

Comparative phytochemistry of peddled yoruba medicinal formulations

Belonwu DC*, Onyeike EN and Oghenekaro Uche Ekere

Department of Biochemistry, University of Port-Harcourt, Choba, Rivers state, Nigeria.

chukkabell2000@yahoo.co.uk, chuka.belonwu@uniport.edu.ng

Abstract

Yoruba formulations are now widely accepted as medicinal formulations in rural and most urban areas among the low income earners. This study was aimed to evaluate the phytochemical constituents of these formulations. Five different formulations were purchased from traditional medical dispensers in Aluu, Choba, Alakahia, Rumuosi and Rumuokoro communities in Obio/Akpor Local Government Area of Rivers state Nigeria. Phytochemical studies revealed the presence of alkaloids, flavonoids, terpenoids, saponins, phenolics and cardiac glycosides. Phylobatanins was absent in all formulations while anthraquinones was absent in the formulation purchased in Aluu community. Quantitative analysis revealed the major components in all five formulations as; Tanins (8.35 ± 0.327 -Aluu; 12.03 ± 0.556 -Choba; 5.67 ± 0.231 - Alakahia; 7.53 ± 0.456 -Rumuosi; 5.63 ± 0.031 - Rumuokoro), Flavonoids (4.24 ± 0.21 -Aluu; 9.06 ± 0.055 - Choba; 5.54 ± 0.051 - Alakahia; 7.35 ± 0.021 - Rumuosi; 8.13 ± 0.062 - Rumuokoro), then alkaloids (2.75 ± 0.07 -Aluu; 0.82 ± 0.020 - Choba; 5.41 ± 0.100 - Alakahia; 3.47 ± 0.023 - Rumuosi; 4.77 ± 0.157 - Rumuokoro). These phytochemicals have been implicated in several medicinal capacities, hence, the acclaimed medicinal properties of these Yoruba medicinal formulations.

Keywords: Phytochemistry, Yoruba formulations

1. Introduction

Yoruba formulations are fast becoming a recurrent site in our societies. According to the World Health Organization (De Silva, 1997), about 80% of the population in many third world countries still uses traditional medicine (e.g. medicinal plants) for their primary health care, due to poverty and lack of access to modern medicine. These formulations are part of the daily drinks of a reasonable fraction of individuals in our communities. These individuals include; low income civil servants, artisans, mechanics, aged members of rural populace, field laborers etc. These formulations are so cheap and readily available and are said to cure all kind of illness. The efficacy of these formulations have not been proven scientifically but only by words of mouth by several users.

Many infectious diseases are known to be treated with herbal formulations throughout the history of mankind (Balasundaram *et al.*, 2011; Nebedum *et al.*, 2009; Joseph & Jini, 2011). The majority of the herb traders which are women with little or no education, but yet, they seem to know the right herbal mixture to administer for any kind of ailment. Stimulated by high population growth rates, rapid urbanization, rural unemployment and the value placed on traditional medicines, these formulations are enjoying greater patronage in Nigeria.

Yoruba medicinal formulations are created from herbs obtained from bushes around the neighborhoods and forests from adjacent states. Recipes for the treatment of common ailments which are reported almost on daily basis such as hypertension, dysentery, low sperm count and weak erection, coated tongue, pile (jedi-jedi), menstrual disorder, malaria, typhoid and fevers are not fully disclosed by the practitioners for patent reasons (Kadiri, 2008).

According to the UNESCO, interest in formulations and medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being. The average cost of malaria treatment based on ACT is estimated to be about ₦1,500 (USD 10.00) inclusive of cost of laboratory tests. This is a princely sum for the average Nigerian in the rural areas which are characterized with low household incomes. A radio-medical therapy for the same ailment will cost on the average ₦200 or could even be procured for free, if the person could collect the medicinal plants and prepare the medicament personally.

1.1 Herbal preparation

Herbal remedies can either be prepared from dry plant “ingredients” or freshly collected samples from the field. It is however affirmed that either plant material is efficient depending on accessibility to plant species as some plants are not easily seen within the locality (kadiri, 2008). Hence, they are collected fresh or bought and preserved dry.

In rural communities, it is common practice for dwellers to prepare herbal remedies in local clay pots. This is strongly preferred to aluminium pots (Olowokudejo & kadiri, 2008).

1.2 Phytochemistry

Phytochemistry is the study of phytochemicals. These are chemicals derived from plants. According to Liu (2004) phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plants foods that have been linked to reducing the risk of diseases. In his report, Anderson, (2004) defined phytochemicals as plant-derived chemicals, which are beneficial to human health and disease prevention. They are chemicals that are not required for the immediate survival of the plant but which are synthesized to increase the fitness of the plant to survive by allowing it to interact with its environment, including pathogens and herbivorous and symbiotic insects (David & Emma, 2011). Inavova et al. (2005) pointed out clearly that medicinal plants constitute the main source of raw pharmaceuticals and healthcare products while Mandal et al. (2007) also reported that extraction and characterization of several active phytochemicals from green plants have given birth to some high activity profile drugs. Such phytochemical screening of various plants had been reported by many workers (Parekh *et al.*, 2008; Soladoye *et al.*, 2008; Siddiqui *et al.*, 2009; Sonibare *et al.*, 2009 and Ashok *et al.*, 2010).

Many phytochemicals normally function as toxins that protect the plants against insects and other damaging organisms. Plants evolved the ability to produce toxic substances and concentrate them in vulnerable regions (the skin, seeds and leaves) in order to dissuade insects and other organisms from eating and killing the plant.

Phytochemical methods are used to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. Modern pharmacological studies indicate that binding to receptors or ion channels on cell membranes is the first step of some drug actions. Phytochemistry of herbal medicines has revealed the presence of various chemical components, such as saponins, alkaloids, volatile oils, flavonoids, anthraquinones etc.

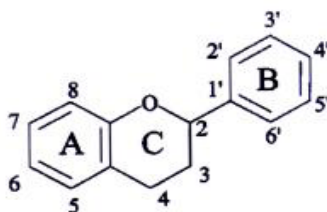
1.3 Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties (Manske, 1965). Also some synthetic compounds of similar structure are attributed to alkaloids (Robert, 1998). In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulphur and more rarely other elements such as chlorine, bromine, and phosphorus. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are, the local anaesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine, nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimetic galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arrhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the anti-malarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste (Rhoades and David, 1979). Most of the known functions of alkaloids are related to protection.

1.4 Flavonoids

Flavonoids represent the most common and widely distributed group of plant phenolics. Their common structure ($C_6-C_3-C_6$) consists of two aromatic rings (A ring and B ring) linked through a three carbon bridge that is usually an oxygenated heterocycle (C ring). Figure 1, shows the basic structure and the system used for the carbon numbering of the flavonoid nucleus. The major flavonoid classes include anthocyanidins, chalcones, flavanols, flavanones, flavones, flavonol, and isoflavones. The variability of the flavonoids is based on the hydroxylation of the pyrone ring, absence or presence of double bond, the number of hydroxyls in the A-ring and B-ring, and/or a double bonded oxygen atom attached to position 4 of the C-ring. Flavonoids may be monomeric, dimeric, or oligomeric. Polymeric flavonoids, known as tannins, are divided into two groups, condensed and hydrolysable. Condensed tannins are polymers of flavonoids while hydrolysable tannins contain gallic acid (Braca *et al.*, 2002).

Fig. 1 Basic structure of flavonoid skeleton (Pietta, 2000)

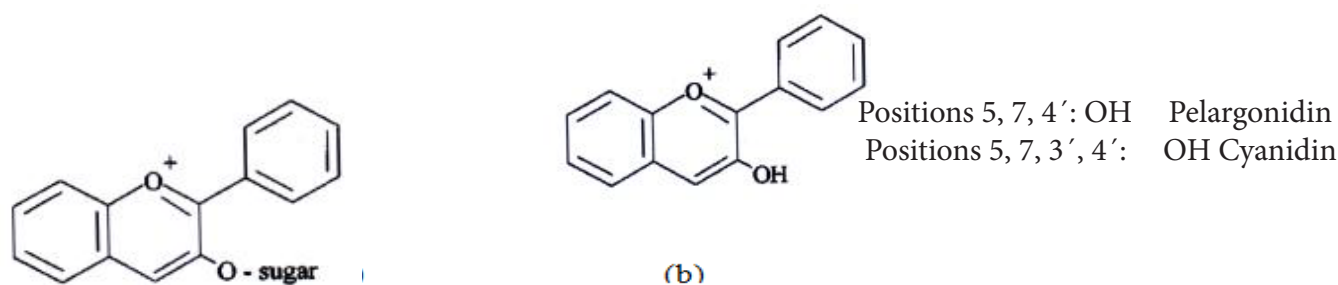


Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological “response modifiers”. *In vitro* studies show that flavonoids also have anti- allergic, anti-inflammatory, anti-microbial (Cushine, 2011 & 2005), anti-cancer (De Sousa *et al.*, 2007) and anti-diarrheal activities (Schuier, 2005).

1.5 Anthocyanins

Anthocyanins are widely distributed among fruits and vegetables. They are one of the main classes of flavanoids. They contribute significantly to the antioxidant activities of the flavanoids (Lapidot *et al.*, 1999). Anthocyanins (Figure 2a) are glycosylated anthocyanidins with sugars generally attached to the 3-hydroxyl position of the anthocyanidin (Figure 2b). According to Pietta (2000), in some cases the sugar residues are acylated by p-hydroxybenzoic, p-coumaric, caffeic, ferulic, sinapic, acetic acid, oxalic acid, malic acid, or succinic acid. Anthocyanidin is an aglycone. This means that there is no sugar group or other functional group attached to the flavan nucleus. Also, the oxygen atom on the C ring has a positive charge on it, and there are two double bonds in the C-ring. In addition to hydroxylated anthocyanidins, such as delphinidin, cyanidin, and pelargonidin, there are also methylated anthocyanidins (malvidin, peonidin, and petunidin)

Fig. 2 Basic structure of (a) anthocyanin and (b) anthocyanidin skeletons Source: Pietta (2000).

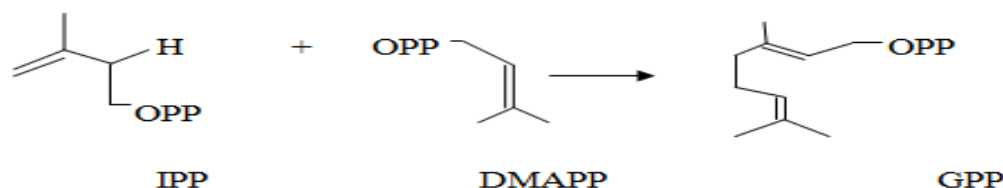


1.6 Tannin

Tannin (also known as vegetable tannin, natural organic tannins or sometimes tannoid, i.e. a type of biomolecule) is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Katie et al 2006). The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine (McGee et al, 2004). Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine.

1.7 Terpenes

They may be defined as a group of molecules whose structure is based on a various but definite number of isoprene units (methylbuta-1, 3-diene, named hemiterpene, with 5 carbon atoms). Terpenoids are extraordinarily diverse but they all originate through the condensation of the universal phosphorylated derivative of hemiterpene, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) giving geranyl pyrophosphate (GPP).



Terpenoids (or isoprenoids), are a subclass of the prenillipids (terpenes, prenylquinones, and sterols), and represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products. Terpenoids can be described as modified terpenes, where methyl groups are moved or removed, or oxygen atoms added.

1.8 How do phytochemicals work?

There are many phytochemicals as seen above and each works differently. These are some possible actions:

Antioxidant: Most phytochemicals have antioxidant activity and protect our cell against oxidative damage and reduce the risk

of developing certain types of cancers. Phytochemicals with antioxidant activity include; allyl, sulfides (onions, leaks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (teas, grapes).

Hormonal action: Isoflavones found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.

Stimulation of enzymes: Indoles, which are found in cabbages, stimulate enzymes that make the estrogens less effective and could reduce the risk of breast cancer. Other phytochemicals, which interfere with the enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

Interference with DNA replication: Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Similarly, capsaicin, found in hot peppers, protect DNA from carcinogens.

Antibacterial effect: The phytochemical allicins from garlic has antibacterial properties.

Physical action: Some phytochemical bind physically to cell wall thereby preventing the adhesion of pathogens to human cells.

To promote the proper use and to determine their potential as sources for new drugs, it is essential to study these medicinal formulations (Parekh & Chanda, 2007).

2. Materials and methods

Formulations were purchased from several local traditional healers peddling them at Omoko in Aluu, Rumuchakara in choba, Alakahia, Rumuosi and Rumuokoro communities in Rivers state. These formulations were those used mainly in treatment of malaria, fever, body and waist pains, typhoid, pile (Jedi-Jedi) and several miscellaneous illnesses as confirmed by several respondents in the rural population.

These were prepared from various herbs and plant materials as well as other additives such as pineapples, lemon, local gin, honey and red potash; which may exert therapeutic activity and also used as adjuncts. The extracts are prepared by various extraction methods and solvents. A higher percentage of those interviewed showed preference to aqueous extract from fermented maize (98%) followed by water (90%) and alcohol (20%). They stated that alcohol is only used for the preparation of remedies consisting mainly hardy plant parts like stem bark, root and seed.

These herbs for herbal therapy were made from a combination of more than one plant (table 1). The combination of these different plants is what's acclaimed to cure several ailments and dysfunctions associated with the body.

According to the practitioners, the dead leaves are usually brown and richer in some active agents than the green leaves. They also assert that the plant would have passed into the dying leaves, certain unwanted metabolites which are required for the medicine.

2.1 Traditional extraction methods

Two main methods in extraction used by these herbal dispensers were; boiling in water or aqueous extract from fermented maize (called ekan ogi or omidun) and soaking in the solvents such as alcohol; with more preference given to boiling than soaking. Boiling is usually done using either water or aqueous extract from fermented maize starch but more preference is given to aqueous extract from fermented maize as this is believed to be more efficient. Alcohol as solvent was never used when boiling herbal "ingredients". Duration of boiling ranged from 1-2 hours on burning fire wood or cooking stove till a change in color of the solvent is observed indicating "full dissolution of active ingredient into the solvents". Soaking, the second choice of preparation is given a far lesser preference unlike boiling. This method is preferred by few herbal dispersers as they believe that the ingredients will be extracted without the "ingredients" from the plant been exposed to heat which they believe may affect the efficacy of the herbal recipes. Plant parts are cut into small piece and soaked in corked bottles or containers for 2-3 days.(Plate 1-4)



(a) Omoko, Aluu



(b) Rumuchakara, choba



(c) Rumuosi, Rumuekini



(d) Alakahia

Plate 1(a-d). Photos of several medicinal formulations purchased.

Table 1: Major plant constituents in each fomulation.

Source of formulation	Plant part used	Botanical name of constituents	Local name
Omoko, Aluu	Stem barks and leaves.	Enantia chloranta Petivera allicea Achyranthes aspera Diospyros mespiliformis	Awopa Aboro, Abora Igi dudu
Rumuchakara, choba.	Stem, bark and Leaves.	Alstonia boonei Abutilon mauritianum Chrysophyllum albidum Alstomia congensis	Ahun, Awun Furu, kawo Agbalumo
Alakahia	Stem and bark	Khaya grandifoliola Khaya ivorensis Aframomum melegueta Capsicum frutescens	Oganwo Ata-ire, atare Ata-ijosi
Rumuosi/Rumuekini	Leaves and roots	Bryophyllum pinnatum Allium sativum Cymbopogon citrates Ocimum basilicum Citrus aurantifolia	Ayuu, Garlic Osan weve
Rumuokoro	Rhizome	Zingiber officinale Tithonia diversifolia Morinda lucida Lawsonia guineensis Azadiractha indica	Ginger Jogbo Agbale Oruwo Dongoyaro

NOTE: This table depicts the major constituents of each formulation and not the only constituents.

The constituent plants for each formulation were identified by the department of Plant science and biotechnology and confirmed by the University of Port-Harcourt Greenhouse staff.

2.2 Methods

2.2.1 Phytochemical screening

Chemical tests were carried out on the sample using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

2.2.2 Qualitative screening

Test for Tannins: About 20 ml of sample was placed in a test tube. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.2.3 Test for Phlobatannins

Deposition of a red precipitate when each sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

2.2.4 Test for Saponin

10ml of the sample was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.2.5 Test for Flavonoids

Two methods were used to determine the presence of flavonoids in the sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the sample followed by addition of concentrated H_2SO_4 . A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each sample. A yellow colouration was observed indicating the presence of flavonoids.

2.2.6 Test for Steroids

2ml of acetic anhydride was added to 10ml of each sample with 2ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

2.2.7 Test for Terpenoids (Salkowski test)

Five ml of each sample was mixed in 2ml of chloroform, and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

2.2.8 Test for Cardiac glycosides (Keller-Killani test)

Five ml of each sample was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.2.9 Test for Alkaloids

5ml of sample was warmed with 2% H₂SO₄ for two minutes. It was filtered and few drops of Drageneloff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

2.2.10 Test for Reducing sugars

Sample was boiled with Fehling's solution A and B for two minutes. An orange red precipitate indicates the presence of reducing sugar.

2.2.11 Test for Anthraquinone

About 5ml of each portion to be tested was shaken with 10 ml of benzene and then filtered. Five millilitres of the 10% ammonia solution was then added to the sample and thereafter the shaken. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase was taken as the presence of free anthraquinones.

2.3 Quantitative screening

2.3.1 Determination of total phenols by spectrophotometric method

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15min. 5ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added.

2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30min for colour development. This was measured at 505 nm.

2.3.2 Alkaloid determination using Harborne (1973) method

5ml of the sample was measured into a 250 ml beaker and 20 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours at 28°C. This was filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH₄OH) was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and measured. Alkaloid content was calculated and expressed as a percentage of the sample analyzed.

2.3.3 Tannin determination by Van-Burden and Robinson (1981) method

5 ml of the sample was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10min.

Swain's method (1979) was also used for the determination of tannin contents of the sample. 5ml of sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80°C for 1hour and stirred with a glass rod to prevent lumping. The sample was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse.

This was made up to mark with distilled water and thoroughly mixed. 1 ml of sample was pipetted into 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na₂CO₃ were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1ml of sample above. The absorbance of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760nm. Percentage tannin was calculated

2.3.4 Saponin determination

The method used was that of Obadoni and Ochuko (2001). The samples were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water-bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

The Spectrophotometric method described by Brunner was used for saponin analysis (1984) was also used. 15ml of sample was measured into a 250 ml beaker and 100 ml Isobetyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of Magnesium carbonate added. The mixture obtained with saturated

MgCO₃ was again filtered through a Whatman No 1 filter paper to obtain a clear colourless solution. 1ml of the colourless solution was pipetted into 50ml volumetric flask and 2 ml of 5% FeCl₃ solution was added and made up to mark with distilled water. It was allowed to stand for 30min for blood red colour to develop. 0-10ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2ml of 5% FeCl solution as done for sample above. The absorbance of the sample as well as standard saponin solutions were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 380nm. The percentage saponin was also calculated

2.3.5 Total Flavonoids determination

Total flavonoid was estimated by Zhisten method. 1ml of the sample was taken in different test tubes containing 4ml of distilled water. At time zero, 0.3ml of 5% sodium nitrite was added to all the tubes. At 5th minute, 0.3ml of 10% aluminium chloride was added to all the tubes. At 6th minute, 2ml of 1M sodium hydroxide were added. The volume in the tubes were immediately diluted with 2.4 ml of distilled water and mixed well. The absorbance of the pink colour developed was read at 510nm against the blank.

This was also determined according to the method outlined by Harbone (1973). 5ml of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through whatman No.42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample. The flavonoid content was calculated and expressed as a percentage of the sample analyzed.

2.3.6 Anthraquinone contents determination

A suspension of sample was heated in water bath at 70°C for one hour. After the suspension was cooled, 50ml of 50% methanol was added to it and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450nm and compared with a standard solution containing 1mg/100ml alizarin and 1mg/100ml purpurin with the absorption-maximum 450nm. The anthraquinone content was calculated and expressed as a percentage of the sample analyzed.

2.3.7 Cardiac glycosides content determination

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy et al (1994). The sample was then purified using lead acetate and Na₂HPO₄ solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

2.3.8 Determination of Cyanogenic glycosides

For this purpose, the method described by Knowles and Watkins (1950) was adopted. Sample was then filtered using double layer of hardened filter paper and distillation was done with Marham distillation apparatus. The extracted sample was transferred into a tow-necked 500ml flask connected with a steam generator. This was steam distilled with saturated sodium bicarbonate solution contained in a 50ml conical flask for 1 hour. 1ml of starch indicator was added to 20ml each of the distillate and was titrated with 0.2N of iodine solution. The percentage hydrocyanide content was calculated.

2.4 Statistical analysis

The results are presented as mean ± standard deviation. The mean values of the various treatment groups were compared using

SPSS version 17 for windows (SPSS Inc USA). The significant level during these tests was set at $p \leq 0.05$.

3. Results

Phytochemical screening of the formulations revealed some differences in constituents. The results of the phytochemical analysis showed that these formulations are rich in several secondary metabolites like alkaloids, saponins, flavonoids as shown in table 2 below. The variation in the percentage composition of these secondary metabolites (table 3) can be attributed to the distinguishable varieties plant specimens used in the preparation of each formulation from table 1.

Table 2. Qualitative Phytochemical analysis of medicinal formulations

	SAMPLE 1 (Aluu)	SAMPLE 2 (Choba)	SAMPLE 3 (Alakahia)	SAMPLE 4 (Rumuosi)	SAMPLE 5 (Rumuokoro)
	+/-	+/-	+/-	+/-	+/-
Alkaloids	++	++	++	++	++
Flavonoids	++	++	++	++	++
Saponins	++	++	++	++	++
Steroids	-	++	+	++	-
Anthraquinones	++	+	++	++	+
Cardiac glycosides	++	++	++	++	++
Phylobatanins	-	-	±	-	-
Tannins	++	++	++	++	++
Phenols/ Phenolics	++	++	++	++	++
Terpenoids	++	++	++	++	++

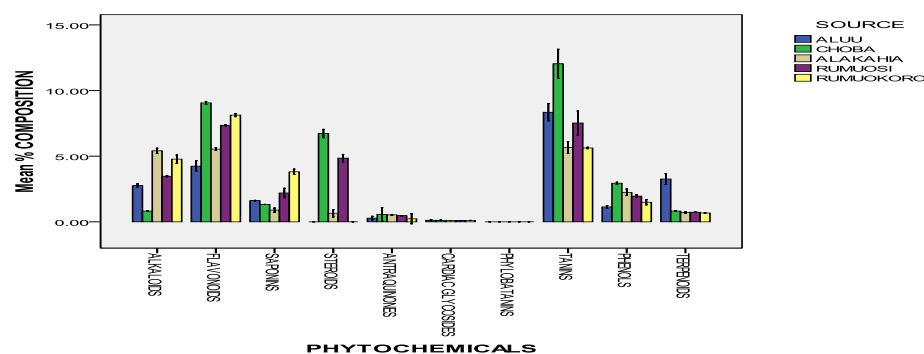
KEY: ++ = High; + = Moderate; - = absent; ± = inconclusive

Table 3. Quantitative phytochemical analysis of medicinal formulations

	SAMPLE 1 (Aluu)	SAMPLE 2 (Choba)	SAMPLE 3 (Alakahia)	SAMPLE 4 (Rumuosi)	SAMPLE 5 (Rumuokoro)
Alkaloids	2.75±0.07	0.82±0.020	5.41±0.100	3.47±0.023	4.77±0.157
Flavonoids	4.24±0.21	9.06±0.055	5.54±0.051	7.35±0.021	8.13±0.062
Saponins	1.61±0.015	1.33±0.006	0.89±0.080	2.20±0.182	3.81±0.104
Steroids	Nil	6.73±0.162	0.66±0.143	4.85±0.144	Nil
Anthraquinones	0.28±0.072	0.57±0.251	0.53±0.020	0.46±0.012	0.23±0.191
Cardiac glycosides	0.12±0.026	0.11±0.015	0.10±0.006	0.09±0.006	0.11±0.006
Phylobatanins	Nil	Nil	NQ	Nil	Nil
Tannins	8.35±0.327	12.03±0.556	5.67±0.231	7.53±0.456	5.63±0.031
Phenols/ Phenolics	1.14±0.053	2.94±0.040	2.24±0.127	1.96±0.05	1.49±0.100
Terpenoids	3.26±0.212	0.82±0.010	0.72±0.029	0.73±0.015	0.67±0.012

%= Percentage composition (Mean ± Standard Deviation).

The graph shows a comparison of each distinguishable phytochemical found all five formulation.



4. Discussion

The qualitative phytochemical analysis of the various Yoruba formulations purchased revealed the presence of some potent bioactive metabolites as seen in table 2 above. The result of phytochemical screening reveals that tannins and flavonoids are high in all formulations. Flavonoids are water soluble phytochemicals and an important plant phenolic which shows antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. This may be responsible for their antibacterial properties (Sofowora, 1993). Pamplona Roger reported that plant extracts containing chemicals with antibacterial properties have been used in treating bacterial and fungal infections.

Steroids were also present in all formulations. Steroids are of importance in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). This may be the reason why this formulation has been implicated in curing sexual impairments.

The presence of terpenoids further buttresses the use of this formulation as herbal medicines (Hayashi et al, 1993). The presence of tannins also, shows that the formulation can be used as a purgative and also in the treatment of cough, asthma and hay fever (Gills, 1992).

The quantitative phytochemical analysis showed that several of these phytochemicals are higher in all formulations; most remarkable are the tannins and flavonoids. The presence of these phytochemicals in these medicinal formulations contribute to the medicinal value of these formulations as phytochemicals have been implicated in treatment of several ailment inflammatory, cardiovascular and also in weight therapy.

The formulations contained an appreciable quantity of phenols and phenolics which imply that they may be used as anti-microbial agents (Okwu et al, 2001). Alkaloids which were also significantly present has been implicated as very important and used in analgesic, anti-spasmodic and bacterial activities.

5. Conclusion

The presence of these phytochemical constituents in the formulations showed that these formulation may be used as a basic medicinal agent such as analgesics, antispasmodic, antibacterial, anti cancer, anti inflammatory and anti oxidants.

6. References

- 1• Anderson GD (2004) Phytochemicals 2004
- 2• Ashok K, Rajkumar P and Kanimozhi M (2010) *J. Sci. Res.* 5(3), 157-162
- 3• Parekh J and Chanda S (2007) *In vitro* antibacterial activity of the crude methanol extract
- 4• of *Woodfordia fruticosa kurz.* flower (Lythraceae). *Braz. J. Microbiol.* 38, 204-207.
- 5• Balasundaram A, Kumar PR, John G, and Selvakumar BN (2011) Antimicrobial activity
- 6• of the leaf extracts of two medicinal plants against MRSA (Methicilin Resistant
- 7• *Staphylococcus aureus*) from Human Urinary Tract Pathogens. *Res. J. Microbiol.*, 6: 625
- 8• 631.
- 9• Braca A, Sortino C, Politi M, Morelli I and Mendez J (2002) Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethanol. Pharmacol.* 79, 379-381
- 10• Cushnie TPT and Lamb AJ (2005) Antimicrobial activity of flavonoids. *Int. J. Antimicrobial Agents* 26 (5), 343-356
- 11• Cushnie TPT and Lamb AJ (2011) Recent advances in understanding the antibacterial properties of flavonoids. *Int. J. Antimicrobial Agents* 38 (2), 99-107
- 12• David O Kennedy and Emma L Wightman (2011) Herbal extracts and phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function. *Brain, Performance and Nutrition Research Centre, School of Life Sciences, Northumbria University, Newcastle*
- 13• De Silva T (1997) Medicinal plants for forest conservation and health care: Industrial utilization of medicinal plants in developing countries. Global initiative for traditional systems (gifts) of health. *Food Agri. Organ. United Nat.* 11, 34-44.
- 14• De Sousa RR, Queiroz KC, Souza AC, Gurgueira SA, Augusto AC, Miranda MA, Peppelenbosch MP, Ferreira CV and Aoyama H (2007) Phosphoprotein levels, MAPK activities and NFkappaB expression are affected by fisetin. *J. Enzyme Inhib. Med. Chem.*

22 (4), 439-444

- 15• Gills LS (1992) Ethnomedical uses of plants in Nigeria. University of Benin press. Nigeria, 276
- 16• Harborne IB (1973) Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, NY. pp: 88-185
- 17• Hayashi T, Okamuka K, Kawasaki M and Morita N (1993) Production of titerpernoids by cultured cells from two Chemotypes *Scoparia Dulcis* Phytochemistry. 35(2), 353-356
- 18• Inavova D, Gerova D, Chervenkov T and Tankova T J (2005) *Ethnopharmacol.* 96, 154-150.
- 19• Joseph B and Jini D (2011) Insight into the Hypoglycaemic Effect of Traditional Indian Herbs Used in the treatment of Diabetes. *Res. J. Med. Plant.* 5, 352-376
- 20• Kadiri AB (2008) Evaluation of medicinal herbal trade (Paraga) in Lagos state of Nigeria. *Enthnobotanical leaflets* 12, 677-681
- 21• Katie E Ferrell and Thorington Richard W (2006) Squirrels: the animal answer guide. Baltimore: Johns Hopkins University Press. pp: 91
- 22• Lapidot T, Harel S, Akiri B, Granit R and Kanner J (1999) pH-dependent forms of red wine anthocyanins as antioxidants. *J. Agricultural and Food Chem.* 47, 67-70.
- 23• Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism cells by extracts of fruits and vegetables. *Food J. Nutr.* 134, 34795-34855
- 24• Mandal V, Mohan Y and Hemalatha S (2007) *Pharmacog. Rev.* 1, 7-18
- 25• Manske RHF (1965) The Alkaloids Chemistry and Physiology. Volume VIII- NY: Academic Press. pp: 673
- 26• McGEE Harold (2004) On food and cooking: the science and lore of the kitchen. New York: Scribner. pp: 714
- 27• Trease GE and Evans WC (2002) Pharmacognosy. 15th Edn. Saunders, pp: 214-393
- 28• Nebedum J, Ajeigbe K, Nwobodo E, Uba C, Adesanya O, Fadare O and Ofusori D (2009) Comparative study of the ethanolic extracts of four Nigerian plants against some pathogenic microorganisms. *Res. J. Med. Plant.* 3, 23-28
- 29• Olowokudejo JD, Kadiri AB and Travih VA (2008) An ethnobotanical survey of herbal markets and medicinal plants in Lagos state of Nigeria. *Ethnobotanical leaflets.* 12, 851-865
- 30• Okwu de (2001) Evaluation of the chemical composition of indigenous spices and flavouring Agents. *Global J. Pure Appl. Sci.* 7(3), 455-459
- 31• Pamploma-Roger GD (1999) The Encyclopedia of medicinal plants. Education and health library, 2nd Edn. Spain, pp:76-97
- 32• Pietta P (2000) Flavonoids as antioxidants. *J. Natural Product.* 63, 1035-1042
- 33• Robert Alan Lewis (1998) Lewis' dictionary of toxicology. CRC Press. pp: 51
- 34• Rhoades David F (1979) Evolution of Plant Chemical Defense against Herbivores
- 35• Schuier M, Sies H, Illek B and Fischer H (2005) Cocoa-related flavonoids inhibit CFTR- mediated chloride transport across T84 human colon epithelia. *J. Nutr.* 135 (10), 2320-2325
- 36• Siddiqui S, Serma A, Rather A, Jabeen F and Meghvansi MF (2009) *Adv. Biol. Res.* 3(5-6), 188-195
- 37• Singha SC (1965) Medicinal Plants in Nigeria. Published by the Nigerian National Press Ltd., Apapa, Lagos, Nigeria.
- 38• Sofowora A (1993) Medicinal Plants and Traditional Medicine in Africa. 2nd Edn. Spectrum Books Limited, Ibadan, Nigeria, pp: 1-153
- 39• Soladoye MO, Sonibare MA and Rosanwo TO (2008) *J. Applied Sciences Asian Net-work for Scientific Information.* 1, 1-6
- 40• Sonibare MA, Soladoye MO and Esan O (2009) *Africa J. Traditional, Complementary and Alternative Medicines (AJTCAM).* 6 (4), 518-525
- 41• Trease GE and Evans WC (1989) A textbook of Pharmacognosy (13th edition) Bacilluere Tinal Ltd, London.
- 42• Trease GE and Evans WC (1983) Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London. pp. 343-383.
- 43• United Nations Educational Scientific and Cultural Organization UNESCO (1997). Culture and health, orientation texts. World decade for cultural dev. 1988-1997. Doc. CLT/DEC/PRO, Paris, France, Pg.129.

44• United Nations Educational Scientific and Cultural Organization (UNESCO) (1998). Terminal Report. Promotion of Ethnobotany and sustainable use of plant resources in Africa. Pg.60, Paris

