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Phytochemical, proximate and antibacterial studies of the ethanol leaf extract of *Abrus precatorius* (Fabaceae)

Mohammed IBRAHIM[1*], Samuel Emmanuel EGGA[1], John Rople GUNGSHIK[1], Victoria Chomini TOSAN[1], Nanribet Geoffrey SER[1*], Wapwera Augustine JIDIMMA[2]

[1]Advanced Materials and Interface Research Group (AMIRG), Department of Chemistry, Faculty of Natural Sciences, University of Jos, Jos. Plateau State, Nigeria. [2]Supreme Bioproducts Nigeria, Jos Office, Nigeria.

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Abstract
The use of herbal/medicine in the treatment of diseases has continued to increase in developing countries. This is due to the high cost, limited accessibility and availability, and effects of some orthodox medicines. Phytochemical, proximate and antibacterial activities of *Abrus precatorius* leaf extract were carried out using standard laboratory procedures to evaluate its medicinal, nutritional and microbial activities. The qualitative and quantitative phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, cardiac glycosides, steroids and saponins, in respective quantities, with flavonoids being the most prominent, while anthraquinones were absent. The proximate analysis showed the following nutritional compositions: Moisture content (11.27 ± 0.01), Ash content (1.31 ± 0.05), Protein (7.62 ± 0.06), Crude fibre (1.98 ± 0.03), Fats (5.20 ± 0.14) and Carbohydrates being the highest (72.62 ± 0.00). Antibacterial analysis revealed that different concentrations of the extract possess activity against *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli.* The minimum concentration of the extract inhibits the growth of these bacteria. FT-IR analysis unveiled some suspected functional groups such as alcohols, alkanes, amides, aromatics and olefins at different absorption bands. *Abrus precatorius* possesses great medicinal potential and can be considered for future drug design and formulations.

Keywords: *Abrus precatorius;* Antibacterial studies; Phytochemical analysis; Proximate analysis; Rosary pea

INTRODUCTION

Globally the sustainability of good health for both human and animals is threatened by certain disease-causing organisms such as, pests, pathogens, bacteria, fungi and viruses [1]. For as long as man has been in existence, the need for good health has been of paramount importance which cannot be over-emphasized [2]. Reports have shown for centuries, plants materials have been used in the traditional treatment of microbial infections while the assemble of knowledge by indigenous people about plants and their products continues use to play an essential role in the health care of the majority of the population is treated with serious concern [3]. Throughout history, humans have sought health solutions through either orthodox medicine (treatment using drugs, radiation, or surgery by healthcare professionals) or

*Correspondence. *E-mail*: nanribetser8@gmail.com *Tel*: +234-8106930061. ISSN 0189-8442

traditional medicine (health practices using plant-, animal-, and mineral-based remedies to treat, diagnose, or prevent illness) [4]. The use of medicinal plants globally has become very important in primary health care especially in developing countries. Hence, several pharmacognostical and pharmacological investigations are being conducted to discover new drugs [5].

Natural compounds extracted from plants, particularly higher plants, have been suggested as alternative sources for antibiotics. The chemical features of these constituents differ considerably among different species because they constitute a potential source of bioactive compounds that have been useful for maintenance of health in humans [8].

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [9].

Abrus precatorius, commonly known as jequirity bean or rosary pea or gunja or *Idon Zakara* in Hausa, is an herbaceous flowering plant in the bean family Fabaceae which is a slender, perennial climber with long, pinnate-leafleted leaves that twines around trees, shrubs and hedges [10]. The *Abrus precatorius* leaf has been used in Nigeria for the treatment of so many diseases including malaria, typhoid, cough, respiratory tract infections and hepatitis [11]. The leaves, seeds and roots of the plants are medicinal and known to cure a vast array of diseases [12]. Rosary pea seeds though toxic, are effective in the cure of various ailments including headache, tuberculosis, painful swellings, snakebites, blennorrhagia, boil, cancer, cold, colic, conjunctivitis, convulsion, cough, diarrhoea, fever, gastritis, gonorrhoea, jaundice, malaria, nightblindness, ophthalmia, rheumatism, diabetes, chronic nephritis and also have a potential of good insecticide [13]. Studies on aqueous, methanolic and chloroform extracts of *Abrus precatorius* leaves was reported to showed great inhibitory activity against a number of disease-causing bacteria such as Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium, and Escherichia coli [14].

This study is aimed at determining the phytochemicals, proximate composition, quantitative evaluation of the phytocompounds and to assess the antimicrobial properties of the ethanolic leaf extract of *Abrus precatorius* (Rosary pea).

EXPERIMENTAL METHODS

Plant collection and preparation. The leaves of *Abrus precatorius* were collected from Ibrahim Badamasi Babangida University, Lapai (IBBUL), permanent site campus, Lapai local government area in Niger state, Nigeria. It was transported to the Federal college of Forestry, Jos, Plateau State, Nigeria where it was identified and authenticated by Mr. Joseph. J. Azila of the Department of Forestry Technology, Federal College of Forestry, Jos, Plateau State, Nigeria. A voucher specimen (No. 175) was deposited in the Herbarium unit of the College. The leaves were carefully picked from the stems, rinsed with distilled water and allowed to shade dry for about 2 months after which it was pulverized into powdery form using an electric blender and stored in an air tight plastic container ready for further use.

Extraction with ethanol. Seventy-five grams (75 g) of pulverized leaf of *Abrus precatorius* was macerated in 750 mL of ethanol in a 1:10 ratio and allowed to stand for about 48 hours after which it was filtered using a porous cloth

and evaporated to dryness using a hot air oven with a temperature of 70°C.

Determination of percentage yield of the extract. The percentage yield of the extract was calculated using the formula:

$$\% \text{ yield} = \frac{\text{WDE}}{WDS} \times 100 \quad \ldots\ldots\ldots\ldots\ldots 1$$

Where: WDE = Weight of dry extract
WDS = Weight of dry sample before extraction

Phytochemical screening. Several qualitative analyses were carried out to test for the presence of certain secondary metabolites such as alkaloids, flavonoids, steroids, terpenes, tannins, saponins, flavones, glycosides and phenols present in *Abrus precatorius*. The presence of these phytochemicals was indicated by colour changes. Quantitate phytochemical analysis of saponins, phenolics, tannins, flavonoids, and alkaloids were also determined using previously established methods [15].

Test for alkaloids. The extract (10 mL) was stirred with 5 mL of 1% HCl on a steam bath. Mayer's and Dragendoff's reagents were added and the appearance of a white and orange precipitate indicates the presence of alkaloids.

Test for tannins. The extract (10 mg) in 10 mL distilled water was filtered, and then the filtrate (3 mL) + 3 mL of $FeCl_3$ solution were mixed. The formation of a dark green precipitate indicates the presence of tannins.

Alkaline (NaOH) test for flavonoids: The extract (5 mL) was put into a test tube followed by a few drops of sodium hydroxide solution and HCl and then shaken vigorously. An intense yellow colour which turned colourless after the dilute acid was added indicates the presence of flavonoids.

Test for saponins. The plant extract (2 g) was shaken with 20 mL distilled water in a test tube. The formation of 1cm layer of froth (foam) indicates the presence of saponin.

Test for cardiac glycosides

Keller-Kiliani Test for cardiac glycosides: The extract (2 mL) + 1 mL acetic acid + $FeCl_3$ + conc. H_2SO_4. The formation of a greenish colour indicates the presence of cardiac glycoside.

Borntrager's test for anthraquinones. The extract (3 mL) was shaken vigorously with 3 mL benzene and filtered. 5 mL of 10% NH_3 solution was added to the filtrate. The formation of a pink, red or violet colour after shaking indicates the presence of free anthraquinones.

Liebermann-Burchard test for steroids. The extract (3 mL) was treated with chloroform, acetic anhydride and few drops of sulphuric acid. A bluish-green formation indicates the presence of steroids.

Molisch's test for carbohydrates: The Molisch's reagent (2 mL) was shaken vigorously with the extract in a test tube and about 2 mL conc. H_2SO_4 was added carefully along the edges of the test tube. The appearance of a reddish-brown ring at the interphase indicated the presence of carbohydrates.

Determination of proximate composition of *Abrus precatorius* leaf. The Association of Official Analytical Chemist (A.O.A.C) (2010) method of proximate analysis was adopted to determine the moisture, ash, crude fibre, crude protein, fats and carbohydrate content of the leaves as described below.

Moisture content. The Abrus precatorius leaf (5 g) was weighed and dried in an oven at about 102°C for 12 hours then the moisture content was estimated by the formula below:

$$\text{Moisture} = \frac{mass\ of\ initial\ sample - mass\ of\ dry\ extract}{mass\ of\ initial\ sample} \times 100 \quad \ldots\ldots 2$$

Ash content. The sample (2 g) was weighed and then ashing by igniting was carried out in a muffle furnace (GALLENKAMP, Model FSL 340-0100, U.K.) at 600°C for over 6 hours

until ashing was complete. The ash content was calculated using the formula:

$$\text{Total ash} = \left(\frac{W2-W1}{W1-W}\right) \times 100 \quad \ldots\ldots\ldots 3$$

Where: W = weight of empty dish in grams; W_1 = weight of the dish + dry matter basis (db) in grams and W_2 = weight of dish + ash in grams.

Crude fat. The gravimetric method was used to determine the crude fat content of the leaves. About 5 g of the sample was weighed and extracted using n-hexane by the Soxhlet extraction method for 4 hours. The formula below was used to determine the crude fat content:

$$\text{Crude fat} = \left(\frac{Wa-Wb}{Wd}\right) \times 100 \quad \ldots\ldots\ldots\ldots 4$$

Where: Wa = weight of extraction flask + fat extracted; Wb = weight of extraction flask and Wd = weight of sample

Crude fibre. The crude fibre content was determined by taking about 2 g as a portion of carbohydrate that resisted sequential digestion with 1.25 % sulphuric acid and 1.25% NaOH followed by sieving through 75 microns, drying at about 130°C for 2hours in an oven and ashing in a muffle furnace until ashing was completed (over 1 hour) and subtracting the ash from fibre. The formula below was used to determine the crude fibre content.

$$\text{Crude fibre} = \left(\frac{W1-W2}{Ws}\right) \times 100 \quad \ldots\ldots\ldots 5$$

Where: W_1 = crucible mass + sample after dying; W_2 = crucible mass + sample after ashing and W3 = initial sample mass (db).

Protein Content. The Kjedahl method was used to determine the protein content. In this method 1 g of the sample was put on a filter paper and introduced into a Kjedahl flask followed by 10 mL of concentrated H_2SO_4 and then digested in a fume cupboard until the solution became colourless. The distillation was carried out with 15 mL of 50% NaOH. The tip of the condenser was dipped into a conical flask containing 6 mL of 4% boric acid in a mixed indicator until a green coloration was observed. Titration was done in a receiver flask wit 0.01 M HCl until the solution turned red [16].

$$X\% = \left(\frac{V1 \times n1 \times f1 \times Mwn}{Ws \times 10}\right) \quad \ldots\ldots\ldots 6$$

$$\text{Protein \%} = N\% \times \text{Factor} \times f2$$

Where: V_1 = volume of 0.1 N HCl; Ws = sample weight; F_1 = Acid factor; Mwn = Molecular weight of nitrogen; F_2 = Dilution factor

Carbohydrate content. The carbohydrate content of the sample was determined by estimating the arithmetic difference method [16] shown in the equation below:

%Carbohydrate = 100 - (%moisture + %fat + %ash + %fibre + %protein).

Source of clinical isolates. Already characterised clinical isolates were obtained from and cultured at the Department of Microbiology in University of Jos. The organisms that were used include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus*.

Standardisation of test organisms. McFarland standardisation was used for the preparation of the inoculations for the optical density of 0.5 mL The reaction gave rise to turbid solution at room temperature and was kept on a bench for use. An aliquot (0.5 mL) of already prepared nutrient was pipetted into sterile test tubes aseptically. The particular test organism was inoculated into each test tube until the bacterial suspensions matched the turbidity of the standard solution [17].

Determination of the antibacterial activity of leaf extract of *Abrus precatorius*. Antimicrobial activity of *Abrus precatorius* extracts was determined using the procedures described by Clinical and Laboratory Standard Institute [18]. A sterilized Muellar-Hinton Agar (HiMedia) was prepared according to the manufacturer instruction and allowed to cool at a temperature of 45°C in a water bath. Subsequently, 0.1 mL of 18 hours old bacterial suspensions adjusted to the 0.5 McFarland standard was dispensed into empty petri dishes correctly labelled. Sterile Agar medium was

dispensed into the plates containing the bacterial suspension, and then left to solidify for about 5-10 minutes. Equidistant wells were bored in the medium using a 5 mm diameter flame sterilized corkborer and labelled accurately. A 100 µg of the test extracts of the different concentrations (500, 250, 125 and 62.5 mg/ mL) and controls were then dispensed into the wells and labelled according to concentration in each well. The plates were incubated at 37°C for 24 hours and diameters of growth of the resulting inhibition zones were measured in millimetres (mm) using a transparent meter rule after incubation. Antimicrobial activity was expressed in terms of the mean value of inhibition zone diameter (mm) of triplicate experiments. Antibiotic gentamicin (10 µg mL^{-1}) was used as a positive control.

Determination of Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) of leaf extract of *Abrus precatorius*. The minimum inhibitory concentration of the extracts was determined for the test organisms in triplicates at varying concentrations of 500, 250, 125, and 62.5 mg/mL. These concentrations were achieved by diluting serially with Mueller-Hinton broth [19]. Sample concentration prepared from the stock solutions were dispensed into the test tubes at different concentrations containing equal volumes of sterile peptone water and tested. The inoculums of test strains prepared from fresh overnight cultures were adjusted to 0.5 McFarland standards, which equals 1.5×10^8 CFU/mL for bacteria. The bacterial suspension of 1 mL was added to each of the tubes and incubated at 37°C for 24 hours. The control tubes contained only the bacteria inoculum. After incubation, the visual turbidity was observed and recorded. The lowest concentration without turbidity was measured as the MIC of the extract.

The minimal bactericidal concentration (MBC) was determined by selecting the tubes that showed no growth during the MIC determination on agar medium and a loopful from each of the tubes sub-cultured on Muellar-Hinton agar plates and incubated for 24 hours at 37°C. The MBC was determined as the least concentration of crude extracts that showed no visible growth [20].

Characterisation using Fourier Transform Infrared Spectroscopy (FT-IR). Characterisation of the functional groups (chemical bonds) present in the sample was done using the FTIR device (Agilent Cary 630/4300 Handheld, America). The ethanolic leaf extract was treated for FTIR spectroscopy IR-Affinity. The samples were run at an infrared region between 400 nm and 4000 nm and the standard DLATGS detector was used at 2.8 nm/sec mirror speed.

RESULTS

Yield of extracts. The quantitative percentage yield of the extracts from 75 g of *Abrus precatorius* leaf powder as macerated with ethanol was 21.3g amounting to 28.4%.

Phytochemical constituents of the extracts. The phytochemical screening of the ethanol extract of *Abrus precatorius* revealed the presence of phyto-constituents including alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, and cardiac glycosides, showing a relative abundance of flavonoids and tannins, followed by alkaloids, saponins, carbohydrates, steroids, and then cardiac glycosides. However, anthraquinones were found to be absent as presented (see in Table 1).

Quantitative analysis of the phytochemicals in leaf extracts. The various classes of constituents based on the percentage quantities obtained in the leaf extract are presented in Table 2 as follows: tannins- 17.55%, alkaloids- 5.97%, phenols- 22.25%, saponins- 8.02%, and flavonoids- 29.93%.

Proximate analysis. The proximate analysis of *Abrus precatorius* leaf extract as presented in Table 3 shows various quantities of primary

metabolites such as protein (7.62%), crude fat (5.20%), crude fibre (1.98%), ash content (1.31%), and a moisture content of 11.27%

Antimicrobial activity of ethanolic leaf extract of *Abrus precatorius*. The *in vitro* disc antimicrobial sensitivity test on some bacteria pathogens for concentrations 500, 250, 125, and 62.5 mg/mL shows that the extract at concentration 62.5 mg/mL, inhibited the growth of *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis* (inhibition zone of 1). *Salmonella typhi* recorded the largest zone of inhibition (13 mm), followed by *Bacillus subtilis* (12 mm), then *Escherichia coli* (10), and *Staphylococcus aureus* the least (9 mm) all at concentration of 500 mg/mL as presented in Table 2.

Minimum inhibitory concentration of the ethanolic leaf extract of *Abrus precatorius* on microorganisms. Table 3 shows the lowest concentration at which the extract that inhibited the growth of the various bacteria. Although the extract showed a higher sensitivity towards the bacteria *Salmonella typhi*, the lowest concentration of the extract which inhibited its

growth was 125 mg/mL The lowest concentration at which the extract inhibited the growth of other bacteria Staphylococcus aureus, Bacillus subtilis, and Escherichia coli was 62.5 mg/mL at the lowest concentration of the extract, 31.25 mg/mL there was no inhibition of the growth of all four bacteria.

Minimum bactericidal concentration of the ethanolic leaf extract of *Abrus precatorius* on microorganisms. Table 4 shows the minimum bacterial concentration of the extract with all being negative which implies bacteriostatic activities of the extract.

Characterisation of the ethanolic leaf extract of *Abrus precatorius* using FT-IR analysis. The FT-IR analysis of the leaf extract shows the presence of various functional groups such as alcohols (O-H), alkanes (C-H), aldehydes (H-C=O), alkenes (C=C), arenes, amides, and aromatics at various absorbance peaks with wave numbers 3454.31, 2953.37, 1734.36, 1618.59, 1476.81, 1297.82, and 743.13 cm-1 respectively as shown in fig.2 and Table 5.

DISCUSSION

The percentage yield of the *Abrus precatorius* leaf gives an idea of the extractability of the plant studied under different conditions and also gives a suitable basis for comparison when other solvent types such as water or N-hexane are used to know which gives a more or lesser yield.

The phytochemicals present in *Abrus precatorius* leaf serves as defence mechanism against pathogens/disease causing organisms [21]. Flavonoids are known for their anticancer, antioxidant, anti-inflammatory, and antiviral properties [24] and are the most prominent phytochemical**s** present quantitatively in the plant studied which implies the plant possess good antioxidant properties.

Alkaloids possess anti-bacterial properties, tannins improve cardiovascular health, and steroids helps to reduce cholesterol level in the human body, cardiac glycosides regulate the cardiac output in patients with heart failures and saponins which lower the risks of cancers [25]. The presence of the phytochemicals in the plant extract is responsible the direct or indirect defensive mechanism against pathogens or harmful ailments in human biological system thereby attesting it potential suitability for medicinal applications [22].

The result of proximate analysis on the *Abrus precatorius* leaf extract**s** showed vital nutrient composition as well as its nutritional significance in terms of proteins, carbohydrates, fats, among others unveiling its potential to serve as a vital source of energy due high carbohydrate percentage and it safety when used in dietary supplements and herbal drugs.

Table 1: Qualitative phytochemical screening of ethanolic leaf extracts *Abrus* of *precatorius*

Constituents	Test Performed	Colour	Relative degree
Alkaloids	Mayer's reagent	Creamy white	+
Saponins	Froth test	Froth formation	+
Tannins	Ferric chloride test	Dark green ring	+
Flavonoids	Alkaline reagent test	Yellow	+
Carbohydrates	Molisch's reagent test	Reddish brown ring	+
Steroids	Liebermann-burchard	Greenish blue	+
Anthraquinones	Bomtrangers' test	No colour	-
Cardiac glycosides	Keller-Killiani's	Greenish	+

+ = Presence; - = Absence

Figure 1: *Abrus precatorius* plant

Table 2: *In vitro* disc antimicrobial sensitivity test of the ethanolic leaf extract of *Abrus precatorius*

Concentration	Test organism/ Diameter (mm) of Zones of inhibition			
(mg/mL)	*S. aureus*	*E. coli*	*S. typhi*	*B. subtilis*
500	9	10	13	12
250	5	7	6	8
125	1	4	3	3
62.5	--	--	--	1

Table 3: Minimum inhibitory concentration of the ethanolic leaf extract of *Abrus precatorius* on the test organisms

Concentration	Test organism			
(mg/mL)	*S. aureus*	*E. coli*	*S. typhi*	*B. subtilis*
250	+	+	+	+
125	+	+	-	+
62.5	+	+	-	+
31.25	-	-	-	-

+ = inhibits growth of bacteria, - = does not inhibit the growth of the bacteria

Table 4: Minimum bactericidal concentration of the ethanolic leaf extract of Abrus precatorius on the test organisms

Concentration	Test organism			
(mg/ ml)	*S. aureus*	*E. coli*	*S. typhi*	*B. subtilis*
250	-	-	-	-
125	-	-	-	-
62.5	-	-	-	-
31.25	-	-	-	-

- = does not kill the bacteria + = kills the bacteria

Table 5: FT-IR Analysis on ethanolic leaf extract of *Abrus precatorius*

Frequency /wavenumber (cm^{-1})	Suspected compound class	Absorption intensity	Group
3454.31	Alcohol	Strong, Broad	O-H Stretching
2953.37	Alkane	Medium	C-H Stretching
2429.08	--	--	--
1734.36	Aldehyde	Strong	C=O Stretching
1618.59	Alkene	Medium	C=C Stretching
1476.81	Arene (aromatics)	Weak	C-H bending
1297.82	Amides	Strong	C-N Stretching
982.44	Alkenes	Strong	C=C Bending
743.13	Aromatic	Strong	C-H Bending

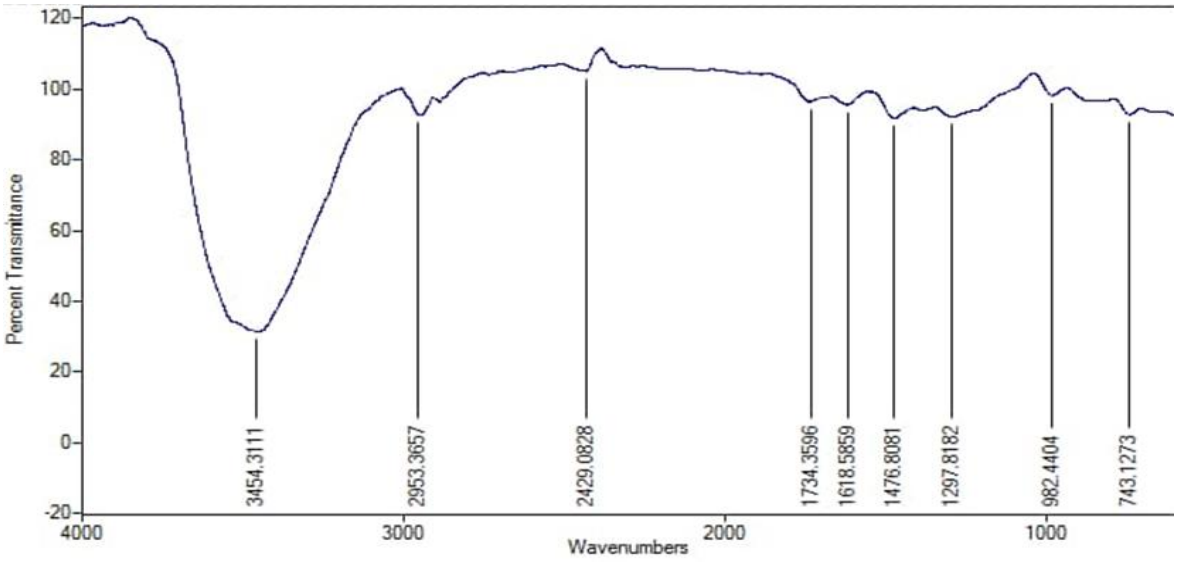

Fig 2: FT-IR Spectrum of *Abrus precatorius* leaf extract

The selectivity of the leaf extracts at against *Staphylococcus aureus*, *Salmonella typhi, Escherichia coli* and *Bacillus subtilis* is to evaluate differential antimicrobial activity at different concentrations. The minimum bactericidal concentration revealed the extract to exalt bacteriostatic activities against the test organisms. This implies that the plant possesses great antibacterial activity for consideration as suitable for antibiotic purposes [23].

The FT-IR spectrum and suspected compounds of the *Abrus precatorius* leaf extract, in Figure 1 and Table 4 showed the presence of more than five (5) absorbance bands in the spectrum indicating the leaf extract consist of a complex molecule. The single bond area falls between the wave numbers 2500-4000 cm^{-1} with a broad absorption band between 3500 and 3000 cm^{-1} indicating the presence of a hydroxyl (O-H) group and a narrow band slightly below 3000 cm^{-1} shows the presence of aliphatic compounds such as the alkane (C-H). Double bonds such as the carbonyl (C=O), amino-group (C=N), azo group (N=N) and olefinic group (C=C) fall in the double bond region 1500 – 2000 cm^{-1} (table 4). At 982.44 cm^{-1}, a vinyl related compound (-CH=CH_2) is identified. A single and strong aromatic absorption band is seen around wave number 743.13 cm^{-1} while a weak band is seen at 1476.81 cm^{-1}. These two regions fall within a unique region called the 'fingerprint' region. The presence of these functional groups could also be responsible for the medicinal properties of the ethanol leaf extract of the plant.

Conclusion. The findings in this study indicated that the leaf of *Abrus precatorius*

plant possesses suitable potentials as a medicinal plant due to the presence of some important phytochemicals, and functional groups. It also possesses antibacterial characteristics with potential to inhibit some persistent disease causing bacterial. Further research should be focused on other parts of the *Abrus precatorius plant* to reveal more on their ethnobotanical and pharmacognostical importance.

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