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Ezekwe Ahamefula Sunday
Department of Medical Biochemistry, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria

Rizwan A Ansari
Department of Biochemistry, Yobe State University, Damatru, Nigeria

Ewa Ogbonnaya
Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria

Corresponding Author:
Ezekwe Ahamefula Sunday
Department of Medical Biochemistry, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria

Phytochemical screening and GC-MS studies of some soup thickeners in southern Nigeria

Ezekwe Ahamefula Sunday, Rizwan A Ansari and Ewa Ogbonnaya

Abstract
The aim of this study was reveal in details the phytochemicals inherent in selected soup thickeners namely *Brachystegia eurycoma* (achi), *Mucuna solannie* (ukpo) *Detarium microcarpuim* (ofor). Matured clean seeds of the aforementioned thickeners processed into flour samples, extracted with methanols and resulting samples extract were subjected to qualitative phytochemical and GC-MS analysis. The phytochemical screened for included tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, phlobactanins, phenolics, proteins, reducing sugars, anthraquinnones, and alkaloids. While saponins, flavonoids and alkaloids were reportedly present in abundance (++), terpenoids and phlobactanins were completely absent (-) in *Brachystegia eurycoma* (achi) flour sample extract. However, analysis performed on ofor flour sample extract, revealed that flavonoids and alkaloids were more abundant (++), while tannins, terpenoids cardiac glycosides, phlotanins, phenolics, reducing surgar were completely absecent (-). Result of the analysis on ukpo flour sample extract indicated that saponins and proteins were more abundant (++), while phlobactanins and anthraquinones were reportedly absent (-). GC-MS analysis performed on achi flour sample extract revealed the presence of 19 compounds and the three most abundant of these reported compounds include n-hexadecanoic acid (26.44%), followed by 9, 12 octadecanoic acid (Z,Z) (18.51%) and Cis vaccenic acid (19.19%). Same analysis carried out on ofor flour sample extract, showed that 17 compounds were present of which two most abundant of them reportedly present were 9,12 octadecadienoic acid (Z,Z) (28.59%) and Cis vaccenic acid (16.54%). However, GC-MS result obtained on analysing ukpo flour sample extract revealed the presence of 34 compounds of which n-hexadecanoic acid (15.17%) and 9, octadecanoic acid (E) - (15.35%) are reportedly the most abundant. The result obtained from this study clearly shows that the aforementioned condiments can deliver therapeutic benefit by virtue of the vital phytochemicals reportedly inherent in them.

Keywords: Thickeners, phytochemicals, saponins, flavonoids and alkaloids

Introduction
Soup is a tasty, popular, nutritious, wholesome and appetite stimulating recipe [1]. Generally, condiments which when introduced into an aqueous mixture boost its viscosity without a substantial modification of its essential qualities such as aroma, taste, and its ability to draw are known as soup thickeners. Thickeners accounts for the soup body, enhances its viscosity and improves the suspension of ingredients added [1] as well as allows for efficient swallowing in medical conditions characterized with impairment of mastication and swallowing of balls. This culminates to reduction in the chances of choking and inhalation of liquids or food particles, thereby averting the possibility of aspiration pneumonia [1].
Brachystegia eurycoma (achi), *Mucuna solannie* (ukpo) *Detarium microcarpuim* (ofor) are inhabitants of the tropical and sub-tropical areas [2] and members of *leguminosae* family and sub-family *caesalpiniacea* [3]. The flours of the three aforementioned thickeners are extensively utilized in soup preparation for eating eba, pounded yam and fufu in states such as Imo, Anambra, Akwa-Ibom and Ondo State. They are equally used as emulsifiers and flavouring agents in traditional soups due to their gum content. The flours when introduced into water swell and subsequently influence the viscosity of the liquid [4]. Although nutritionally, ofo, ukpo and achi have been identified as important and economic sources of protein, carbohydrates, calories, vitamins and minerals [5, 6], detailed information on the supposedly medicinal factors "the phytochemicals" are lacking. Thus, it is imperative to expand the information base of these very critical condiments in other to maximize use and benefits.

Materials and Methods
Collection of raw materials and flour production
The raw materials *Brachystegia eurycoma* (achi), *Mucuna solannie* (ukpo) *Detarium microcarpuim* (ofo) were procured from a popular local market within Port Harcourt metropolis,

Rivers State. Seeds of *Brachystegia eurycoma* (achi), *Mucuna solannie* (ukpo) *Detarium microcarpuim* (ofo) were thoroughly cleaned to eliminate dirt. Achi seeds were roasted at 150 ^{0}C for 10 min. However, ukpo seeds were roasted for 30 min at the temperature of 150 ^{0}C. Meanwhile, ofo seeds were roasted at 100 ^{0}C for a period of 10 min. All pretreatment preceded dehulling after which dehulled seed were separately milled with the aid of a disc attrition mill prior to being packaged in airtight containers [7].

Extraction of condiments flour

Exactly 10mg each of the flour samples was extracted using 100ml of methanol at 25 ^{0}C for 24 h after which the mixture was filtered with the aid of filter paper. The residue was then extracted with additional 100 ml of methanol as following the initial extraction procedure. The extracts generated from the 1st and 2nd extraction attempts were combined. Exactly 10 ml of the mixture was evaporated at 50 ^{0}C by oven drying and the resulting extract stored away from light prior to use [8].

Qualitative phytochemical analysis

Tests for reducing sugar by Fehling's test

Exactly 0.5 g of each of the flour samples extract was dissolved in distilled water and filtered after which the filtrate was subjected to heating with 5 ml of equal volumes of Fehling's solution A and B. Appearance of red precipitate of cuprous oxide showed that reducing sugars were present [9].

Test for protein by xanthoproteic test

Few drops of nitric acid were mixed with the extract of each of the flour samples. Formation of yellow color was a proof that protein was present [10].

Test for tannins

Exactly 0.5 g each of the flour samples extract was suspended and stirred in 10 ml of distilled water and afterwards filtered. Few drops of 1% ferric chloride solution were subsequently introduced to 2 ml of the filtrate. Formation of a blue-black, green or blue green precipitate showed that tannins were present [11].

Test for saponins

Precisely 1 g of each of the flour samples extract was boiled with 5 ml of distilled water and afterwards filtered. To the filtrate, about 3 ml of distilled water was introduced and shaken vigorously for about 5 min frothing which persisted on warming indicated the presence of saponins [10].

Test for alkaloids by Mayer's test

Exactly 50 mg each of flour sample extract devoid of solvent is stirred in 2 ml of dilute hydrochloric acid (HCl) and filtered. To the filtrate, few drops of Mayer's reagent were introduced by the side of the test tube. Formation of white or creamy precipitate indicated the presence of alkaloids [12].

Test for flavonoids by Shinoda's test

Exactly 0.5 g each of the flour samples extract was allowed to dissolve in ethanol warmed and then filtered. Three pieces of magnesium chips were added into the filtrate. This was followed by the addition of few drops of conc. HCl. Appearance of pink, orange, or red to purple colour showed that flavonoids were present [11].

Test for terpenoids by Salkowski test

Exactly 2 ml of chloroform was introduced into a test tube holding 0.5 g of the samples extract. After which of 3 ml conc. H_2SO_4 was added to form a layer. The appearance of a reddish brown colour at the interface showed that terpenoids were present [12].

Test for phenols by ferric chloride test

Few drops of neutral 5% ferric chloride solution were introduced into a test tube containing 50 mg each of the flour samples extract dissolved in 5 ml of distilled water. A dark green colour suggested that phenolic compounds were present [13].

Test for steroids

Exactly 5 ml of distilled water was added into a test tube containing 0.5 g each of flour sample extracts and the mixture shaken vigorously and observed for a stable persistent froth. The resulting froth was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion showed that steroids were present [11].

Tests for cardiac glycosides by Keller Killiani's

Exactly 100 mg each of the flour samples extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of concentrated H_2SO_4 acid. A brown ring obtained at the interface showed that cardiac glycosides were present [11].

Test for free anthraquinones

Exactly 2 mg each of the flour samples extract was introduced into a dry test tube, after which 5 ml of chloroform was added and shaken for at least 5 min. This was filtered and the filtrate was added into an equal volume of 10% ammonia solution, and was shaken again. The presence of bright pink colouration in the aqueous upper layer was a pointer that free anthraquinones were present [11].

Test for phlobatannins

To determine the presence of phlobactannins, 0.5mg each of the flour samples extract was boiled with 1 % aqueous hydrochloric acid. Deposition of a red precipitate was an indication that phlobactannins were present [10].

GC-MS of analysis of flour samples

An agilent 7890B Gas Chromatography (GC) system fitted with a 30 m× 250 µm × 0.25 µm Rtx-5MS capillary column coupled to Agilent 5977A Mass Spectrometric (MS) was employed at a temperature of 325 ºC. Ultra-high purity helium (99.99%) formed the mobile phase at constant flow rate of 1.0 cm^3/min. The injector, transfer line and ion source temperature were set at 290 ºC. The ionizing energy was 70ev. Electron multiplier voltage was obtained from auto tune. The oven temperature was programmed from 60 ºC for 2 mins, then 10 ºC /min to 110 ºC/min and then 280 ºC at the rate of 5 ºC/min. The samples were diluted with appropriate acetone (1/100 v/v), filtered and 1 µL was injected into the inlet. All data were obtained by collecting the total ions currents (TIC). The percentage composition was determined from calibration curve (0-0.9g/cm^3). The sample and the standard were prepared in like manner. The standard was processed separately before being spiked into the sample and signal of the sample was obtained from the difference of the spiked sample and that of the standard. The experiment was repeated more than six times. As a quality control, percentage relative standard (%RSD) was estimated by comparing coefficient of determination (R2) values of calibration curves using both standard signal and spiked sample signal [14].

Results

Table 1: Result on the qualitative phytochemical analysis on selected soup thickeners

Thickeners			
Phytochemicals	**Achi**	**Ofor**	**Ukpo**
Tannins	+	-	+
Saponins	++	+	++
Flavonoids	++	++	+
Steroids	+	+	+
Terpenoids	_	-	+
Cardiac glycosides	+	-	+
Phlobactanins	-	-	-
Phenolics	+	-	+
Proteins	+	+	++
Reducing sugars	+	-	+
Anthraquinnones	+	+	-
Alkaloids	++	++	+
Key: +: abundant, ++: more abundant, -: absent			

Table 2: Result on Gc-Ms analysis on Achi

RT	Name of Compound	Molecular Formula	MW	Peak Area %
3.258	1-Hexanamine, 3,5,5-trimethyl-	$C_9H_{21}N$	143	1.32
6.441	2-Penten-1-ol, (Z)-	$C_5H_{10}O$	86	3.18
6.478	1-Dodecanamine	$C_{12}H_{27}N$	185	0.84
8.227	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	1.17
8.441	.alpha.-Methyl 4-O-methyl-D-mannoside	$C_8H_{16}O_6$	208	1.22
8.569	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.78
9.053	(5R,8aR)-5-Propyloctahydroindolizine	$C_{11}H_{21}N$	167	1.64
9.634	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.55
9.816	cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	2.29
10.008	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	26.44
10.720	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	$C_{19}H_{34}O_2$	294	1.20
10.762	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	1.57
11.089	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	18.51
11.121	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	17.19
11.228	Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.26
11.955	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.82
12.228	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	4.86
13.035	Methyl 11-docosenoate	$C_{23}H_{44}O_2$	352	1.65
13.298	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	4.53

Table 3: Result on GC-MS analysis On of or

RT	Name of Compound	Molecular Formula	MW	Peak Area %
2.386	1,2-Propanediol, 3-chloro-	$C_3H_7ClO_2$	110	7.06
7.088	Dodecanoic acid	$C_{12}H_{24}O_2$	200	3.69
8.227	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	1.13
8.580	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	5.21
9.495	Pentadecylamine	$C_{15}H_{33}N$	227	0.90
9.634	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.79
9.805	Pentadecylamine	$C_{15}H_{33}N$	227	1.54
9.971	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.79
10.720	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	3.07
10.762	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	`4.10
11.089	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	28.59
11.121	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	16.54
11.217	Octadecanoic acid	$C_{18}H_{36}O_2$	284	4.68
11.955	Methyl 13-eicosenoate	$C_{21}H_{40}O_2$	324	1.57
12.217	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	2.68
13.041	Methyl 11-docosenoate	$C_{23}H_{44}O_2$	352	1.64
13.287	Erucic acid	$C_{22}H_{42}O_2$	338	2.02

Table 4: Result on GC-MS analysis on UKPO

RT	Name of Compound	Molecular Formula	MW	Peak Area %
2.381	2,4(1H,3H)-Pyrimidinedione, dihydro-5-hydroxy-	$C_4H_4N_2O_3$	128	0.70
2.504	2,4(1H,3H)-Pyrimidinedione, dihydro-5-hydroxy-	$C_4H_4N_2O_3$	128	3.12
2.868	H2N(CH2)7NH2	$C_7H_{18}N_2$	130	1.20
3.039	L-Alanine, N-glycyl-	$C_5H_{10}N_2O_3$	146	0.63

3.563	Ethylene oxide	C_2H_4O	44	0.89
4.044	Ethylene oxide	C_2H_4O	44	0.49
4.323	1H-Pyrrole-2,5-dione	$C_4H_3NO_2$	97	0.86
4.504	Ethylene oxide	C_2H_4O	44	0.55
4.954	N,N'-Dimethylsulfamide	$C_2H_8N_2O_2S$	124	0.65
6.457	Acetamide, N-butyl-	$C_6H_{13}NO$	115	10.08
8.227	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	2.08
8.553	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.63
9.217	1-Methylpropylhydroxylamine	$C_4H_{11}NO$	89	0.50
9.489	9-Hexadecenoic acid, methyl ester,(Z)-	$C_{17}H_{32}O_2$	268	1.64
9.634	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	7.91
9.800	Pentadecylamine	$C_{15}H_{33}N$	227	2.25
9.955	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.17
10.720	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.85
10.762	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	4.78
10.794	2-(3-Methylguanidino)ethanol	$C_4H_{11}N_3O$	117	0.84
10.917	Methyl stearate	$C_{19}H_{38}O_2$	298	1.36
11.062	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	15.35
11.190	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.76
11.950	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	3.24
12.212	Oleic Acid	$C_{18}H_{34}O_2$	282	4.21
12.335	Allantoic acid	$C_4H_8N_4O_4$	176	0.57
12.917	β-Alanine, N-methyl-, ethyl ester	$C_6H_{13}NO_2$	131	0.65
13.035	Methyl 11-docosenoate	$C_{23}H_{44}O_2$	352	3.67
13.142	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	0.57
13.287	Erucic acid	$C_{22}H_{42}O_2$	338	4.55
13.966	2-Methylaminomethyl-1,3-dioxolane	$C_5H_{11}NO_2$	117	0.61
14.078	Oleic Acid	$C_{18}H_{34}O_2$	282	1.54
15.057	2-Amino-1-(o-hydroxyphenyl)propane	$C_9H_{13}NO$	151	0.62
19.144	3-fluoroamphetamine	$C_9H_{12}FN$	153	0.47

Discussion

Phytochemicals are biologically active compounds in plants that wield mainly medicinal potentials [15]. This is evident by the fact that phytochemicals have been identified through research effort as the key factors that determine not only the therapeutic potential of a particular medicinal plant, but its strength as well. Table 1.0 shows the outcome of the qualitative phytochemical analysis performed on the methanolic flour extract of the three thickeners studied (*Brachystegia eurycoma* (achi), *Mucuna solannie* (ukpo) *Detarium microcarpuim* (ofor)). The phytochemical screened for were tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, phlobactanins, phenolics, proteins, reducing sugars, anthraquinnones, and alkaloids. While saponins, flavonoids and alkaloids were reportedly present in abundance (++), terpenoids and phlobactanins were completely absent (-) in *Brachystegia eurycoma* (achi) flour sample extract. However, analysis carried out on ofor flour sample extract, revealed that flavonoids and alkaloids were more abundant (++), while tannins, terpenoids cardiac glycosides, phlotanins, phenolics, reducing surgar were completely absent (-). Result of the analysis on ukpo flour sample extract indicated that saponins and proteins were more abundant (++), while phlobactanins and anthraquinones were reportedly absent (-). This result is consistent with the work of Ezekwe *et al*. [16] which reported similar degree of abundance for some of the phytochemicals reportedly present in castor seed (ogiri Igbo) a condiment of a kind. GC-MS was carried out with the assistance of the National Institute Standard and Technology (NIST) database which has over 62,000 patterns. Spectrum obtained on unknown compounds was compared with that of known compounds deposited in the NIST library. The name, molecular weight and structure of the compounds were determined. GC-MS analysis performed on achi flour sample extract showed the presence of 19 compounds and the three most abundant of these compounds included n-hexadecanoic acid (26.44%), followed by 9, 12 octadecanoic acid (Z,Z) (18.51%) and Cis vaccenic acid (19.19%). Same analysis performed on ofor flour sample extract, indicated the presence of 17 compounds of which two most abundant of them present were 9, 12 octadecadienoic acid (Z, Z) (28.59%) and Cis vaccenic acid (16.54%). However, GC-MS result obtained on ukpo flour sample extract clearly revealed the presence of 34 compounds of which n-hexadecanoic acid (15.17%) and 9, octadecanoic acid (E) - (15.35%) are reportedly the most abundant.

Conclusion

The results generated from this work indicate clearly that the aforementioned condiments (*Brachystegia eurycoma* (achi), *Mucuna solannie* (ukpo) *Detarium microcarpuim* (ofor)) wield the potential to deliver therapeutic benefit by virtue of the inherent vital phytochemicals present in them.

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