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Phytochemistry of *Anchomanes difformis* stem, *Dioscorea bulbifera* stem bark, *Fadogia cienkowskii* leaf, *Hannoa klaineana* stem and *Vitex simplicifolia* leaf.

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ABSTRACT

The phytochemical investigation of *Anchomanes difformis* stem*, Dioscorea bulbifera* stem bark*, Fadogia cienkowskii* leaf*, Hannoa klaineana* stem bark and *Vitex simplicifolia* leaf using gas chromatography flame ionization detector (GC-FID) revealed that they possess adequate secondary metabolites (Sparteine, Phytate, Oxalate, Phenol, Tannin, Naringerin, Lunamarin, Ribalinidine, Catechin, Epicatechin, Rutin, Kaemferol, Anthocyanin and Sapogenin). In *Anchomanes difformis* plant, Lunamarin had the highest concentration (429.89 µg/ml) while Sparteine had the lowest concentration (0.023 µg/ml). In *Dioscorea bulbifera* stem bark, Rutin had the highest concentration (28.64 µg/ml), while Phytate had the lowest concentration (0.78 µg/ml). In *Fadogia cienkowskii* leaf, Anthocyanin had the highest concentration (943.68 µg/ml), while Phytate had the lowest concentration (1.55 µg/ml). In *Hannoa klaineana* stem*,* Anthocyanin had the highest concentration (66.34 µg/ml), while Sparteine had the lowest concentration (0.04 µg/ml). In *Vitex simplicifolia* leaf, Anthocyanin had the highest concentration (344.39 µg/ml), while Sparteine had the lowest concentration (0.01 µg/ml) respectively when compared to other active components present in the selected medicinal plants. Hence, the presence of these active components in high amount could be responsible for their therapeutic activity as antimalaria and anticancer plants.

Keywords: GC-FID, Phytochemical, Antimalaria and Anticancer.

Aims Research Journal Reference Format:
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1. INTRODUCTION

One of the common practices in the world is the extensive use of plants in the management of diseases and minority of these plants has received scientific investigation. Phytochemicals are bioactive compound found in plant diets such as fruits, vegetables, grains, beans that are responsible for disease protection. The research carried out on the use of plants in medicine showed that a combination of these plants are used for the management of some diseases like gonorrhoea, liver infection, diabetes, malaria, typhoid fever, cancer etc. (Aiyeloja and Bello, 2006). *Anchomane*s *difformis* (Blume) is an herbaceous plant belonging to the genus *Anchomane*s with prickly stem having huge divided leaf and spatial that arises from a horizontal tuber occurring in the West Africa forest, which contains some biological active chemical that produce definite physiological and biological actions in the human body. (Burkill, 1985). It is used traditionally to treat oedemas, difficult child-birth, as a poison antidote, and as a strong diuretic for treating urethral discharge; jaundice and kidney-pains.

Dioscorea bulbifera is a medicinal plant with the genus *Dioscorea* that has been generally known as a potential source of natural antioxidants. It is commonly known as yam or air potato which can be used in the treat gastric cancer, goiter, sore throat etc. Traditionally, it can be used as antioxidant, antitumor and anti-inflammatory.

Hannoa klaineana is a plant with the genus *Hannoa* that can be used traditionally to treat malaria and fever (François *et al.,* 1998). It can also be used to treat intestinal diseases and constipation. It can also be used in South Eastern Nigeria for the treatment of malaria.

Fadogia cienkowskii is flowering plants which belong to the family Rubiaceae. It belongs to the genus *Fadogia. Rytigynia* and *Fadogia* produce a great clade but none of them is monophyletic *(Lantz and Bremer 2005).* It can be used to treat diarrhea, inflammation and male sexual impotence. In Nigeria, it is traditionally used to treat malaria in the Nsukka community (Enugu State, Eastern Nigeria).

Vitex simplicifolia is a plant with the genus known as *Vitex*. Young twigs are used as tooth-sticks in Nigeria. The decoction of the bark of the plant can be used as a lotion in Ivory Coast and for the treatment of oedemas, skin troubles and for toothache.

Studies have shown that in rats, the aqueous extract oral administration at different doses exhibited neither acute toxicity nor any abnormal behavioral change which suggest that it could be used for therapeutic purposes (Adedapo, *et al.* 2009). Research has also shown that the aqueous stem bark of this plant investigated in albino rat possess some analgesic and anti-inflammatory properties.

2. MATERIALS AND METHODS

Materials: GC-FID (Buck 530), Refrigerator (Thermocool, China), Water bath (Pyrex) and Electronic weighing balance (England).

Chemicals: Potassium hydroxide (KOH), acetic acid, quercetin, ferric chloride (FeCl3+), Hydrochloric acid, Sulphuric acid, Million's reagent were all purchased from Sigma Chemical, USA.

Plant identification and collection

The selected medicinal plants such as *Fadogia cienkowskii, Vitex simplicifolia, Hannoa klaineana, Dioscorea bulbifera, Anchomanes difformis* were identified and collected in International Centre for Ethnomedicine and Drug Development (InterCEDD) in Nsukka, Enugu State, Nigeria. The specimen were washed to discard impurities and placed thinly on the flat clean tray (to prevent spoilage by moisture condensation) and permitted to dry at room temperature for seven days (Sofowora 1982). The leaves of the plants were pulverized into fine powder with wooden pestle and mortar respectively however; the stems were grounded into powder form by means of an electric mill and stored until the need arises.

Qualitative Phytochemical Analysis

According to the methods of Harborne (1973); Trease and Evans (1989), the test for phytochemicals are as shown below:

Test for Tannins

0.5g Pulverized plant sample was boiled in a test tube with distilled water of 20ml and Whatman No. 1 filter paper was used for filtering. The filtrate was added to 0.1% $FeCl_3$ and revealed a brownish green colouration within some time which signifies the presence of tannins.

Test for Saponins
Pulverized plant sample of 2g was boiled with distilled water of 20ml and Whatman No. 1 filter paper was used for filtering. 10ml of the filtrate was fixed in a test tube with distilled water of 5ml and mixed together to form a stable standard froth. In addition, three drops of olive oil was mixed with froth and allowed to stand to form emulsion, this indicates the presence of saponins.

Test for Resins
0.5g pulverized plant material was extracted using 15ml of 96% ethanol. The extract from ethanol was poured into a beaker containing 20ml of distilled water. An observed resinous precipitate revealed the presence of resins. Also, Chloroform extraction was done using 0.12g and it was concentrated to dryness. The residue formed was further dissolved again in 3ml acetone and concentrated HCL was added respectively. In addition, for 30 minutes the mixture was kept on a water bath. The formation of a pink colour which changes to red colouration indicates the presence of resins.

Test for Alkaloids
Pulverized plant material of 0.5g was added to 5ml of 1% HCL and boiled for 5min in a water bath. 1ml of the filtrate was used to filter and treat the sample with some drops of Wagner's Draggendorf's and Mayer's reagent respectively. The observation of the colouration of creamy white precipitates and reddish brown displayed the presence of alkaloids.

Test for Glycosides
To 0.5 g of pulverised plant samples, 10 ml of distilled water was added and boiled for 5 min. This was filtered and 2 ml of the filtrate hydrolysed with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. A reddish–brown precipitate showed the presence of glycosides.

Test for Flavonoids
0.2g Pulverized plant sample and 10ml of ethyl acetate were heated for 30min in a water bath. The heated solution was filtered and a filtrate of 4ml was added to 1ml of 1% aluminum chloride solution and examined for some time. The formation of yellow colour in the ethyl acetate layer showed the presence of flavonoids.

Test for Steroids and Terpenoids
1g pulverized plant material was added to 9ml of ethanol and refluxed for some minutes. The filtrate in a heated water bath was concentrated to 2.5ml and 5ml of hot water was added. The mixture was observed for 1hour and filtered off the waxy matter. 2.5ml of chloroform was used to extract the filtrate by the help of a separating funnel. 0.5ml of the chloroform extract was added to concentrated sulphuric acid of 1ml to form a lower layer in a test tube. The formation of a reddish brown colouration interface reveals the presence of steroid. In addition, another chloroform extract of 0.5ml was evaporated to dryness and heated for 10 minutes with 3ml of concentrated sulphuric in a water bath. The formation of grey color shows the presence of terpenoids.

Test for Proteins
5ml of distilled water was added to 0.1g pulverized plant sample and allowed for observation for three hours before filtering. 0.1ml of millon's reagent was added to 2ml portion of the filtrate and shook the solution vigorously and allowed for observation. The observation of yellow precipitate formation indicated the presence of proteins.

Test for Carbohydrates
Addition of two drops of α-naphthol solution to 2ml of the sample in a test tube and 1ml of concentrated H_2SO_4 was poured on the sides of the tube down to form two layers. The observation of change of colour to brown at the intersection of the two layers shows the presence of carbohydrates.

Preparation of extract and Phytochemical determination using GC-FID method

The pulverised sample was prepared by cold maceration technique (O'Neill, et al., 1985). The plant material was soaked for three uninterrupted days at room temperature. The rotary evaporator was used to concentrate at a low pressure with a maximum temperature of 45˚C to produce the crude extracts and the extracts were stored at 4^{o}C until it was used. The phytochemical analysis was carried using an auto system buck 530 chromatographer in gas phase equipped with an on-column automatic injector, flame ionization detector, with Hp88 capillary column (100m × 0.25mm), injector temperature 220^{o}C, detector temperature 250^{o}C, over temperature to 180^{o}C, injection volume 1µl sample, hydrogen was used as a carrier gas (24 pound per square inch (PSI). The concentration of each active component was determined based on area peaks and retention time.

3. RESULTS AND DISCUSSION

The result of the qualitative analysis carried out on the selected medicinal plants (*Anchomanes difformis* stem*, Dioscorea bulbifera* stem bark*, Fadogia cienkowskii* leaf*, Hannoa klaineana* stem bark *and Vitex simplicifolia* leaf) revealed the presence of essential phytochemicals such as alkaloid, glycoside, saponin, terpenoid, protein, carbohydrate, steroid, resin and flavonoid.

Table 1 Phytochemical constituents in the six selected medicinal plants

Extract	*Anchomanes difformis*	*Fadogia cienkowskii*	*Vitex simplicifolia*	*Hannoa klaineana*	*Dioscorea bulbifera*
Alkaloids	++	+	++	++	++
Flavonoids	++	+	+++	++	+++
Glycoside	++	+	++	+	+
Tannins	-	++	+++	+	++
Terpenoid	++	++	-	++	+
Saponins	++	++	+++	++	+++
Resin	-	++	-	++	+++
Proteins	+	+	++	+	++
Carbohydrate	++	+	++	+	+
Steroids	++	+	+++	+++	+++

KEY

+ means present in trace amount
++ means present in high amount
+++ means present in very high amount
- means absent

Quantitative analysis on Phytochemical Constituents

The Gas Chromatography Flame Ionization Detector (GC-FID) isolation of *Anchomanes difformis* revealed twelve (12) components with in which Lunamarine had the highest concentration of 429.89 µg/ml while Spartein had the lowest concentration of 0.02 µg/ml. The presence of Lunamarine displaying the highest concentration could suggest the high effectiveness of this plant in the treatment of cancer (*Manu and Kuttan 2009). Also,* the high composition of Lunamarine (alkaloid) in *A. difformis* confirms the findings of (Odeghe *et al* 2012) that the use of this plant traditionally in the management of malaria could be due to the presence of Lunamarine. It also agrees with Ikewuchi and Ikewuchi, (2008) that medicinal plants that rich in moderate amount of Lunamarine (Alkaloid) and tannin may possess potential health promoting effects.

Table 3.2: Quantitative analysis on *Anchomanes difformis* constituents

S/NO.	Component	Retention time	Concentration (µg/ml)
1.	Sparteine	2.53	0.02
2.	Phytate	3.66	1.42
3.	Oxalate	5.77	3.06
4.	Phenol	14.87	32.48
5.	Tannin	16.19	61.31
6.	Naringerin	18.01	25.10
7.	Lunamarine	18.80	429.89
8.	Ribalinidine	30.14	0.07
9.	Catechin	31.17	94.85
10.	Epicatechin	32.46	40.47
11.	Rutin	32.93	288.63
12.	Kaempferol	35.01	66.21

The Gas Chromatography Flame Ionization Detector (GC-FID) isolation of *Fadogia cienkowskii* revealed seven (7) components in which Anthocyanin had the highest concentration of 943.68 µg/ml while Phytate had the lowest concentration of 1.55 µg/ml. The high composition of Anthocyanin (Flavonoid) in *F. cienkowskii* plant agrees with the report of Harbone and Baxter (1999), that this plant possesses numerous essential properties such as anti-inflammatory activity, enzyme inhibition and antimicrobial activity.

Table 3.4: Quantitative analysis of *Fadogia cienkowskii* constituents

S/NO.	Component	Retention time	Concentration (µg/ml)
1.	Phytate	*4.91*	1.55
2.	Anthocyanin	*7.60*	943.68
3.	Tannin	*12.61*	*61.41*
4.	Naringerine	*18.49*	18.75
5.	Ribalinidine	*25.89*	*2.12*
6.	Catechin	*30.95*	77.32
7.	Kaempferol	*35.87*	*20.84*

The Gas Chromatography Flame Ionization Detector (GC-FID) isolation of *Vitex simplicifolia* revealed nine (9) components in which Anthocyanin had the highest concentration of 344.39 µg/ml while Spartein had the lowest concentration of 0.0024 µg/ml. The increased Anthocyanin (Flavonoid) concentration present in the plant when compared to other active components confirms the report of Middleton and Chithan (1993) that this plant can be used in the management of oxidative stress and other pain related diseases.

Table 3.5: Quantitative analysis of *Vitex simplicifolia* constituents

S/NO.	Component	Retention time	Concentration (µg/ml)
1.	Sparteine	1.88	0.00
2.	Phytate	4.60	0.15
3.	Anthocyanin	8.74	344.39
4.	Tannin	12.67	29.84
5.	Naringerine	18.70	19.18
6.	Ribalinidine	29.12	2.01
7.	Catechin	31.41	62.04
8.	Epicatechin	32.35	26.50
9.	Kaempferol	39.25	25.34

The Gas Chromatography Flame Ionization Detector (GC-FID) isolation of *Hannoa klaineana* revealed eight (8) components in which Anthocyanin had the highest concentration of 66.35 µg/ml while Tannin had the lowest concentration of 0.01 µg/ml. The high composition of Anthocyanin (flavonoid) present in the plant supports the report of Harborne and Williams (2000) that this plant possesses cytotoxic antitumour properties.

Table 3.6: Quantitative analysis of *Hannoa klaineana* constituents

S/NO.	Component	Retention time	Concentration (µg/ml)
1.	Anthocyanin	0.59	66.35
2.	Catechin	1.56	18.25
3.	Epicatechin	7.86	10.15
4.	Tannin	7.97	0.01
5.	Naringerine	15.23	22.43
6.	Ribalinidine	18.34	2.01
7.	Phenol	25.12	0.24
8.	Phytate	26.17	0.26

The Gas Chromatography Flame Ionization Detector (GC-FID) isolation of *Dioscorea bulbifera* revealed seven (7) components in which Rutin had the highest concentration of 28.64 µg/ml while phytate had the lowest concentration of 0.78 µg/ml. The increased composition of Rutin (Glycoside) supports the report of Fatoba et al, (2003) that this plant could be used in the management of heart diseases that may result from malaria severity.

Table 3.7: Quantitative analysis of *Dioscorea bulbifera* constituents

S/NO.	Component	Retention time	Concentration (µg/ml)
1.	Lunamarine	3.02	4.25
2.	Phytate	3.86	0.78
3.	Ribalinidine	4.77	5.20
4.	Tannin	12.23	3.31
5.	Phenol	18.45	1.23
6.	Rutin	27.23	28.64
7.	Kaempferol	28.90	7.28

CONCLUSION

This research shows that the phytochemistry of the selected plants revealed that a component of *F. cienkowskii* displayed the highest concentration when compared with the remaining plant components in dose manner. The presence of these plants active components in high concentrations could be a promising opportunity in designing a novel drug with high efficacy to slow down the rate at which malaria parasite and tumour cells proliferate.

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