red color	-	+	-	-	-	+
Emodol	Borntrager's	Red color	-	-	-	-	-	-
Flavones aglycones	Shibata's	Red to Orange	-	-	-	-	-	-
Terpenoids	Lieberman B.	Pink to Red colour	+	+	+	+	+	-
Alkaloids	Mayer's	Buff ppt	-	+	+	+	+	-
	Wagner's	Dark brown ppt	-	+	+	+	+	
	Dragendoff's	Orange red ppt		+	+	+	+	+
Flavonoids	Shinoda	Dee red	-	+	+	+	-	-
	Tetraoxosulphate (vi) acid	Deep yellow	-	+	+	+	-	-
Cardiac glycoside	Legal's	Deep red colour	-	+	-	+	_	+.
	Kedd's	Violet colour	-	+	-	+	_	+
	Keller – kilanis	Reddish brown	-	+	-	+	--	+
	Baljet	Orange to Deep red	-	+	-	+	_	+
	Liberman	Bluish green	-	+	-	+	_	-

Key: + = Present, - = Absent, Hex = N-Hexane, M_E = Methanol, Cl = Chloroform, E_{ta} = Ethyl acetate, n-But = n-butanol and Aq = Residual aqueous portion.

DOI: 10.56892/bima.v6i03.49

Tannins have been reported to inhibit the growth of microorganisms by precipitating out the microbial protein hence depriving them growth (Deghima, et al., 2021; Huang, et al., 2018; Mujeeb et al., 2014). Tannins were present in the methanol, ethyl acetate and n-butanol fractions of the extract. This explains the good antimicrobial activity of the extract on tested pathogens (Huang et al., 2018). The presence of carbohydrate and reducing sugars in the extract of *A. indica* seed indicate that energy content was high and could be a source of raw material for food and drug industries that utilized carbohydrates reducing sugars (Deghima, et al., 2021). Saponin was detected in some portions of the extract and was found to produce antifungal activity against *C. albican* (Porte et al., 2022; Działo et al., 2016).

The antibacterial activity of the n-butanol fraction of the seed extract was found to be fairly good against gram (+) bacteria e.g. *S. aureus* and gram (-ve) bacteria e.g. *K. pneumonia* and *E. coli.* The antifungal activity of ethyl acetate and n-butanol fraction of the seed extract was found to have little activity against *P. notatum.* The antimicrobial sensitivity test indicated that, the extracts inhibited the growth of *S. aureus S. pyogenes P. aeruginosa, E. coli, S. typhi, C. albicans and P. digitali.* The extracts did not inhibit the growth of *B. subtilis, A. niger and F. oxysorum.* The increase in concentration of the extract also increases the zone of growth inhibition of some of the micro-organism. The highest growth inhibition of 42mm,38 mm,31 mm mm,39 mm diameter, was exhibited by (4µl/ disk), 80mg/ml of compound MND against *S. aureus, S. pyogenes P. aeruginosa S. typhi* and *E. coli* respectively. The lowest zone of growth inhibition was observed with 20 mm and 21 mm diameter as against *C. albicans* and *P. notatum* from the MND (4µl/disk) and (80mg/ml). The highest minimum inhibition concentrations of the extracts on the test isolate was exhibited against *P. aeruginosa, E. coli* and *S. aureus* from n-butanol (10 mg/cm^3). The lowest MIC recorded was against the test Microbes from the n-hexane fraction of the methanol extract.

Table 3: Antimicrobial Assay of some microorganisms against MND and various seed extracts of *A. indica* with their zone of inhibition (mm)

Extract	Sa	Sp	Pv	Pa	Kp	Ec	St	Bs	Pd	Ca	An	Fo	Pn
n-hex (80mg/ml)	11	12	13	10	13	12	12	-	9	16	-	-	4
$CHCl_3$ (80mg/ml)	18	20	15	18	18	19	15	-	10	10	-	-	12
EtoAc (80mg/ml)	25	27	21	24	22	26	22	-	12	13	-	-	13
N – BuOH (80mg/ml)	32	35	22	29	32	32	27	-	28	24	-	-	22
Aq (80mg/ml)	20	23	20	19	16	20	19	-	11	17	-	-	18
Compound MND (4ul/disk)	42	38	31	39	34	30	26	-	27	21	-	-	20
Ampiclox	47	42	38	43	43	35	30	-	37	28	-	-	25

Key:-n-hex = n-hexane, $CHCl_3$ = Chloroform, EtoAc = Ethylacetate, n - BuOH = n-Butanol, Aq = Aqueous, Sa = *S. aureus*, Sp = *S. pyogenes*, Pv = *P. vulgaris,* Pa = *P. aeruginosa*, Kp = *K. pneumonia*, Es = *E. coli*, St = *S. typhi*, Bs = *B. subtilis*, Ca = *C. albicans*, An = *A. nigar*, FO = *F. oxysorum*, Pn = *P. notatum*.

DOI: 10.56892/bima.v6i03.49

Table 4: Minimum Inhibition Concentration of MND and the various partition portion of the extracts against microorganisms

Microoganisms	MND (mg/cm^3) 80 40 20 10 5	n-hex (mg/cm^3) 80 40 20 10 5	EtoAc (mg/cm^3) 80 40 20 10 5	n-BuoH (mg/cm^3) 80 40 20 10 5	Aq (mg/cm^3) 80 40 20 10 5
(+) *S. aureus*	- - - 0x +	- 0x + ++ +++	- - - 0x +	- - - 0x +	- 0x + ++ +++
(+) *S. pyogenes*	- - - 0x +	- 0x + ++ +++	- - - 0x +	- - 0x + ++	- - 0x + ++
(-) *P. vulgaris*	-	-	-	-	-
(-) *P. aeruginosa*	- - - 0x +	- 0x + ++ +++	- - - 0x +	- - - 0x +	- - - 0x ++
(-) *K. pneumonioe*	- - - - 0x	- - 0x + ++	- - - 0x +	- - - - 0x	- - 0x + ++
(-) *E. coli*	- - - 0x +	- 0x + ++ +++	- - - 0x +	- - - 0x +	- - 0x + ++
S. typhi	- - - 0x +	- 0x + ++ ++	- - - 0x +	- - 0x + ++	- - 0x + ++
B. subtilis	-	-	-	-	-
P. digitalis	- - 0x + ++	- - 0x + ++	- - 0x ++ +++	- - 0x + ++	- 0x + ++ +++
C. albicans	- - 0x + ++	- 0x + ++ ++	- - 0x + ++	- - - 0x +	- - 0x + ++
A. niger	-	-	-	-	-
F. oxysonum	-	-	-	-	-
P. notatum	- - 0x + ++	- 0x + ++ +++	- - 0x + ++	- - 0x + ++	- 0x + ++ +++

Key:-n-hex = n-hexane, $CHCl_3$ = Chloroform, EtoAc = Ethylacetate, n - BuOH = n-Butanol, Aq = Aqueous, Sa = *S. aureus*, Sp = *S. pyogenes*, Pv = *P. vulgaris,* Pa = *P. aeruginosa*, Kp = *K. pneumonia*, Es = *E. coli*, St = *S. typhi*, Bs = *B. subtilis*, Ca = *C. albicans*, An = *A. nigar*, FO = *F. oxysorum*, Pn = *P. notatum*.

Table 5: Minimum Bactericidal/Fungicidal concentrations of MND and various extract against test microorganism

Test Organisms	MND(mg/ml) 80 40 20 10 5	n-hex (mg/ml) 80 40 20 10 5	EtoAc (mg/ml) 80 40 20 10 5	n-BuoH (mg/ml) 80 40 20 10 5	Aq (mg/ml) 80 40 20 10 5
(+) *S. aureus*	- - - - 0x	0x + ++ +++ +++	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++
(+) *S. pyogenes*	- - - 0x +	- 0x + ++ +++	- - 0x + ++	- - 0x + ++	- 0x + ++ +++
(+) *P. vulgaris*	-	-	-	-	-
(-) *P. aeruginosa*	- - - - 0x	- 0x + ++ +++	- - 0x + ++	- - 0x + ++	- 0x + ++ +++
(-) *K. pneumonioe*	- - - - 0x	- - 0x + ++	- - - 0x +	- - - - 0x	- - - 0x +
(-) *E. coli*	- - - 0x +	- 0x + ++ +++	- - 0x + ++	- - 0x + ++	- - 0x + ++
S. typhi	- - - 0x +	- 0x + ++ +++	- - 0x + ++	- - 0x + ++	- - 0x + ++
B. subtilis	-	-	-	-	- - 0x + ++
P. digitalis	- 0x + + ++	0x + ++ +++ +++	- - 0x + ++	- - 0x + ++	- 0x + ++ +++
C. alnilans	- 0x + + ++	- 0x + ++ +++	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++
A. Niger	-	-	-	-	-
F. oxysonum	-	-	-	-	-
P. notatum	- - 0x + ++	- 0x + ++ +++	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++

Key: - = No turbidity (No growth), 0x =MIC, + = light turbid (light growth), ++ = Moderate turbid, +++ = High turbidity

The antimicrobial effects of the extracts observed on the micro-organisms could be attributed to the presence of aforementioned secondary metabolites present in the plant (Huang, et al., 2018). Tannins are known to exhibit great potential in phytomedicines, as astrigent as well as anti-parasitic propertry (Deghima, et al., 2021). Terpenes have been reportedly used as anti-tumor and an antiviral agent; some are known to be cytotoxic to tumor cells. The eusdesmane sesquiterpenes are reported to exhibit high antibacterial properties (Działo, et al., 2016; Tanko, et al., 2020). Saponins, have anti-oxidant, anti-cancer, anti-inflammatory and anti-viral properties (Agidew, 2022) while flavonoids

DOI: 10.56892/bima.v6i03.49

are known to exhibit anti-inflammatory properties (Panche et al., 2016). The large zone of inhibition exhibited by the MND against *S. aureus, S. pyogenes, P. aeruginosa, E. coli,Kleb. P. and S. typhi* justifies the use of *A. indica* by traditional healers. The presence of alkaloids also add to the medicinal importance of *A. indica* as significant quantities have been used locally as analgesic, anti-malarial and as stimulants (Sharifi-Rad et al., 2019). Phenolic compounds which represent varieties of natural antioxidants, have been used as nutraceuticals and also in control of human pathogenic (Mutha et al., 2021).

E.coli have been known to be a common cause of diarrhea, infant death and other diarrheagenic infections in humans. The moderate growth inhibition against *E. coli* might be attributed for the use of the seed portion of the extract to treat diarrhea and dysentery. (Diggle and Whiteley, 2020; Kumar and Goel, 2019). The high MIC of *S. aureus* is important in the health care sector, this is because it could be used as an alternative to orthodox antibiotics for treatment of infections caused by the microbes (Gnanamani et al., 2017). *S. aureus* is also known to play an important role in causing skin diseases including superficial and deep follicular lesion. The MIC exhibited by MND extract against *S. aureus* can be important in health care delivery, since it can be used as alternative to Orthodox antibiotics in the treatment of infections caused by these microbes (Tong et al., 2015). The use of these extracts and MND against *S. aureus, E. coli, K. pneumoniea and S. typhi* would reduce the cost of healthcare, since *A. indica* seeds are readily available and affordable (Shaba et al., 2013).

CONCLUSION

The presence of the studied metabolites in the seed portion of *A. indica* suggests great potential in the extract as source of phytomedicines. The zone of inhibition shown by the various extracts and MND against *S. aureus, P. pyogenes, K. Pneumoniea, E. coli and S. typhi,* justifies its use in traditional medical practice for the treatment of sores, boils and dysentery. The strong activity of the isolate and n-butanol extract on the test microbes, shows that, the seed extract of *A. indica* can be a good source of compounds that are effective against some infectious diseases causative agents. The isolated MND have exhibited impressive activity against some of the test microbes, especially when the isolate is less combine with other secondary metabolites. Therefore, the observed antimicrobial properties of the seed extract of *A. indica* corroborate its use in the ethno medicine.

Acknowledgment

The authors wish to express their gratitude to the Tertiary Education Trust Fund (TETFund) for funding this study and the management of IBB University, Lapai for creating an enabling environment for research to thrive.

REFERENCES

Aarthy, T., Mulani, F. A., Pandreka, A., Kumar, A., Nandikol, S. S., Haldar, S. and Thulasiram, H. V. (2018). *Tracing the biosynthetic origin of limonoids and their functional groups through stable isotope labeling and inhibition in neem tree (Azadirachta indica) cell suspension. BMC Plant Biology,* 18(1),230.

Abubakar I., Mann A. and Mathew J. T. (2015a). Phytochemical composition, Antioxidant and Anti-nutritional properties of root-bark and leaf methanol extracts of *Senna alata L.* Grown in Nigeria. *African Journal of Pure and Applied Chemistry*, 9(5),91-97.

DOI: 10.56892/bima.v6i03.49

Abubakar I., Mann A. and Mathew J. T. (2015b). Evaluation of Phytochemical, Anti-nutritional and Antioxidant Potentials of Flower and Seed Methanol Extracts of *Senna alata* L. Grown in Nigeria. *American Journal of Applied Chemistry*. 3(3),93-100.

Abu-Reidah, I.M. and Taamalli, A. (2022). Phenolic Compounds: Extraction, Optimization, Identification and Applications in Food Industry. *Processes*, 10,12.

Adamu, S. S. and Sajo, I. Y. (2021). Antibacterial Activity of Cow Ghee, Urine, And Milk On Some Pathogenic Organisms (Escherichia coli and Staphylococcus aureus). *Bayero Journal of Medical Laboratory Science,* 6(2),36–44.

Adem C., Demet G., Aydin T. and Birteksöz, A. S. (2011). Synthesis, Spectral Characterizations and Antimicrobial Activity of Some Schiff Bases of 4-Chloro-2-Aminophenol. *Bull. Chem. Soc. Ethiop*., 25(3),407-417.

Benarba, B. and Pandiella A. (2020). Medicinal Plants as Sources of Active Molecules Against COVID-19. *Front. Pharmacol*. 16,15-28.

Agidew, M.G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bull Natl Res Cent* 46,87.

Brown, G.D. (2003). ^{13}C-^{2}H Correlation NMR Spectroscopy studies of the In vivo Transformation of Natural products from *Artemisia annua*. *Journal of Phytochemistry Research*. 5,45–59.

Chóez-Guaranda, I., Viteri-Espinoza, R., Barragán-Lucas, A., Quijano-Avilés, M. and Manzano, P. (2022). Effect of solvent-solvent partition on antioxidant activity and GC-MS profile of *Ilex guayusa* Loes. leaves extract and fractions. *Natural product research*, 36(6), 1570–1574.

Deghima, A., Righi, N., Rosales-Conrado, N., León-González, M. E., Baali, F., Gómez-Mejía, E., Madrid, Y. and Bedjou, F. (2021). Anti-inflammatory activity of ethyl acetate and n-butanol extracts from Ranunculus macrophyllus Desf. and their phenolic profile. *Journal of ethnopharmacology*, 265,113347.

Diggle, S. P. and Whiteley, M. (2020). Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. *Microbiology (Reading, England)*, 166(1),30–33.

Działo, M., Mierziak, J., Korzun, U., Preisner, M., Szopa, J. and Kulma, A. (2016). The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders. *International Journal of Molecular Sciences*. 17(2),160.

Francis, A. C. (2003). *Organic Chemistry.* Mcgraw Hill University of Virginia Fifth Edition. N. York. U.S.A. Pp. 1011-1346.

Gnanamani, A., Hariharan, P. and Paul-Satyaseela, M. (2017). Staphylococcus aureus: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach. In S. Enany, & L. E. C. Alexander (Eds.), Frontiers in Staphylococcus aureus. IntechOpen.

Guimarães, H. A., Braz-Filho, R. and Vieira, I. J. C. (2012). *1H and 13C-NMR Data of the Simplest Plumeran Indole Alkaloids Isolated from Aspidosperma Species. Molecules,* 17(3),3025–3043.

Hostettmann, K. and Wolfende, J. L. (2004). Application of Liquid Chromatography/UV/MS and Liquid Chromatography/NMR for The On-Line Identification of Plant Meatbolites. In: C. Tringali (Eds.) *Bioactive Compounds from Natural*

DOI: 10.56892/bima.v6i03.49

Sources Isolation, Characterization and Biological Properties. e-Library, Taylor and Francis. Pp. 32-68.

Huang, Q., Liu, X., Zhao, G., Hu, T. and Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Animal nutrition (Zhongguo xu mu shou yi xue hui)*, 4(2),137–150.

Hussein, R. A. and El-Anssary, A. A. (2018). Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. In (Ed.), Herbal Medicine.

Perveen, S. and Al-Taweel, A. (2019). *Pharmacognosy - Medicinal Plants Natural Products in Drug Discovery.* 1-19.

Imanuddin, R., Hidayat, A., Rachmat, H.H., Turjaman, M. P., Nurfatriani, F., Indrajaya, Y. and Susilowati, A. (2020). Reforestation and Sustainable Management of *Pinus merkusii* Forest Plantation in Indonesia: A Review. *Forests*. 11,1235.

Jayawardene, K., Palombo, E. A. and Boag, P. R. (2021). Natural Products Are a Promising Source for Anthelmintic Drug Discovery. *Biomolecules*, 11(10), 1457.

Kumar, N. and Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology reports (Amsterdam, Netherlands)*, *24*, e00370.

Lin, M., Yang, S., Huang, J. and Zhou, L. (2021). Insecticidal Triterpenes in Meliaceae: Plant Species, Molecules and Activities: Part (*Aphanamixis-Chukrasia*). *International Journal of Molecular Sciences*, 22(24),13262.

Moin, M.S., Siddiqui, J.I., Alam, M.A., Khatoon, F., Khan, S. and Minhajuddin, A. (2021). Ethnomedicinal potential of widely used plant Azadirachta indica A. Juss: A comprehensive review. *J Phytopharmaco*, 10(6),456-467.

Mohammed, M. (2022). Isolation of bioactive constituents from the ethylacetate leaf extract portion of Gmelina arborea (Verbenaceae). *Journal of Pharmacognosy and Phytochemistry,* 11(1),71-77.

Mohammed, M., Jajere, U.M., Danmalam, A., Kolo, M. T. and Babakano, M. (2019). Isolation and structure elucidation of Ipolamiide from the stem bark of stachytarpheta angustifolia mill vahl (VERBENACEAE). *Bima Journal of Science and Technology,* 3(1),96-107.

Mohammed, M., Bugaje, I. M. and Garba, M. A. (2015). Three iridoid glycosides from the Root extract of *Stachytarpheta angustifolia* Mill (Vahl) Verbenaceae. American Research Institute for Policy Development. *Journal of Chemistry and Biochemistry*, 3(1)47-62.

Mottaghipisheh, J. and Iriti, M. (2020). Sephadex® LH-20, Isolation, and Purification of Flavonoids from Plant Species: A Comprehensive Review. *Molecules (Basel, Switzerland)*, 25(18),4146.

Mujeeb, F., Bajpai, P. and Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed Research International*, 14, 497606.

Mutha, R.E., Tatiya, A.U. and Surana, S.J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Futur J Pharm Sci* **7**, 25.

Ndamitso, M.M., Musah, M., Mohammed-Hadi, Z., Idris, S., Tijani O.J., Shaba

DOI: 10.56892/bima.v6i03.49

E.Y. and Umar, A. (2013). Analysis of Phytochemical Content and Antibacterial Activity of Tapinanthus dodoneifolius Extracts. *Researcher*, 5(5),54-59.

Ohikhena, F. U., Wintola, O. A. and Afolayan, A. J. (2017). Evaluation of the Antibacterial and Antifungal Properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques. *The Scientific World Journal*, 20, 9658598.

Oyvind, M. Anderson, K. and Kenneth, R. M. (2006). Flavonoids, Chemistry, Biochemistry and Applications, CRC Press, Tyylorandfrancis group, Boca Raton, London, New York.

Panche, A. N., Diwan, A. D. and Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47.

Petruzzello, M. (2022). *Azadirachta indica,* nim. https://www.britannica.com/plant/neem-tree.

Porte, S., Joshi, V., Shah, K. and Chauhan, N.S. (2022). Plants' steroidal saponins - A review on its pharmacology properties and analytical techniques. World J Tradit Chin Med 8:350-85.

Shaba, E. Y., Mathew, J. T., Inobeme, A., Mustapha, S., Tsado, A.N. and Amos, J. (2013). Phytochemical and Antimicrobial Screening of the Fruit Pulp of *Canarium schweifurthii* (ATILE). *Nigerian Journal of Chemical Research,* 18(1),6-10.

Sharifi-Rad, J., Kobarfard, F., Ata, A., Ayatollahi, S. A., Khosravi-Dehaghi, N., Jugran, A. K., Tomas, M., Capanoglu, E., Matthews, K. R., Popović-Djordjević, J., Kostić, A., Kamiloglu, S., Sharopov, F., Choudhary, M. I. and Martins, N. (2019). *Prosopis* Plant Chemical Composition and Pharmacological Attributes: Targeting Clinical Studies from Preclinical Evidence. *Biomolecules*, 9(12),777.

Sofi, M. S. and Nabi, S. (2018). Induction of caspase-3 dependent apoptosis, cell cycle arrest and cytotoxicity in breast cancer cells by abrus precatorius. *International Journal of Pharmacy and Pharmaceutical Sciences,* 10(8),29.

Tanko, E., Dauda, B.E.N., Mann, A., Oyeleke, S.B., Fadipe, L. A. and Mathew J.T. (2020). Phytochemical and Antibacterial Studies of *Ensete gilletii* (E.A.J. De Wildman) Stem Extract and Fractions. *Nigerian Research Journal of Engineering and Environmental Sciences*, 5(1), 390-398.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. and Fowler, V. G., Jr (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28(3),603–661.

Torres-Contreras, A.M., Garcia-Baeza, A., Vidal-Limon, H.R., Balderas-Renteria, I., Ramírez-Cabrera, M.A. and Ramirez-Estrada, K. (2022). Plant Secondary Metabolites against Skin Photodamage: Mexican Plants, a Potential Source of UV-Radiation Protectant Molecules. Plants, 11,220.

Tsado A. N, Abdulkadir A., Shaba A. M., Mathew J. T., Umar A. M. and Chirama D. N. (2018a). Phytochemical, Antioxidant and Antimicrobial Potentials of Methanol Seed Extracts of *Carica Papaya*. *World Wide Journal of*

DOI: 10.56892/bima.v6i03.49

Multidisciplinary Research and Development, 4(3), 24-28.

Tsado, A. N., Saidu, T. B., Santali, E. S., Shaba, A. M., Gana, E. N. and Mathew J. T**.** (2019b). Phytochemicals and Hypoglycemic Properties of Methanol Leaf Extract of *Phyllanthus amarus* Conference Proceedings/Paper. *Assumption University-ejournal of Interdisciplinary Research*, 4(1), 68–75.

Twaij, B.M. and Hasan, M.N. (2022). Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses. *Int. J. Plant Biol. 13*,4-14.

Welz, A.N., Emberger-Klein, A. and Menrad, K. (2018). Why people use herbal medicine: insights from a focus-group study in Germany. *BMC Complement Altern Med*., 18,92.

Wylie, M.R. & Merrell, D.S. (2022). The Antimicrobial Potential of the Neem Tree *Azadirachta indica.* Front. Pharmacol. 23,1-32.

Zheng – Xiang X., Dan – Dan Z., Shuang L. Y. – Z., Zhang, H., Hong – Sheng T., Shi – Lin C. and Hong – Xi X. (2012). Bioassay – Guided isolation of prenyiatedXanthones and polycylicAcylphloroglucinols from the leaves of *Garcinia nujiangensis*, *J. Nat. Prod.* 75,(8),1459 – 1464