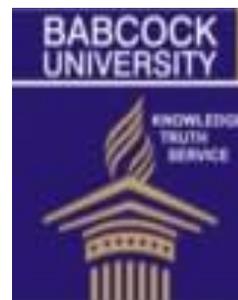




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Research

Isolation of a Pentacyclic Triterpene from *n*-hexane extract of *Abrus precatorius* Linn leaves

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Abstract

Abrus precatorius is widely used as traditional medicine for treatment of various ailments. The leaves were investigated with the aim of evaluating phytochemical properties with a view to determining its suitability for medicinal use(s). The results showed that the leaves contain phenols (1.57 ± 0.07 ; 3.12 ± 0.33 ; 7.49 ± 0.03), saponins (0.01 ± 0.01 ; 0.01 ± 0.01 ; 0.02 ± 0.02), alkaloid (0.80 ± 0.20 ; 0.93 ± 0.31 ; 1.27 ± 0.11), glycosides (0.50 ± 0.02 ; 6.05 ± 0.18 ; 5.85 ± 5.02), flavonoids (0.60 ± 0.01 ; 2.19 ± 1.90 ; 3.42 ± 0.43) and steroids (12.53 ± 0.22 ; 36.04 ± 1.73 ; 50.62 ± 2.87) mg/100 g *n*-hexane, ethylacetate and ethanol extracts respectively. The leaves were subjected to sequential extraction with solvents of increasing polarity from *n*-hexane, ethylacetate and ethanol to obtain crude extracts. These went through thin layer chromatography where the *n*-hexane extracts gave the best TLC profile. The *n*-hexane extract was gradiently eluted in a silica gel column that led to the isolation of an orange solid (R1) subjected to spectroscopic techniques (NMR and FTIR). The isolated compound was identified as (3 β)-3-formylphenoxyoleane)-12-en-28-oic acid. In general, results showed the plant leaf contains phytochemicals which could be exploited for drug discovery.

Keywords: *abrus precatorius*, *n*-hexane, (3 β)-3-formylphenoxyoleane)-12-en-28-oic acid.

Introduction

Researchers, government and other organisations charged with food and nutrition have shown great concern on the nutritional status of the general population more especially children, pregnant and lactating mothers habiting developing countries as a result of natural disasters, bad economic policies, political instability, population explosion, high price of food commodities, poor implementation of agricultural policies and restriction in food importation are major factors that contribute to the burden of inadequate nutritional food intake among average people (Hassan and Umar 2006). Inadequate nutrition could be the possible cause of malnutrition among the population and in order to arrest the situation, more interest has been geared towards the exploitation and utilisation of unusual food plants to complement our daily diets (Hassan *et al.*, 2011). Diet rich in fruits, vegetables, grains and legumes reduces the risk of diseases such as cancer, heart diseases, high blood pressure, diabetes and obesity (Uruquiaga and Leighton, 2000). In addition, they help in the prevention of several micronutrient deficiencies like anaemia, goitre, etc. prevalent in less developed countries (Oyeyemi *et al.*, 2015).

Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic effects (Gill *et al.*,

2011). Plant extracts as well their primary and secondary metabolites have important therapeutic role in the treatment of many human diseases (Gill *et al.*, 2011b). Natural products mainly from plant kingdom offer a wide range of biologically active compounds that act as natural antioxidants with recognized potential in drug discovery and development (Mishra *et al.*, 2008).

Materials and methods

Plant material

The plant material of *Abrus precatorius* was collected from Argungu Local Government Area of Kebbi State in July, 2018 during raining season and transported to Sokoto in a paper envelope. It was identified and authenticated in the Herbarium section of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto with voucher number UDUSH/ANS/0249.

The fresh leaves of *A. precatorius* were sorted, de-stalked and rinsed in water to remove dust and dirt. The samples were air dried and pulverized using a wooden pestle and mortar. The powdered sample was the stored in the laboratory in clean, air-tight polythene bag until required for use.

Extraction of plant material

The sample (200.00 g) was transferred to a separating funnel with the aid of a spatula. *n*-hexane (800.00 cm³) was added and allowed to stand for 24 hours. An aluminium foil was used to cover the funnel. After 24 hours, the *n*-hexane was drained out of the separating funnel and the marc was then re-extracted with 150.00 cm³ *n*-hexane successively until it was colourless. The marc was then removed out of the separating funnel and then air dried for an hour. The solvent was evaporated using rotary evaporator at 45.00 °C for 20 minutes for the *n*-hexane to evaporate. Then weighed after drying and the mass recorded.

The process was repeated with ethyl acetate (500.00 cm³) and ethanol (450.00 cm³), in each case the solvent were evaporated using rotary evaporator at 45.00 °C for 20 minutes (Sofowora, 2008).

PHYTOCHEMICAL ANALYSIS

The following methods were used to conduct quantitative and qualitative phytochemical examination for the presence and quantity of various secondary metabolites on the extract of *Abrus precatorius*.

A. Qualitative phytochemical analysis

1. Tests for alkaloids

Dragendorff and Mayers' test

The extract (0.05 g) of each portion was stirred with 5.00 cm³ of 10.00 % aqueous HCl on water bath and then filtered. To the filtrate, 1.00 cm³ was poured into 2 test tubes. To the first portion, few drops of dragendorff's reagent was added. To the second 1.00 cm³, Mayer's reagent was added and observed (Sofowora, 2012).

2. Tests for steroids/triterpenes

Salkowski test

The extract (0.05 g) was dissolved in 2.00 cm³ of chloroform; sulphuric acid was carefully added and observed (Sofowora, 2012).

3. Test for anthraquinones (Borntrager's test)

The extract (0.05 g) was shaken with 10.00 cm³ CHCl₃. It was then filtered and 5.00 cm³ of 10.00 % ammonia solution was added to the filtrate. The mixture was shaken and observed (Sofowora, 2012).

4. Tests for flavonoids

Ferric chloride test – Test solution was treated with few drops of ferric chloride solution and observed (Evans, 2005).

5. Tests for tannins

Ferric chloride test

The extract (0.05 g) was stirred with 10.00 cm³ of distilled water, filtered and ferric chloride reagent was added to the filtrate and observed (Evans, 2005).

6. Test for saponins

Frothing test

The extract (0.10 g) was mixed with water and shaken and observed for the formation of froth; observation was made for its stability in 15 minutes for a positive result (Bhandary *et al.*, 2012).

7. Test for cardiac glycosides

Keller-killiani's test

The extract (0.05 g) was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was carefully added and observed. (Sofowora, 2008).

B. Quantitative Phytochemical analysis.

1. Determination of alkaloids

Procedure

Five grams of sample was extracted with 100.00 cm³ of methanol:water (1:1) and solvent evaporated. The resultant residue was mixed with 20.00 cm³ of 0.0025 M H₂SO₄ and partitioned with ether to remove unwanted

materials. The aqueous fraction was basified with strong NH₃ solution and then extracted with excess chloroform to obtain the alkaloids fraction. The chloroform extraction was repeated several times and the extract was concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to initial weight of sample (Day and Underwood, 1986). Percentage alkaloid was calculated using equation 1.0

$$\% \text{ Alkaloid} = \frac{\text{weight of alkaloid}}{\text{weigh of Sample}} \times 100 \quad 1.0$$

2. Determination of tannins

Extraction: 0.10 g of the sample was put into a 100.00 cm³ conical flask and 50.00 cm³ of distilled water was added. The flask was gently heated to boiling for 1 hour and filtered while hot.

Procedure: the filtrate was collected in a 50.00 cm³ volumetric flask. The residue was washed several times and the combined solution made to the volume with distilled water. To 0.00, 1.00, 2.00, 3.00, 4.00, and 5.00 cm³ of the standard tannic acid and 10.00 cm³ of the sample solution in a 50.00 cm³ volumetric flask, 2.50 cm³ of Follin-Dennis reagent and 10.00 cm³ Na₂CO₃ solutions were added and made to the volume with distilled water. The flask was

allowed to stand for 20 minutes and the optical density was measured at 760 nm (Day and Underwood, 1986). The concentration of tannic acid in the sample was calculated using equation 1.1

$$X = \frac{\text{Absorbance of sample}}{\text{absorbanc of standard}} X \text{ conc}100 \text{ mg/dl} \quad 1.1$$

3. Determination of saponins

Procedure: from the plant extract, 5.00 g was placed in a 250.00 cm³ flask containing 30.00 cm³ of 50.00 % alcohol. The mixture was boiled under reflux for 30 minutes and was immediately filtered while hot through a coarse filter paper. Two grams (2.00 g) of charcoal was added, the content was boiled and filtered while hot. The extract was cooled (some saponins may be separated) and an aqueous volume of acetone was added to complete the precipitation of saponins. The separated saponins were collected by decantation and dissolved in the least amount of boiling 95 % alcohol and filtered while hot to remove any insoluble matter.

The filtrate was allowed to cool at room temperature thereby resulting in the precipitation of saponins. The separated saponins were collected by decantation and suspended in about 2.00 cm³ of alcohol and

filtered. The filter paper was immediately transferred to desiccator containing anhydrous calcium chloride and the saponins were left to dry. They were weighed with reference to extract used (Day and Underwood, 1986).

The percentage saponin was calculated using equation 1.2

% Saponin

$$= \frac{\text{weight of filter paper + Sample} - \text{weight of filter paper}}{\text{weight of sample}}$$

4. Determination of glycosides

Procedure: 1.00 g of the extract was extracted in 10.00 cm³ of 70.00 % alcohol and mixture was filtered. From the filtrate, 8 cm³ of the mixture was added to 8.00 cm³ of 12.50 % lead acetate (to precipitate resins, tannin and pigments). The mixture was shaken well, completed to volume (100.00 cm³) distilled water and filtered. The filtrate (50.00 cm³) was pipette into another 100.00 cm³ volumetric flask and 8.00 cm³ of 4.70 disodium hydrogen phosphate solution (to precipitate excess lead) was added. The mixture was made up to the volume with distilled water and mixed. The mixture was filtered twice through a Whatman No. filter paper. Baljet reagent (10.00 cm³) was added to 10.00 cm³ of purified filtrate. A blank sample of 10.00 cm³ of distilled water

was also added to 10.00 cm³ of Baljet reagent. The 2 were allowed to stand for 1 hour (time maximum for colour development). The intensity of the colour was read at 495 nm spectrophotometer against a blank (20.00 cm³ distilled water). The colour was stable for several hours (Day and Underwood, 1986).

Percentage glycosides is calculated using equation 1.3

$$\% \textit{ glycoside} = \frac{A}{17} \times 100 \dots\dots\dots 1.3$$

5. Determination of flavonoids

Five (5.00 g) of the sample was hydrolysed by boiling in 100.00 cm³ of HCl solution for 30 minutes. They hydrolysate was filtered to recover the extract (filtered). The filtrate was treated with ethylacetate drop wise until in excess, the precipitated flavonoid was recovered by filtration using a weighed filter paper after drying in the oven at 100.00 °C for 30 minutes, cooled in desiccator and reweighed. The difference in weight face the weight of flavonoid which was expressed as a percentage of the weight of the sample analysed as seen in equation 1.4 (Day and Underwood, 1986).

$$\% \textit{ flavonoid} = \frac{\textit{ weight to filter paper + sample} - \textit{ weight of empty fi}}{\textit{ weight of sample}}$$

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was carried out using TLC silica gel (mesh size 60-120) pre-coated aluminium plates using ascending technique. Spotting was carried out manually using capillary tubes and developed in an air tight chromatographic tank at room temperature.

Preliminary TLC of all the extracts was conducted and the solvent systems used are [Hexane: Ethyl acetate (9:1, 9:2, 9:3, 8:2, 5:5, 2:8, 4:6, 1:9) and Ethanol: Chloroform: Water (6:2:2)]. Developed chromatograms were air dried and visualised under normal light, UV light (254 and 366 nm) and by spraying with 10.00 % sulphuric acid followed by heating at 105 °C for 5 – 10 minutes in an oven.

Column chromatography

The *n*-hexane extract was successively subjected to repeated silica gel Sephadex LH-20 to yield compounds R1 (Figure 1).

Characterization Of Compound R1

The Physical appearance, solubility and Spectral analysis was conducted on R1

Results And Discussion

results

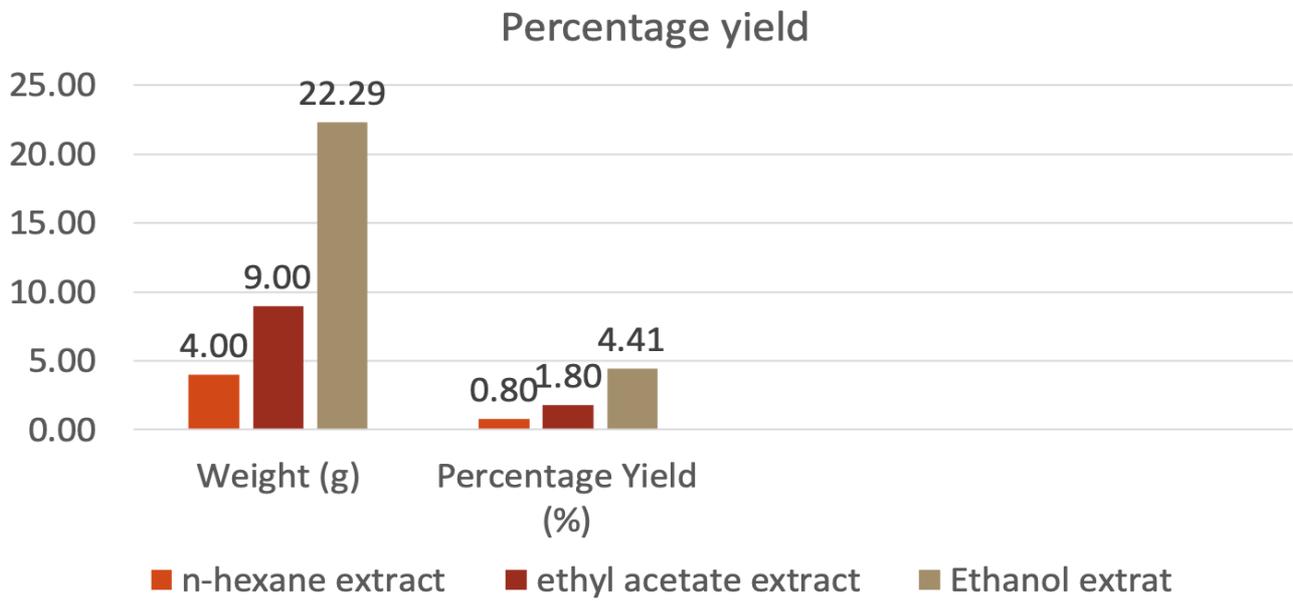


Plate 1: Percentage yield of *A. precatorius* leaves

PHYTOCHEMICAL SCREENING**Qualitative Phytochemical Screening.**Table 1: Qualitative phytochemical analysis of *A. precatorius* leaves

Constituents	Test	Observation	Inference		
			<i>n</i> -hexane extract	Ethylacetate extract	Ethanol extract
Alkaloid	Dragendroff's	White-yellowish	+	ND	+
	Hager's	ppt			
Steroids/ Triterpenoids	Salkowki's	Reddish-brown color	+	ND	+
Anthraquinones	Bontrager's test		ND	ND	ND
Flavonoids					
	Alkaline reagent test	Intense yellow color	ND	+	+
Saponins	Frothing/foaming	Froth persists for 15 minutes	ND	ND	+
Cardiac Glycocides	Keller-Killianis's test	Reddish brown ppt	+	+	+
Phenols	Ferric chloride test	Red-brown color	+	+	+
Reducing Sugar	Fehling's test	Brick-red ppt	+	+	+
Volatile oil	Sudan test		ND	ND	ND

Key: += detected; ND= not detected

Quantitative phytochemical analysis

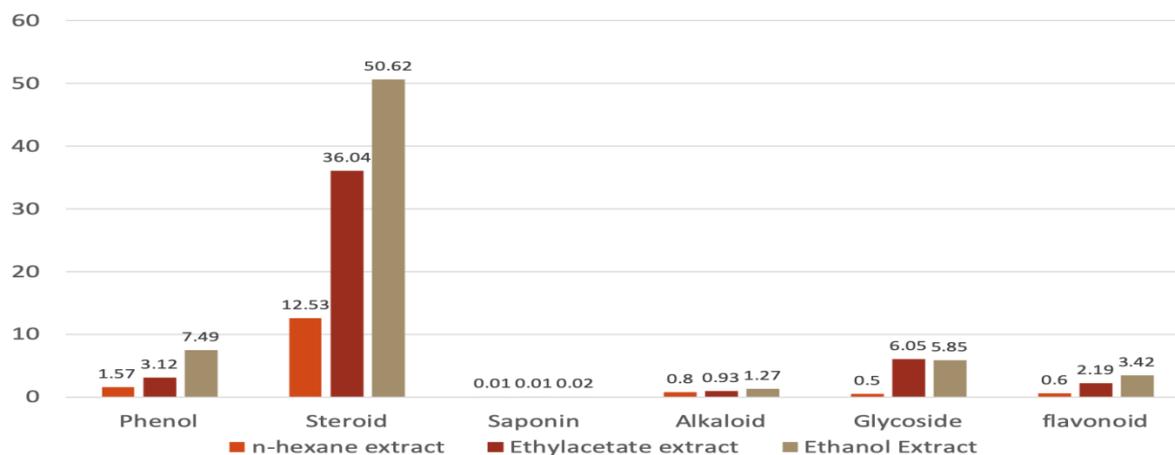


Plate 2: Quantitative phytochemical analysis of the leaves of *A. precatorius*

Physiochemical And Phytochemical Properties Of R1

Table 2: Physiochemical and phytochemical properties of R1

Parameter	Test	Observation	Inference
Solubility			
I.	<i>n</i> -hexane	Sample dissolves completely	Soluble
II.	Ethyl acetate	Sample dissolves completely	Soluble
III.	Chloroform	Sample dissolves completely	Soluble
Color			Orange
Physical state			Crystalline
Terpenoid	Libermann	Reddish-violet color	Positive
	Buchard's test		

Spectral Analysis Of R1Table 3: ¹³C, ¹H and DEPT NMR Data of R1 Compared with Literature Values (300 MHz, CDCl₃)

	¹³ C NMR of R1 (δ ppm, 300 MHz, CDCl ₃)	¹ H NMR of R1 (δ _H ppm, 300 MHz, CDCl ₃)	DEPT	¹³ C NMR *	¹ H NMR*
1	37.37	-	CH ₂	33.41	0.94
	-	-	-	-	1.58
2	27.16	1.67	CH ₂	27.16	1.57
3	-	4.06	CH	78.98	3.21
4	37.44	-	C	38.72	-
5	-	-	CH	55.18	0.71
6	19.76	1.65	CH ₂	18.29	1.50
	-	1.32	-	-	1.32
7	32.68	-	CH ₂	32.69	1.40
	-	1.28	-	-	1.23
8	39.38	-	C	39.25	-
9	-	1.49	CH	47.58	1.49
10	36.64	-	C	36.98	-
11	22.71	2.01	CH ₂	23.37	1.84
12	118.18	5.35	CH	122.47	5.29
13	143.85	-	C	143.66	-
14	-	-	C	41.66	-
15	27.99	1.04	CH ₂	27.61	1.02
	-	1.62	-	-	1.57
16	22.63	2.02	CH ₂	23.03	1.98
	-	1.64	-	-	1.63
17	-	-	CH	46.72	-
18	-	2.33	CH	41.36	2.90
19	-	1.64	CH ₂	45.85	1.64
	-	0.92	-	-	1.13
20	29.73	-	C	30.68	-
21	34.43	-	CH ₂	33.83	1.18
	-	-	-	-	1.34
22	31.95	1.62	CH	32.35	1.54
	-	1.71	-	-	1.71
23	28.67	-	CH ₃	28.08	0.98
24	16.37	0.88	CH ₃	15.56	0.88
25	14.13	-	CH ₃	15.28	0.77
26	19.71	-	CH ₃	16.85	0.61
27	25.86	-	CH ₃	25.86	1.13
28	174.02	-	C	177.42	-
29	32.81	0.90	CH ₃	33.09	0.90
30	24.04	0.92	CH ₃	23.63	0.92
C1'	155.34	-	C	-	-
C2'	-	6.46	CH	-	-
CHO	195.33	9.48	CH	-	-

* ref. (Robert and Werner, 2002)

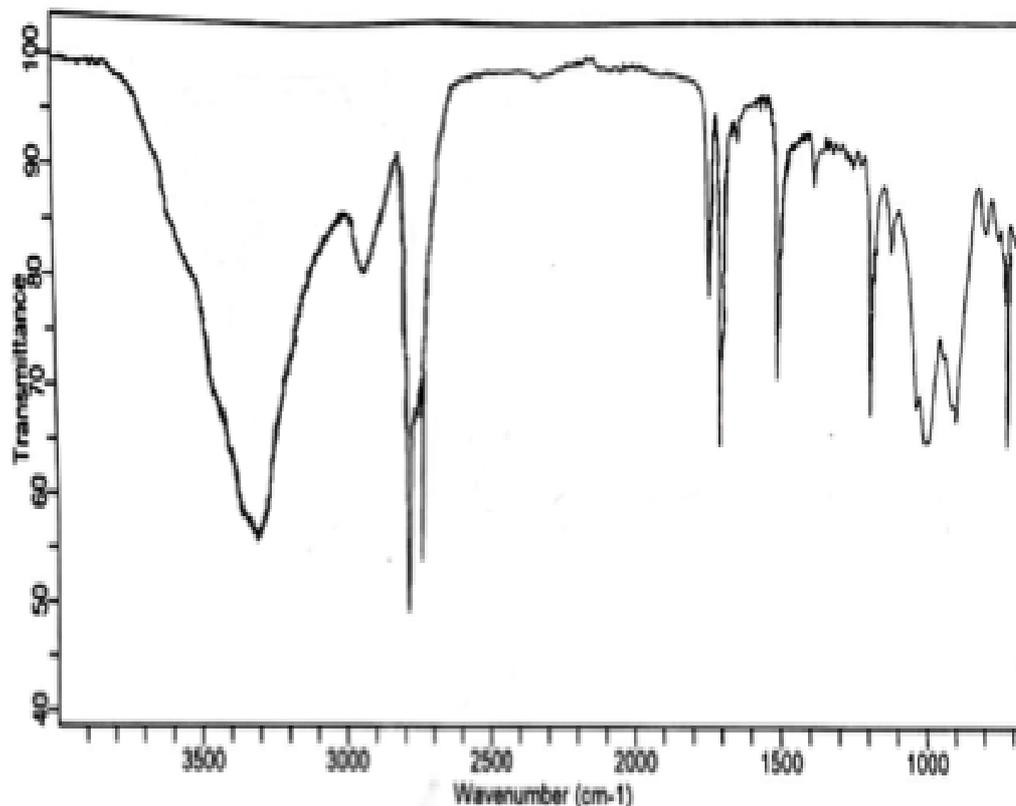


Plate 3. FTIR Spectrum of R1

Discussions

Percentage Yield

The results of the solvent extraction of *Abrus precatorius* leaves showed an incomparable outline in yield from the various solvents used for the extraction. It could be observed from the results (Plate 1) that the *n*-hexane had lowest yield while (0.80 %) ethanol has the highest percentage yield (4.41 %). This is due to the variation in the polarity of the solvents. This is in line with the report of Halilu *et al.* (2013).

Phytochemical Screening

Qualitative phytochemical screening:

Qualitative phytochemical analysis of the *n*-hexane, ethyl acetate and ethanol extract of *Abrus precatorius* revealed the presence of steroids/triterpenoids, saponins, cardiac glycosides, anthraquinones, flavonoids, alkaloids, phenols and reducing sugar (Table 1).

Quantitative phytochemical analysis:

Saponins: The saponin content (Plate 2) was discovered to be highest (0.02 ± 0.02 %) in the

ethanol extract and lowest (0.01 ± 0.01 %) in *n*-hexane extract. From the study, the leaves of *A precatorius* cannot be regarded as a very good source of saponin.

Phenolic: The result showed that the concentration of phenol is least (1.57 ± 0.07 %) in *n*-hexane extract and highest (7.49 ± 0.03 %) in the ethanol extract (Plate 2). However, the values are lower than the values (22.84, 17.08 and 10.87 %) reported for the leaves, seeds and roots of *A precatorius* by Ojo et al. (2016).

Alkaloids: The results showed that the concentration of alkaloid is highest in the ethanol extract (1.27 ± 0.11 %) and lowest in the *n*-hexane (0.80 ± 0.20 %) and ethyl acetate extract (0.93 ± 0.31 %) is as shown in (Plate 2). This signifies that majority of alkaloid compounds in the leaves are polar.

Steroids: Preliminary findings showed that the leaves of *A precatorius* contain high number of triterpenes in the ethanol extracts (50.62 ± 2.87 %) followed by ethyl acetate (36.04 ± 1.73) and *n*-hexane (12.53 ± 0.22 %) with the lowest (Plate 2). This could explain why the plant is used for anti-bacterial, anti-inflammatory and anti-neoplastic purposes. Although, the values reported in this study are

higher than those reported for the leaves (4.27 %), seed (5.17 %) and root (2.08 %) extract of the *A precatorius* by Ojo et al. (2016).

Flavonoids: The flavonoid content was found to be lowest in the *n*-hexane and highest in ethanol extract. This shows that the compounds are more soluble in a polar environment. Therefore, in trying to isolate a flavonoid in the leaves of *A precatorius*, it is recommended from this study to use ethanol for extraction as well as in trying to test for activities like inflammation, tumour and free radicals because it has the highest concentration of flavonoids. A study conducted by Ojo *et al.* (2016) reported similar values (3.73, 3.68 and 1.41 %) for the leaves, seed and root of *A precatorius* respectively.

Alkaloids: The concentration of alkaloid is highest in the ethanol extract and lowest in the *n*-hexane and ethyl acetate extract. This signifies that majority of alkaloid compounds in the leaves are polar. Although, these values are lower than the values (6.18 %), (4.06 %) and (2.18 %) reported for the leaves, seeds and roots respectively (Jang *et al.*, 2010). This could explain the why the plant has a tendency

to become weedy and invasive (Jang *et al.*, 2010).

The presence of above secondary metabolites in the leaves of *A precatorius* may contribute to its medicinal value. Some of these compounds are well documented to exhibit anti-inflammatory, antioxidant, anti-diabetic and anti-fertility and hypoglycaemic activity in animals (Akhtar *et al.*, 1981).

Thin layer chromatography (TLC) of the extracts

From Plate 3 and Table 2, the extract of *n*-hexane has the best separation and hence was chosen for column chromatography.

Isolation of compound R1

Compound R1 was isolated as an orange crystalline solid substance; which gave a positive result to Libermann Buchard's test indicating a triterpene (Sofowora, 2012). R1 was found to be soluble in *n*-Hexane, Ethyl Acetate and Chloroform (Table 2). It was subjected to spectroscopic techniques (FTIR and NMR).

The $^1\text{H-NMR}$ spectrum of the compound R1 revealed the presence of several signals between δ_{H} 0.86 and 9.38 (Table 3) which are

attributed to overlapping methyl, methylene and methine protons.

A doublet-doublet of one proton at δ_{H} 2.33 (d, H, J=60 Hz) and a triplet of one vinyl proton at δ_{H} 5.35 (t, H, J=30 Hz) were assigned to H-18 and H-12, respectively. One methine proton at δ_{H} 9.38 showed that R1 has at least one aldehyde group.

The $^{13}\text{C-NMR}$ spectrum (δ_{C} (ppm), CDCl_3 300 MHz) showed recognizable signals at δ_{C} 118.18, 143.85, 174.02 and 195.33 (Table 3). The peak at 118.18 ppm was assigned to C-12 while that at δ_{C} 143.85 was assigned to C-13, these are indicative of olefinic carbons at C-12 and C-13. The signal at δ_{C} 174.02 ppm was attributed to the carbonyl carbon of a carboxylic acid at C-28 which is a characteristic of oleanane skeleton. A carbonyl carbon of an aldehyde at δ_{C} 195.33 ppm was assigned to C-7' ppm and a quaternary carbon of a benzene group at δ_{C} 155.34 ppm attributed to C-1'.

The DEPT spectrum of compound R1 (Table 3) showed positive signals due to methine (CH) and methyl (CH_3). The negative signals were seen due to methylene (CH_2). The expected signal for δ_{C} 37.44, 39.38, 36.64, 29.73 and 174.02 ppm for C4, C8, C10, C20

Conclusions And Recommendations

Conclusions

This study evaluated the phytochemical properties and isolation of compound from the leaves of *A. precatorius* using standard methods.

The leaves was found to contain several phytochemicals including phenols (1.57 ± 0.07 ; 3.12 ± 0.33 ; 7.49 ± 0.03), saponins (0.01 ± 0.01 ; 0.01 ± 0.01 ; 0.02 ± 0.02), alkaloid (0.80 ± 0.20 ; 0.93 ± 0.31 ; 1.27 ± 0.11), glycosides (0.50 ± 0.02 ; 6.05 ± 0.18 ; 5.85 ± 5.02), flavonoids (0.60 ± 0.01 ; 2.19 ± 1.90 ; 3.42 ± 0.43) and steroids (12.53 ± 0.22 ; 36.04 ± 1.73 ; 50.62 ± 2.87) mg/100 g for *n*-hexane, ethylacetate and ethanol extracts respectively.

In an attempt to explore new chemical substance(s) from the leaves of *A. precatorious*, the results of sequential extraction on the powdered leaves (4.41 %, 1.80 % and 0.80 % for *n*-hexane, ethylacetate and ethanol extracts respectively) went through thin layer chromatography where the *n*-hexane extracts emerged with best profile and was gradiently eluted in a silica gel column. This led to the isolation of an orange crystalline solid (R1) that went through spectroscopic analysis (NMR and FTIR) and

was named as (3 β)-3-formylphenoxyoleane)-12-en-28-oic acid.

Recommendations

This work has been able to evaluate the phytochemicals and isolated a triterpene from the leaves extract of *Abrus precatorius*. In view of this, the following recommendations are made:

- i. The toxicity profile of the whole plant should be investigated.
- ii. Biological activity of the isolated compound should be investigated.

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