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ANTIOXIDANT, COMPOSITIONAL EVALUATION AND BLOOD PRESSURE MODULATING POTENTIALS OF *BRYOPHYLLUM PINNATUM* (LAM.), *VISCUM ALBUM* (L.) AND *ARTOCARPUS ALTILIS* (PARKINSON) LEAVE EXTRACTS

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ABSTRACT

Bryophyllum pinnatum (Lam.), *Viscum album* (L.) and *Artocarpus altilis* (Parkinson) are medicinal plants widely used based on their ethnomedicinal properties in the regulation of blood pressure. This study was designed to evaluate the antioxidant activities and compositional constituents of these plants. The antioxidant potentials were analyzed using DPPH and FRAP assays, while Folin-Ciocalteu method was employed in the determination of the total phenolic antioxidant contents. Compositional analyses of the leave extracts were determined using Gas Chromatograghy-Mass Spectrophotometer (GC-MS). The total phenolic contents in *Bryophyllum pinnatum, Artocarpus altilis* and *Viscum album* were revealed as; 659.50 ±0.02, 1667.50 ±0.03, 1232.00 ±0.02 mg GAE.100 g^{-1} respectively. Considering the antioxidant activities, *Artocarpus altilis* leaf extract showed inhibitory activity on DPPH with IC_{50} of 2.24 ±0.26 $mg.mL^{-1}$, *Bryophyllum pinnatum* and *Viscum album* with IC_{50} values 3.63 ±0.07 and 4.65 ±0.06 $mg.mL^{-1}$ respectively.The FRAP in $mg.GAE^{-1}$ for *Artocarpus altilis*, *Bryophyllum pinnatum* and *Viscum album* revealed; 2505.20 ±0.04, 1561.80 ±0.01 and 1698.00 ±0.03 respectively. GC-MS identified some vital phenolic components and essential fatty acids in the plants. The findings therefore suggest that; the plants if properly utilized, it could serve as alternatives in regulating blood pressure.

Keywords: antioxidant activity; benzesterol; phenol-1, 3-Dodecanol; piscofuranine

INTRODUCTION

Occurrence of diseases have been increasing at a regular rate and claiming millions of lives in spite of the huge improvements in modern medicine all over the world. High blood pressure is one of such diseases. A disease of the heart and blood vessels that is defined as blood pressure persistently above 140/90 mmHg is called hypertension and persistently below 120/80 mmHg is called Hypotension. Hypertension is the most common cardiovascular disease and constitutes a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease and renal insufficiency **(Chobanian et al., 2003; Lans, 2006)**. Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly great in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance **(Abubakar et al., 2010)**. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs **(Borris,1996)**. Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons **(Hosseinzadeh et al., 2015)**.Various herbal plants have been used to combat hypertension.*Bryophylum pinnatum* (*B. pinnatum*), *Viscum album* (*V. Album*) and *Artocarpus altilis* (*A. Altilis*) are examples of the various medicinal plants basically used individually in ethno-medicine for the regulation of blood pressure and in the treatment of hypertension.

A. altilis belongs to the family, Moraceae. It is commonly referred to as breadfruit as it is similar to freshly baked bread. Breadfruit is a tropical fruit and the breadfruit tree produces fruits from March to June and from July to September **(Akanbi et al., 2009)**. *A. altilis* leaves have been reported to be used as anti-hypertensive drug **(Lans, 2006)**. The yellowing leaf is brewed into a tea and taken to reduce high blood pressure **(Orwa et al., 2009)**. *B. pinnatum* is an erect, succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and also

Figure 1 *Bryophyllum pinnatum* (Lam.) leaf.

Figure 2 *Viscum album* (L.) leaves.

Figure 3 *Artocarpus altilis* leaves.

vegetatively from leaf bubils **(Okwu and Josiah, 2006)**. It belongs to the family, *Crassulaceae*. It has a tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers **(Okwu and Josiah, 2006)**. *B. pinnatum* leaves have tested positive for antihypertensive activity **(Nwali et al., 2012)**. European mistletoe (V. *album*) is an evergreen, hemi-parasitic plant, normally found growing on a variety of trees, especially pine, poplar, apple trees, locust trees among others. It belongs to the family, *Moraceae*. *V. album* has been recognized for its use as anti-inflammatory, anti-diabetic and anti-hypertensive activities **(Sengul et al., 2009)**.

Despite the efficacy and wide usage of herbal plants, not all of the herbal plants reported to be useful are harmless **(Lans, 2006)**. Bioactive compounds derived from medicinal plants can be useful but might have serious dose-related side effects. Thus there is a need for compositional evaluation of herbal plants to see if their health benefit outweighs the risk of adverse effect and to detect if these plants contain other important bioactive compounds.

Scientific hypothesis

The correlation between antioxidant capacity, total phenolic content, unsaturated fatty acid content to hypertension is a little shady even though folk medicine practices uphold the correlation to be effective. From literature, 40 mg of Lisinopril, an active compound for hypertension control and regulation is the maximum allowed dose to be used, similarly, little amount of these leaves are needed to be consumed to provide the needed phenolic compounds and antioxidant compounds for blood pressure regulation and control in the estimated range of 0.024, 0.032 and 0.061 g for *A. altilis*, *V. album*, *B. pinnatum* leaves respectively for phenolic compounds and range 0.016, 0.024 and 0.026 g for *A. altilis*, *V. album*, *B. pinnatum* leaves respectively for antioxidant compounds for the regulation of blood pressure.

The aim of this research is to evaluate the plant constituent, total phenolic content and antioxidant power of the plant leaves.

MATERIAL AND METHODOLOGY

Material: All the chemicals and reagents used in this study were of analytical grade and were products of Sigma Aldrich, USA. The water used was glass distilled.

Sample collection and identification

Fresh samples of *A. altilis*, *B. pinnatum* and *V. album* leaves were collected from different locations in Nigeria. *A. altilis* was collected from Ajilosun area in Ado-Ekiti, Ekiti State. *B. pinnatum* was collected from sango ota, Ogun State. *Viscum album* was detached from a *Gmelina arborea* tree at Stadium area of Ogbomoso, Oyo state. The plant leaves were identified and authenticated at the Department of Biology herbarium, Ladoke Akintola University of Technology. The leaves were detached from the stem, washed with distilled water to remove dirt and other contaminants, and air dried to reduce moisture

content and then oven dried at 45 °C to constant weight. The samples were grinded and stored in an air tight container for further analysis.

Preparation of the Extracts

The grinded samples (1 g) were soaked in 50 mL of the solvents methanol for 72 h, after which the samples were filtered using whatman's filter paper. The filtrates were concentrated using water bath at 50 °C according to **Afolabi et al. (2014)**.

GC-MS Analysis

One gram of the dried samples each was soaked in 50 mL hexane for two days. The extract was concentrated in a water bath. Hexane extract of the leaves were screened by GC- MS. The GC-MS system used was GCMS-QP2010SE SHIMADZU.

Phytochemical content assay

Estimation of Total Phenolic Content

The total phenolic contents assay was carried out using the method described by **Sengul et al. (2009)**. In a 1.5 mL Eppendorf tube, 790 µL of distilled water, 10 µL of diluted sample and 50 µL of Folin-Ciocalteau reagent were added and the mixture vortexed. After 1 min, 150 µL of aqueous sodium carbonate (20%) was added and the mixture vortexed and allowed to stand in the dark at room temperature for 2 h. The absorbance was read at 750 nm using UV-visible spectrophotometer. The total polyphenol concentration was calculated from a calibration curve (100 – 500 $\mu g.mL^{-1}$) and the results were expressed as mg of gallic acid equivalents (mg $GAE.g^{-1}$) dry sample.

Antioxidant activities assay

Estimation of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activities of the extracts were determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method according to **Brand-Williams et al. (1995)**. A fresh 0.002% solution of DPPH was prepared in methanol and its absorbance was recorded at 515 nm. 50 µL of pure leaf extracts was mixed with 3 mL solution of DPPH and allowed to stand in darkness for 15 min. The absorbance was again recorded at 515 nm.

Ferric reducing antioxidant power (FRAP) assay

Determination of antioxidant properties was carried out using the method of **Chan et al. (2007)**. About 1 mL appropriate dilutions of methanolic leaf extract was dispensed into different test tubes. 2.5 mL phosphate buffer was added, followed by 2.5 mL K_2FeCN solution and then incubated at 500 °C for 20 min. 2.5 mL TCA solution was added to the mixture to stop the reaction. Reaction mixture was separated into aliquots of 2.5 mL and each diluted with 2.5 mL of distilled water and allowed to stand in the dark for 30 min for color development. The absorbance was read at 700 nm against a reagent blank. Ferric reducing antioxidant power was expressed as gallic acid equivalent (mg $GAE.g^{-1}$ sample).

Statistical analysis

The data were subjected to one-way ANOVA to analyze the significant difference in all data and Duncan's Multiple Range Test (DMRT) ($p \leq 0.05$) to analyze the significant difference between Mean values of samples using SPSS 18 software (SPSS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The total phenolic content of the methanolic leave extracts are presented in Table 1 as 659.5, 1667.5 and 1232.0 mg $GAE.g^{-1}$ dry extract for *B. pinnatum, A. altilis* and *V. album* respectively. *A. altilis* has the highest phenolic content followed by *V. album* and then *B. pinnatum*. Polyphenols are considered to have beneficial effects on human health and provide protection against several chronic diseases, such as cardiovascular diseases (CVD) **(Manach et al., 2005)**. All the herbal plant leaves understudy have high phenolic content. This could justify their use as anti-hypertensive herbs.

Analysis of the scavenging activities of the methanolic of three herbal plants on 1,1-diphenyl-2-picrylhydrazyl radical as reported in fig.1indicated that the *A.altilis* has the highest scavenging capacity against DPPH, with IC_{50} value of 2.24. *B. pinnatum* showed a higher scavenging power with IC_{50} value of 3.63 than *V. album* with IC_{50} value of 4.65. Higher percentage inhibition indicates better scavenging activity or antioxidant potentials. *A. altilis* showed the highest

percentage inhibition on DPPH. Therefore, *A. altilis* is the most potent out of the plant leaves studied and expected to have the highest blood pressure reducing ability, anti-aging, anti-cancer and anti-atheriosclerosis properties.

Table 1 Total phenolic contents (TPC) of the methanolic leave extracts of *A. altilis, B. pinnatum* and *V. album*.

	Concentration (mg $GAE.g^{-1}$)
Bryophyllum pinnatum	659.5 ±0.03
Artocarpus altilis	1667.5 ±0.02
Viscum album	1232.0 ±0.02

Table 2 Ferric reducing antioxidant power (FRAP) expressed as $mg.GAE^{-1}$ of the methanolic extract of *A. altilis, B. pinnatum* and *V. album* leaves mg $GAE.g^{-1}$.

Sample	**mg $GAE.g^{-1}$**
Bryophyllum *pinnatum*	1561.8 ±0.01
Artocarpus *altilis*	2505.2 ±0.04
Viscum *album*	1698.0 ± 0.03

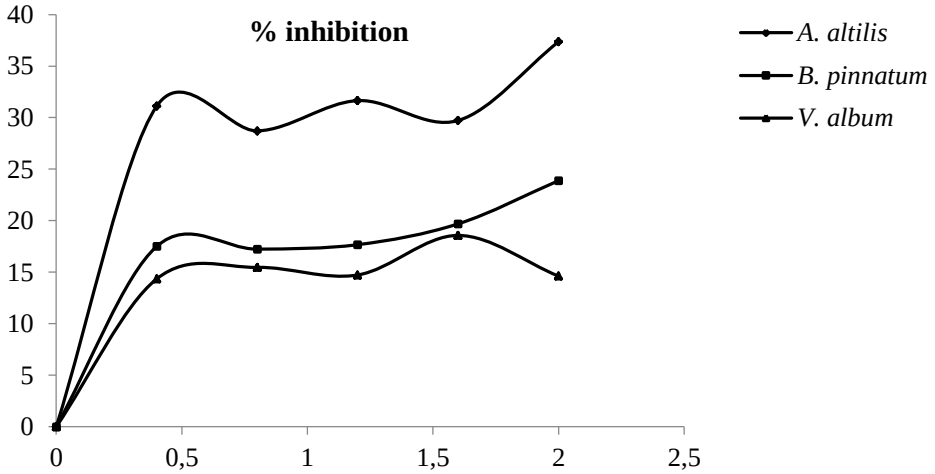


Figure 4 The Percentage (%) inhibitory potentials of *A. altilis, B. pinnatum* and *V.album* leave extracts on 1,1-diphenyl-2-picryl-hydrazyl (DPPH).
Keys: *A. altilis = Artocarpus altilis*; *B. pinnatum = Bryophyllum pinnatum; V. album = Viscum album.*

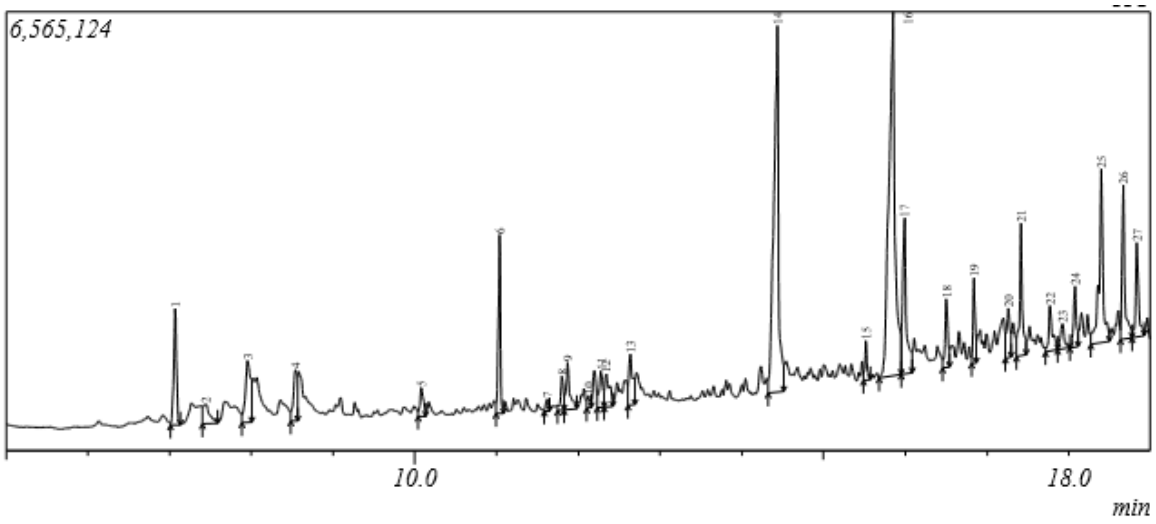


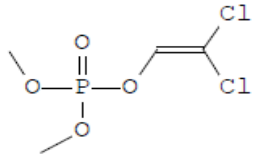
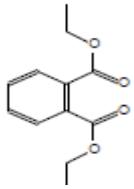
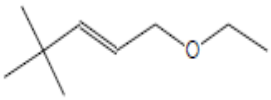
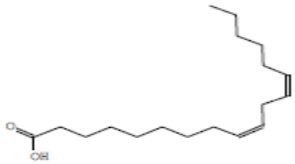
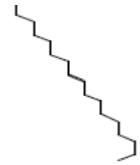
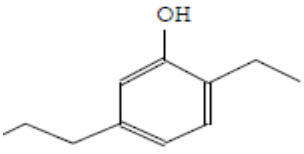
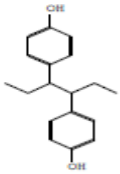
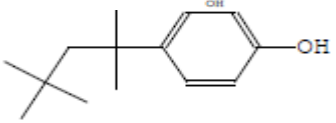
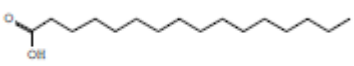
Figure 5 Chromatogram of *A. altilis* extract.

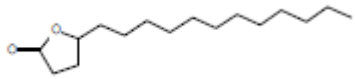
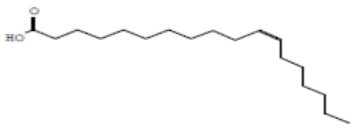
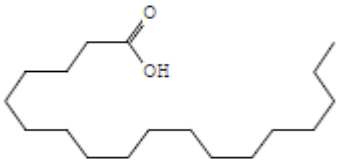
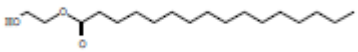
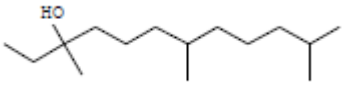
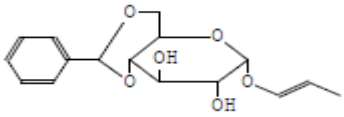
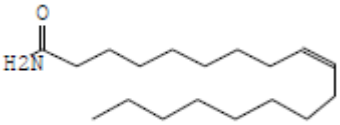
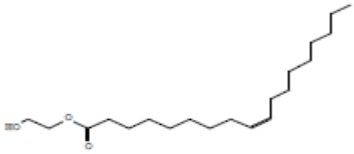
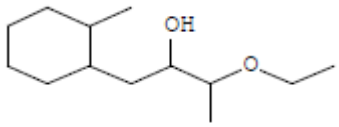
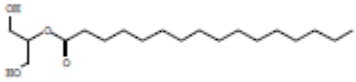
Plants with high antioxidant properties have been reported to also be efficient in treating coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, cataracts and inflammations **(Huang et al., 2005)**.

Also, Table 2 shows the reducing activities of various extracts when compared with gallic acid as the standard. The higher the absorbance of the reaction mixture, the higher would be the reducing power. At mg/ml concentration, the methanolic extract of *B. pinnatum* had the minimum reducing power of 1561.8 ±0.01 mg GAE.g^{-1}; *V. album* had 1698.0 ±0.03 mg GAE.g^{-1} while *A. altilis* showed the highest reducing ability with 12505.2 ±0.04 mg GAE.g^{-1}. This result compliments the one obtained from the DPPH assay as shown in Figure 4.

These various results indicate the highest anti-oxidant potential of *A. altilis* out of the three plants examined. This result is also justified by their phenolic compound where *A. altilis* had the highest phenolic compound (Table 1). Studies have shown that dietary phenolic compounds have a protective role against cardiovascular risk due to the numerous chemical and structural properties, and biological effects including high antioxidant capacity *in vitro* and *in vivo*, anti-inflammatory and anti-hypertensive effects, and improved endothelial functions **(AFNS, 2008)**. However, twenty five compounds were detected in the chromatogram of *A.altilis* as shown in Figure 5 and the probable structures of the components were presented in Table 3. In the results, cis vaccenic acid (22.98%) is the major fatty acid present in *A.altilis* with Palmitic acid (18.07%), stearic acid (5.77%), Linoleic acid (0.90%) also detected. The phenolic compounds detected were phenol (2.03%), 3-Dodecanol (1.88%) and Benzesterol (1.39%). These compounds could probably be responsible for the total phenolic contents shown in this plant extract (Table 1) as determined by the folin ciolcateau's method. A 2008 study at the University of Alberta suggests that vaccenic acid feeding in rats over 16 weeks resulted in lowered total cholesterol, lowered low density lipoprotein (LDL) cholesterol and lower triglyceride levels **(Stebins, 1945)**. Vaccenic acid, Alpha d glucopyranose, oleic acid amide and tetradecanoic acid content of *A. altilis* could be responsible for the observed antihypertensive activity because of the antihypertensive active sites detected in these compounds **(Wang et al., 2013)**.

Table 3 Various compounds detected in *A. altilis* extract with probable structure.

Peak	Retention time (min)	Concentration ($g.100g^{-1}$)	Name	Structures
1	7.069	3.48	Dichloros	
2	7.440	1.36	2-Nonenal, (E)-	
3	7.954	3.60	2,4-Decadienal, (E,E)-	
4	8.540	2.18	2-Undecenal	
5	10.079	1.07	2-methyltetracosane	
6	11.035	4.00	Diethyl Phthalate	
7	11.615	0.31	1-ethoxy-4,4-dimethyl-2-pentene	
8	11.801	0.90	Linoleic acid	
9	11.869	1.86	8-Heptadecene	
10	12.123	0.36	Myristic aldehyde	
11	12.277	1.39	2-Ethyl-5-n-propylphenol	
12	12.341	1.52	Hexestrol	
13	12.635	2.03	Phenol,4-(1,1,3,3-tetramethylbutyl)-phenol	
14	14.433	18.07	Palmitic acid	

15	15.518	0.93	Furanone	
16	15.848	22.98	cis-Vaccenic acid	
17	15.989	5.77	Stearic acid	
18	16.503	1.97	2-hydroxyethyl ester	
19	16.841	1.88	3-Dodecanol, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	
20	17.260	1.68	alpha.d-Glucopyranose	
21	17.416	3.96	Oleic acid amide	
22	17.771	1.69	9-Octadecenoic acid	
23	17.923	0.99	Tetradecanoic acid,	
24	18.075	1.73	3- Ethoxy-1-(2-methyl-cyclohexyl)-butan-2-ol	
25	18.400	6.99	Beta-monoglyceride	

In the chromatogram for *B. pinnatum* (Figure 6), fourteen compounds were detected and their probable structures shown in Table 4.

Oleic acid (30.41%) is the major fatty acid present in *B. pinnatum*. Palmitic acid (22.66%) and 9-Octadecenoic acid (7.78%) were also detected. Oleic acid is the major source of omega 9 fatty acid. Research has shown that omega-9 fatty acids can help to reduce the risk of cardiovascular disease and stroke. Because omega-9 fatty acids have been known to increase high density lipoprotein (HDL) good cholesterol and decrease low density lipoprotein (LDL) bad cholesterol, also, they help eliminate plaque buildup in the arteries, which causes heart attack and stroke. The stereoisomer of oleic acid is called elaidic acid or trans-9-octadecenoic acid. Elaidic acid, the most abundant trans-fatty acid in diet, appears to have an adverse effect on

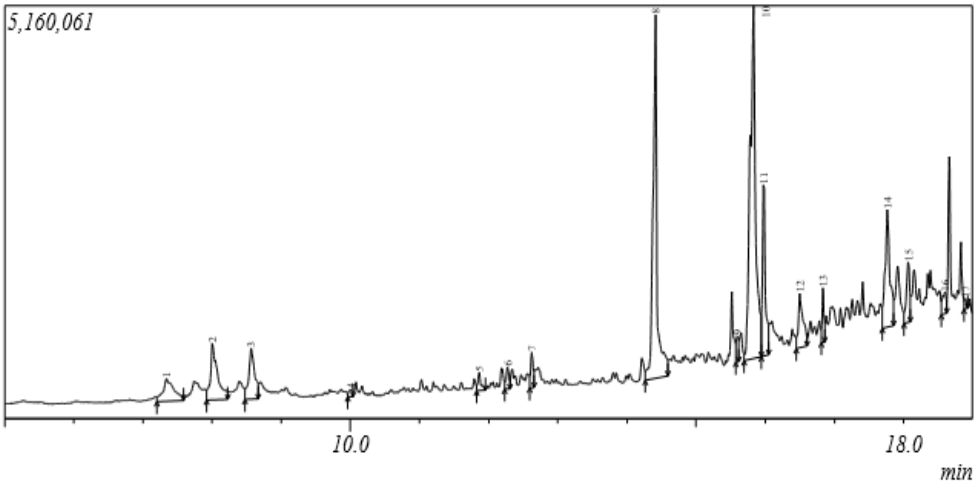
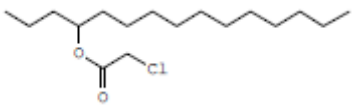
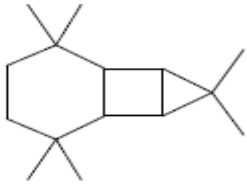
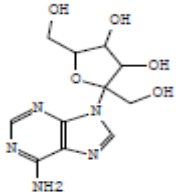
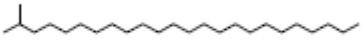


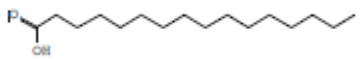
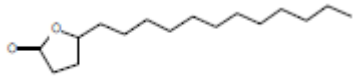
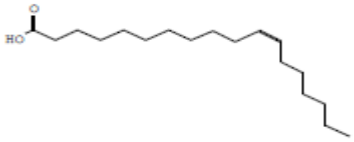
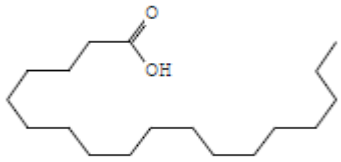
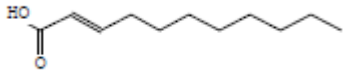
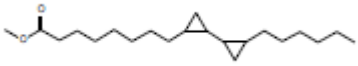
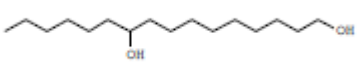
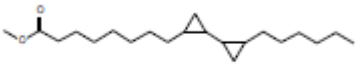
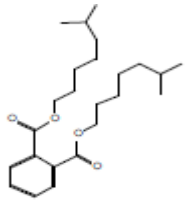
Figure 6 Chromatogram of *B. pinnatum* Extract.

health **(AFNS, 2008)**. Piscofuranine (1.41%) was the only phenolic compound present. The active site responsible for anti-hypertensive activity is the carbon of the carboxylate group for angiotensin 1 inhibitor, therefore piscofuranine, bis-ethlenetherephthalate and oleic acid content of *B. pinnatum* could be responsible for the observed antihypertensive activity.

Eighteen compounds which correspond to nineteen peaks in the chromatogram (Figure 7) were detected in the extract of *V. album* as presented in Table 5. Cis vaccenic acid (33.68%) is the major fatty acid detected in *V. album*.

Table 5 Compounds detected in *V. album* extract with probable structures.

Peak	Retention Time (min)	Concentration ($g.100g^{-1}$)	Name	Structures
1	8.012	4.59	2,4-Decadienal, (E,E)-	
2	8.565	3.83	2-Undecenal	
3	9.015	1.12	2-Nonen-1-ol, (E)-	
4	11.026	0.53	Chloroacetic acid.	
5	11.098	0.59	2-methyltetracosane	
6	12.271	0.63	Tricyclo[4.3.0.0(7,9)]nonane	
7	12.621	1.19	Psicofuranine	
8	12.915	0.25	Methyltetracosane	
9	13.844	0.26	13-Tetradecenal	

10	14.414	24.16	Palmitic acid	
11	15.592	1.65	Furanone.	
12	15.826	33.68	cis-Vaccenic acid	
13	15.975	10.66	Stearic acid	
14	16.499	2.99	trans-2-undecenoic acid	
15	16.834	1.02	[1,1'-Bicyclopropyl]-2-octanoic acid.	
16	17.762	3.73	1,10-Hexadecanediol	
17	18.067	1.77	[1,1'-Bicyclopropyl]-2-octanoic acid	
18	18.658	5.84	Diisooctyl phthalate	

However, palmitic acids (24.16%), stearic acid (10.66%) and trans-2-undecenoic acid (2.99%) were also detected. The presence of fatty acids especially Cis vaccenic acid in combination with pscofuranine, diisooctylphthalate could be responsible for the observed anti-hypertensive activity **(Sengul et al., 2009)**.

Also, stearic acid has been shown to be detrimental to human health, however, subsequently, study has also revealed that if studied in isolation, it actually contributes to a decrease in LDL levels leading to an improved overall cholesterol ratio **(Wong et al.,2006)**.

CONCLUSION

In this finding, A. altilis showed the highest anti-hypertensive potential because of the high antioxidant capacity, total phenolic content and its high blood pressure lowering fatty acids contents, while B. pinnatum showed the least anti-hypertensive potential. These antioxidants may achieve their antihypertensive effects by reducing aldehyde conjugate/AGE formation and oxidative stress by improving insulin-resistance and endothelial function, or by normalising calcium channels and peripheral vascular resistance (Grynberg, 2007; Beg et al., 2011). With this current work, it is clearly shown that the active sites involved in the process of regulating blood pressure for groups of anti-hypertensives are found in the structures of the compounds detected in these herbs, for example the structure of Lisinopril a popular anti-hypertensive has carboxylic and amine functional groups which is also present in palmitic acid, stearic acid and piscofuranine all present in the herbs. This could justify the observed antihypertensive activity of these herbs.

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