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Isolation and Characterization of Lupeol from the Root Bark of *Pterocarpus erinaceus* Poir and its *In-vitro* Antimicrobial Assay

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ABSTRACT

Lupeol, a pentacyclic triterpenoid was isolated from the root bark extract of *Pterocarpus erinaceus.* Traditionally, the plant used is some parts of Nigeria and Africa for the treatment of inflammation, cancer, hemmorrhoid, wounds, worm expulsion and abdominal pain. Silica gel column chromatography of the root bark extract led to the isolation of lupeol (15 mg). The compound appeared as white needle-like crystals with melting point of 217-218 ^{o}C. Spectral data were obtained from Fourier transform infrared spectrophotometer (FTIR) and Gas Chromatography-Mass spectrophotometer (GCMS). Antimicrobial bioassay carried out showed that the isolated compound (Lupeol) is sensitive to *Methicillin resistant Staphylococcus aureus, Vancomycin resistant enterococci, Staphylococcus aureus, Klebsiella pneumonia, Helicobacter pylori, Campylobacter fetus, Proteus mirabilis, Candida albicans* and *Candida tropicalis* with zones of inhibition of 27 – 29 mm for bacteria species and 24 – 29 mm for fungi species. These results are comparable to that of standard drugs, Ciprofloxacin and Fluconazole, with zone of inhibition 30 – 39 mm and 32 – 35 mm respectively. Minimum Inhibitory Concentration (MIC) of the compound was observed between 12.5-25.0 µg/ml while the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) ranged from 25.0 to 50.0 µg/ml. This study revealed that the antimicrobial activity against the selected pathogens is a result of the presence of Lupeol and other bioactives in the plant.

Keywords: Antimicrobial assay, FTIR, GC-MS, Lupeol and *Pterocarpus erinaceus.*

INTRODUCTION

Bioactive compounds from plants have played an important role in the development of new or novel drugs. These compounds are synthesized by plants during their metabolic activities and they are synthesized when the plant needs to adapt to certain changes within its environment. These compounds have very complex chemical structures and they are known as secondary

metabolites. Triterpenes are an example of a class of plant secondary metabolites. Research has proven that they play an important role in exerting various physiological actions in humans and animals [1].

Lupeol is a pentacyclic triterpene reported to have important physiological and therapeutic effects in human health issues. Studies in the past have shown several important pharmacological activities of lupeol. In various *in-vitro* and *in-vivo* models, lupeol has been tested for its therapeutic efficiency against conditions such as inflammation, wounds, arthritis, diabetes, cardiovascular diseases, renal disorder, hepatic toxicity, microbial infections and cancer [2]. This phytosterol is mostly found in fruits, and vegetables. It has been found to be pharmacologically effective in treating various diseases under preclinical settings (in animal models) irrespective of the routes of administration such as, topical, oral, intra-peritoneal and intravenous. Lupeol has been reported to selectively cure unhealthy human cells, while sparing normal and healthy cells [3]. Because of its non-toxicity to normal cells and tissues, chemoprevention with lupeol is relatively a new area of oncology [4].

Pterocarpus erinaceus also known as Senegal rose wood or African barwood belongs to the Papilionaceae family. *Pterocarpus erinaceus* is a medium sized tree that grows to between 12-15 mm tall with trunk diameter averaging 1.2 and 1.8 m. It is predominantly found in Senegal, the Gambia, Nigeria, Benin, Sudan and Ghana. The brownish black bark is scaly and thick and also produces a red sap when cut. The leaves of *P. erinaceus* are alternate and grow up to 3 cm in length, the flowers are irregular, yellowish in colour and the petals are 10-12 mm long. The seed of *P. erinaceus* are enclosed in a green pod when fresh but this turns brown when ripe and dry, the pods are usually 5 cm – 8 cm long and contain 3-5 kidney shaped seeds [5]. The medicinal uses of *P. erinaceus* include the use of the leaves as a febrifuge, the bark is used for tooth and mouth troubles; the bark resin formed into decoction is used as astringent for severe diarrhea and dysentery. The grated root bark is mixed with tobacco and smoked in a pipe as a remedy against cough and fever [6]. The decoction or infusions of the stem and root bark are used for treatment of tumors, urethral discharges, ring worm of the scalp, wounds and chronic ulcer [7]. The leaves, stem bark and roots of *P. erinaceus* are used in Burkina Faso to treat inflammatory diseases, ulcer, rheumatism, fever, bacterial infections, malaria and stomach ache. Leaf decoctions are used to treat syphilis, fever and are also used as aphrodisiac [8].

This paper, reports the novel isolation, characterization and antimicrobial assay of lupeol from the root bark of *Pterocarpus erinaceus* Poir.

MATERIALS AND METHODS

Sample collection and identification of the plant

The plant *P. erinaceus* was collected fresh from Taraba State, Nigeria in 2023. It was authenticated at the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria. A specimen voucher number 39185 was deposited at the Herbarium for reference purposes. The root bark was separated, air- dried and pulverized using a wooden mortar and pestle.

Extraction

Plant material (750 g) was extracted successively using 1.5 L of n-hexane, ethyl acetate and methanol respectively by cold maceration for 14 days in each case. The extracts were concentrated *in-vacuo* using rotary evaporator at 40 ^{o}C. The ethyl acetate fraction was then subjected to antimicrobial studies [9] and after subsequent isolation, the isolated compound was subjected to spectral analysis.

Isolation procedure/column chromatography

One hundred and twenty grams (120 g) of silica gel (60-120 mesh) was made into slurry with 100% n-hexane and was packed into a 2.5 × 63 cm glass column and allowed to stand for about one hour to attain stability. Approximately 6 g of ethyl acetate extract was pre-adsorbed on 6 g of silica gel and loaded onto the column. The loaded sample was eluted gradiently starting with n-hexane (100%), n-hexane: ethyl acetate (99:1), n-hexane: ethyl acetate (98:2), n-hexane: ethyl acetate (97:3) and n-hexane: ethyl acetate (96:4). A total of 80 fractions of 50 ml each were collected and monitored by TLC and by spraying with 10% sulphuric acid. The fractions were regrouped into four namely B1 (9 mg), B2 (20 mg), B3 (10 mg), B4 (12 mg) and B5(10 mg) based on their TLC spot profile. Fraction B2 was found to have a significant proportion of the compound of interest so it was subjected to further purification by preparative TLC using the solvent system Hexane / Ethyl acetate (9:1). A single homogenous spot was obtained on TLC with two different solvent systems Hexane/Ethyl acetate (9:1) and (8:2). The compound (15 mg) and labeled (PEB2), appeared as white needles – like compound and was subjected to spectral analyses.

Structural determination of the isolated compound PEB2

The structure of the compound was determined using GC-MS (Agilent 19091S-433UI) and Infrared spectrum (IR) was recorded on FTIR- 400s (Shimadzu) in CCl_4. The isolated compound was weighed (1 mg) and dissolved in 200 µL chloroform (HPLC grade) in a glass vial and then injected into the GC - MS for analysis. For the IR analyses, the pure compound was weighed (3 mg) and mixed with 5 mg of KBr and then ground to a very fine powder. The powder was compressed under high pressure using a press to produce pellets of the compounds to be analyzed.

Triterpenoid test for isolated compound PEB2

The isolated compound (1 mg) was placed in a test tube, a few mg of Liebermann- Buchard reagent (glacial acetic acid + Conc.H_2SO_4) was added. The formation of red violet color was observed at the interface of test tube indicates the compound is a triterpenoid.

Determination of antimicrobial bioassay of the isolated compound PEB2

The test organisms used for this analysis were clinical isolates of bacteria and fungi obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. The isolates were: Methicillin resistant *Staphylococcus aureus*, *Vancomycin Resistant enterococci*, *Staphylococcus aureus, Campylobacter fetus, Escherichia coli*, *Helicobacter pylori*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Candida albicans, Candida krusei* and *Candida tropicalis*. Antimicrobial activity was measured using agar well diffusion method according to the Committee for Clinical Laboratory Standard [10].

RESULTS AND DISCUSSION

The powdered root bark of *P. erinaceus* was extracted successively with n-hexane, ethyl acetate and methanol. The masses and percentage yields revealed 22.5 g (4.5%) of methanol crude extract, 15.6 g (3.1%) of crude ethyl acetate extract and 12.3 g (2.5%) of n-hexane crude extract. The higher yield of the methanol extract is due of it high polarity. Dhawan and Gupta [11] reported that highly polar solvents are better for extraction of plant material than others. Result of the thin layer chromatography analysis of the root bark extract indicated five phyto-constituents. Their R_f values and their various colours in the developing reagent 10% H_2SO_4 are presented in Table 1. The results of fractions obtained from the column chromatography (Table 2) showed five fractions coded B1 to B5 at various solvent systems and their corresponding number

of spots. Fraction B2 was found to have a significant proportion of the compound of interest and was subjected to further purification by preparative TLC using the solvent system Hexane / Ethyl acetate (9:1) to obtain a white-needle-like compound that weighs (15 mg). The compound had melting point of 217 – 218 °C and retention factor (Rf) of 0.52 respectively (Table 3). The FTIR spectrum of the compound **PEB2** (Figure 1) revealed an intense broad absorption frequency peak at 3302.4 cm^{-1} which is typical of the O-H bond of a hydroxyl group. A fairly intense band at 2922.2 cm^{-1} that can be assigned to an aliphatic C-H (stretch) was observed. The C=C vibration was observed at 1640.0 $cm^{-`1}$. Other peaks present are 1457.4 and 1379.1 cm^{-1} due to bending vibrations of alkane and alkenes respectively (Table 5). The IR absorbance values are in concordance with Silverstein *et al*., [12]. The MS spectrum was used to analysed and confirm the chemical structure. The molecular ion peak of the spectrum was identified with m/z 426 and it was found to be equal to the calculated molecular weight of the compound lupeol with molecular formula $C_{30}H_{50}O$. Fragmentation of the molecular ion by removal of a methyl group gave the fragment peak at *m/z* 411. This peak is characteristic of a pentacyclic triterpene with an isopropenyl group [13]. When it fragments by losing ethene (CH_2=CH_2) gas, the fragment at *m/z* 383 is produced [14]. Fragment *m/z* 383 further loses -$C_{13}H_{22}$ and -H_2O (or -$C_{13}H_{24}O$), which gives fragments *m/z* 207 (allocates the hydroxyl group at C3 position) and *m/z* 189 [15]. These fragments may also arise from the cleavage between C-8/C-14 and C-12/C-13 bonds (with proton transfer) and is usually confirmatory that such a compound possess a lupane or hopane skeleton [16]. The fragments at *m/z* 189 and *m/z* 218 indicate that compound **PEB2** is a pentacyclic triterpene. Other fragments at *m/z* 43, 81, 107, 147, 175, 257 and 315 are also associated with lupeol [17; 18]. The results of characterization with MS based Library: NIST14.Lib data and comparison with the above cited literatures lead to the suggestion of the compound **PEB2** as lupeol. The compound also gave a positive test using Buchard -Liebermann test for triterpenoid (Table 4). The structure of the isolated compound (Lupeol) and it mass spectrum is as shown in Figure 2.

Table 1: Thin layer chromatography of the ethyl acetate extract

Compound spots	R_f Values	Colour in 10 % H_2SO_4
1.	0.57	Purple
2.	0.54	Violet
3.	0.64	Violet
4.	0.32	Purple
5.	0.16	Brown

Key: $R_f \Rightarrow$ Retention factor

Table 2: Fraction from column chromatography of Ethyl acetate extract

Fractions	Eluting solvents	Number of major spots
B1	Hexane; Ethyl acetate (95:5)	3
B2	Hexane; Ethyl acetate (90:10)	2
B3	Hexane; Ethyl acetate (85:5)	4
B4	Hexane; Ethyl acetate (80:20)	4
B5	Hexane; Ethyl acetate (70:30)	3

Table 3: TLC profile of the isolated compounds PEB2

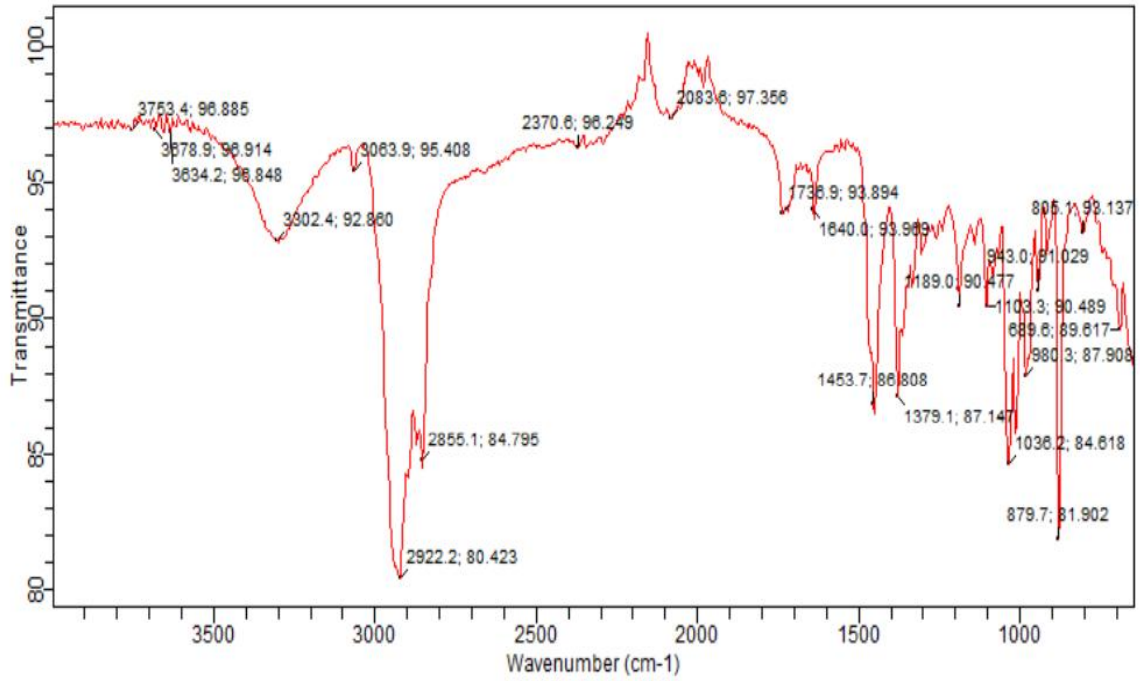
Compound code	solvent system	Observed No of spot	R_f Value	Melting point (°C)
PEB2	Hexane ethyl acetate (9:1)	1	0.52	216-218

Table 4: Buchard -Liebermann test for triterpenoid on the Isolated Compound PEB2

Isolated compound	Test	Result	Inference
PEB2	Liebermann's test	+	Terpenoid present

Table 5: Characteristic IR intensities of compound PEB2

Functional groups	O-H (stretch) alcohol	C-H (stretch) alkanes	C=C (Stretch) alkenes	C-H Ben. vib (Alkanes)	C-H Ben.vib (alkenes)
Frequency cm^{-1}	3302.3	2922.7	1640.0	1457.7	1379.1



. Figure 1: IR Spectrum of PEB2 (Lupeol) isolated from the root bark of *Pterocarpus erinaceus*

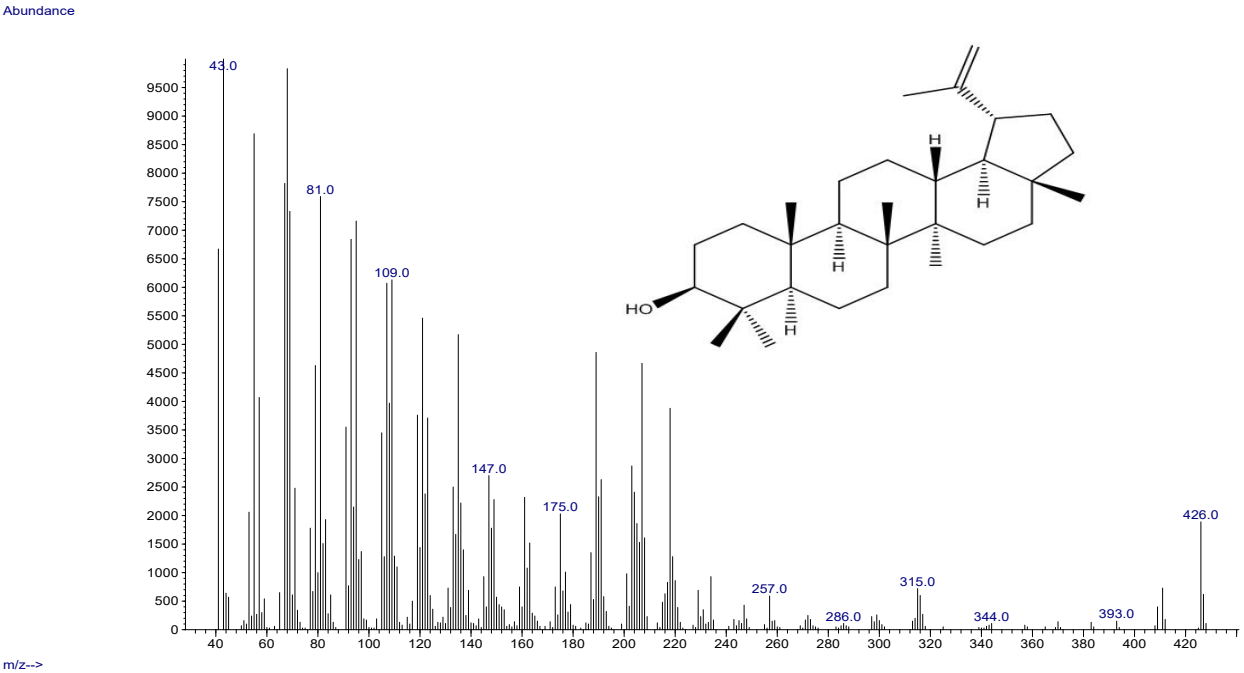


Figure 2: Mass spectrum of PEB2 (lupeol) and its structure

The isolated compound PEB2 was screened for antimicrobial activities using eleven pathogenic microbes. Results (Table 6) shows that the isolated compound was sensitive to Methicillin resistant *Staphylococcus aureus*, Vancomycin Resistant *Enterococci*, *Staphylococcus aureus*, *C. fetus*, *Proteus mirabilis* and *Candida tropicalis whereas Escherichia coli, Helicobacter pylori, K. pneumonia and Candida krusei* were resistant against the isolated compound. These shows that the isolated compound PEB2 exhibits broad spectrum activity. This was in line with results reported by Benziane *et al*., [19] and Ghada *et al*., [20]

Table 6: Antimicrobial activity of the isolated compound PEB2

Test organisms	PEB2	CIP	FLU
Methicillin resistant *Staphylococcus aureus*	S	R	R
Vancomycin resistant *enterococci*	S	S	R
Staphylococcus aureus	S	S	R
Escherichia coli	R	S	R
Helicobacter pylori	R	S	R
Campylobacter fetus	S	S	R
Klebsiella pneumonia	R	0	R
Proteus mirabilis	S	S	R
Candida albicans	S	R	S
Candida krusei	R	R	S
Candida tropicalis	S	R	S

Key; S: ⇒ Sensitive, R ⇒ Resistance CIP ⇒ Ciprofloxacin, FLU ⇒ Fluconazole

Table 7: Zones of inhibition of the compound against the test microorganisms (mm)

Test organisms	PEB2	CIP	FLU
Methicillin resistant *Staphylococcus aureus*	28	0	0
Vancomycin resistant *enterococci*	29	32	0
Staphylococcus aureus	25	35	0
Escherichia coli	0	39	0
Helicobacter pylori	0	34	0
Campylobacter fetus	28	30	0
Klebsiella pneumonia	0	0	0
Proteus mirabilis	27	30	0
Candida albicans	29	0	35
Candida krusei	0	0	32
Candida tropicalis	24	0	32

Key; CIP ⇒ Ciprofloxacin, FLU ⇒ Fluconazole

Table 8: Minimum inhibitory/ minimum bactericidal concentration of Lupeol against the test organisms (µg/ml)

Test organisms	MIC	MBC/MFC
Methicillin resistant *Staphylococcus aureus*	12.5	25
Vancomycin resistant *enterococci*	12.5	25
Staphylococcus aureus	25	50
Escherichia coli	-	-
Helicobacter pylori	-	-
Campylobacter fetus	12.5	25
Klebsiella pneumonia	-	-
Proteus mirabilis	12.5	50
Candida albicans	12.5	
Candida krusei	-	-
Candida tropicalis	25	50

The result of the zones of inhibition (Table 7) showed that the isolated compound exhibited comparable zone of inhibition with the test drug ciprofloxacin against VR*E, S. aureus*, *C. fetus* and *Proteus mirabilis* with inhibition range of 30 – 39 mm. Fluconazole had zone of inhibition against *C. albicans, Candida krusei and C.* tropicalis with the range of 32 – 35 mm. These results are comparable to that of isolated compound PEB2 with zone of inhibition between 27 – 29 mm for bacteria and 24 – 29 mm for fungi hence; the extract is as effective as the test drugs. The result was in agreement with Anas *et al*., [21] where they reported zones of inhibition of between 24-30 mm for lupeol and 32-40 mm for standard control drugs ciprofloxacin and fluconazole. The result of the MIC and MBC/MFC of the isolated compound PEB2 (Table 8) revealed that the compound had MIC of 12.5 µg/ml against *MRSA*, *VRE*, *C. fetus*, *P. mirabilis* and *C. albicans* respectively while *S. aureus* and *C. tropicalis* had MIC of 25 µg/ml. This implies that the isolated compound is active and since it was able to inhibit the growth of the organisms at much lower concentration than ciprofloxacin and fluconazole (5 mg/ml) which are standard drugs for the treatment of these infections. This finding was in line with report of Jean De Dieu *et al*., [22] who observed the MIC of 12.5 µg/ml in lupeol, stigmasterol and β-sitosterol against *Streptococcus pyogens, Candida tropicalis* and *Salmonella. typhi*. The result of the MBC/MFC for the isolated compound also revealed that MR*SA,* VR*E, C. fetus and C. albicans* had MBC at a concentration of 25 µg/ml whereas *S. aureus and P. mirabilis* had MBC/MFC of 50 µg/ml respectively. These results also imply that the isolated compound has a higher activity and would therefore kill the organisms; it also means that the isolated compound would be very effective in curing diseases caused by any of these microorganisms. This finding is in agreement with results reported by Bako *et al*., [23] which demonstrated the importance of Triterpenoid in the antimicrobial activity of *Pterocarpus erinaceus.* Lupeol or 3-hydroxylup-20(29)-ene), is a

powerful bioactive compound present in different medicinal plants [24]. A wide range of bioactivities and bioassays of lupeol have been reviewed [25] which suggest its useful medicinal properties with a diversity of action against various diseases. The presence of lupeol could be the linked to the antimicrobial properties observed in the plant this is because it has been reported to be antiangiogenic, antioxidative, antimicrobial, Antimalarial and anti-inflammatory in nature [26].

CONCLUSION

From the results, plant *Pterocarpus erinaceus* contains Lupeol that was detected by chemical test, thin layer chromatography/column chromatography and confirmed by Gas Chromatography-Mass spectroscopy and Fourier transform infrared spectroscopy. The antimicrobial bioassay suggests its useful medicinal properties with a diversity of action against various pathogens. The plant extracts of *Pterocarpus erinaceus* could therefore, be seen as a potential source of useful drugs and this justifies the claim by the traditional healers.

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REFERENCES

[1]. David, J. P. (2001). Phytochemistry and medicinal plants. *Phytochemistry*, *56*, 237-243.

[2]. Chaturvedi, P. K., Bhui, K., & Shukla, Y. (2008). Lupeol, Connotations for chemoprevention, *Cancer Letters,* 263, 1-13.

[3]. Hifzur, S., & Mohammad S., (2011). Beneficial health effects of lupeol triterpene: A review of preclinical studies, *Life Sciences*, *88*, 285–293

[4]. Palanimuthu, D., Baskaran, N., Silvan, S., Rajasekaran, D., & Manoharan, S. (2012). Lupeol a bioactive triterpene prevents tumor formation during 7,12- dimethyl Benz(a) anthracene-induced oral carcinogenesis, *Pathology and Oncology Research*, *18*: 1029–1037.

[5]. Orwa, C., Mutua, A., Kindt, R., Jamnadas, R., & Simons, A. (2009). Agroforestry database: A tree species reference and selection guide version. World Agroforestry centre International Centre for Research in Agroforestry, Nairobi, Kenya P. 340

[6]. Irvin, F.R. (2005). Woody plants of Ghana. Oxford University Press. London. P.868

[7]. Dalziel, J. M (2J007). The useful plants of west tropical Africa. The crown agents d\or the colonies, London P. 612

[8]. Nadembega, P., Boussim, J. J., Nikiena, J. B., Poli, F., & Antogeni, F. (2011). Medicinal plants in Baskoure, Kourittenga province, Burkina Faso: an ethnobotanical study. *Journal of ethnopharmacology, 133*, 378-395

[9]. Sofowora, A (2008). Phytochemical screening. Medicinal Plants and Traditional Medicine in Africa. 3rd ed. Spectrum books limited, Ibadan, Nigeria, 199-204.

[10]. National Committee for Clinical Laboratory Standards(NCCLS) (2002). Performance Standards for antimicrobial susceptibility testing. 8th Informational supplement. *Nat. Comm. Clin. Lab. Stand,* Villanova, Pa.

[11] Dhawan, D., & Gupta, J. (2017). Comparison of different solvents for extraction potential from *Datura metal* plant Leaves. *International Journal of Biological Chemistry*, *11*, 17-22

[12]. Silverstein, R. M., Bassler, G. C., & Morrill, T. C., (1999). Spectrometric Identification of organic Compounds, 5th Ed., Wiley.

[13]. Pereira, F. B. M., Domingues, F. M. J., & Silva, A. M. S. (1996). Triterpenes from *Acacia dealbata*. *Natural Product Letters*, *8,* 97–103

[14]. Carvalho, T.C., Polizeli, A. M., Turatti, I. C. C., Severiano, M. E., Carvalho. C. E., & Ambrosio, S. R. (2010). Screening of filamentous fungi to identify biocatalysts for lupeol biotransformation. *Molecules 15*(9), 6140–6151

[15]. El Sayed, A. M. (2016). Leaves of *Schinus polygamous* (Cav.) Cabrera (Anacardiaceae) are a potential source of hepatoprotective and antioxidant phytochemicals. *Journal of Medicinal Plants Research*, *10*, 223–231

[16]. Ogunkoya, L. (1981). Application of mass spectrometry in structural problems in triterpenes. *Phytochem 20*, 121–126

[17]. Kiria, M. J. (2018). Antimicrobial Activity and Constituents of the Root Bark of *Corpuscular eriocalyx*. MSc Thesis, University of Nairobi, Nairobi, Kenya

[18]. Leite, S. P., Adami, T. B., Bjerk, T. R, dos Reis Souza, M. R., Cardoso, C. A. L., Krause, L. C. & Caramao, E. B. (2020). Ultrasonic assisted extraction of bioactive compounds from different parts of *Hancornia speciosa* Gomes. *Journal of Medicinal Plants Research 14*(7), 300–308

[19]. Benziane, M.M, Bouzidi, K, Bellahouel, N, Merah, S., Fortas, B., Soulimani, Z., Saidi, R., & Derdour, S.A. (2012). Antimicrobial Activity of the Alkaloids and Saponin Extracts of *Anabasis articulata*. *Journal of Biotechnology Pharmacy Research*, *3*(3), 54–57.

[20]. Ghada, G., Kamilia, A., Abo, E., & Ghada, A. (2021): Triterpenoids from the Aerial Parts of *Anabasis articulata* (Forssk): Gastroprotective Effect *In-vivo* with in Silico Studies, Cytotoxic and Antimicrobial Activities. *Natural Product Research,* 1-10

[21]. Anas, A., Ahmed, A., Umar, S., Jajere, U.M., Mshelia, E. H. & Natasha, O.B. (2017). Inhibitory Effect of Isolated Lupeol from Stem Bark of *Diospyros mespiliformis* Horsch (Ebenaceae) against some Microbial Pathogens. *Journal of Pure and Applied Sciences*, *10* (1), 293 – 299

[22]. Jean De Dieu, T., Jules, R.K., Mathieu, T., Timothée, J., Kenla, N. & Pierre, T. (2011). The Antimicrobial Activities of Extract and Compounds Isolated from *Brillantaisia lamium*: *Iranian Journal of Medical Sciences*, *36* (1), 23-37

[23]. Bako, B., Ushie, O. A., & Malu, S. P. (2023). Lupeol and Lauric Acid Isolated from Ethyl Acetate Stem Extract of Justicia secunda and Their Antimicrobial Activity. *Journal of Chemistry Society of Nigeria*, *48* (1), 081-094

[24]. Cammareri, M. Consiglio, M. F., Pecchia, P. Corea, G. Lanzotti, V. Ibeas, J. I., Tava, A., & Conicella, C. (2008). Molecular characterization of α –amyrin synthase from *Aster sedifolius* L. and triterpenoid saponin analysis, *Plant Science. 175,* 255- 261.

[25]. Gallo, M. B. C., & Sarachine, M. J. (2009). Biological activities of lupeol. *International Journal of Biomedical and Pharmaceutical Science, 3,* 46–66

[26]. Sudhahar, V., Kumar, S. A., & Varalakshmi, P (2008). Role of lupeol and lupeol linoleate on lipemicoxidative stress in experimental hypercholesterolemia. *Life Science*. *78,* 1329-1335