Original Research Article

Phytochemical Composition of Ethanol Extract of a cocktail Herbal Mixture (Aju Mbaise)

ABSTRACT

Aim: This study was carried out to determine the phytochemical constituent of ethanol extract of Aju Mbaise herbal mixture.

Study design: In the course of the experiment, fresh samples of the plants that make up Aju Mbaise were collected and identified as *Cnestis ferruginea*, *Xylopia aethiopica*, *Uvaria chamae*, *Palisota hirsuta*, *Scleria sp*., *Napoleona imperialis*, *Dialium guineense*, *Combretum racemosun*, and *Heterotis rotundifolia* respectively. The fresh plants were air-dried, cut into small pieces and blended before the extraction process. Ethanol was used as the extraction solvent.

Place and Duration of Study: The study was carried out in the Research Laboratory of the Department of Biochemistry, Faculty of Science, University of Port Harcourt, in July 2018.

Methodology: The qualitative phytochemical analysis was determined by Standard methods for testing alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids, while the quantitative phytochemical was estimated by spectrophotometric method.

Results: The phytochemical result showed the presence of alkaloids (8.69%), flavonoids (19.10%), glycosides (6.86%), hydrogen cyanide (0.92%), phenols (31.56%), saponins (0.83%), steroids (0.94%), tannins (16.80%), and terpenoids (14.31%).

Conclusion: The study showed that ethanol extract of Aju Mbaise herbal mixture contains tremendous amount of phytochemicals.

Keywords:

Aju Mbaise, Extraction, Phytochemical, Spectrophotometric and *Xylopia aethiopica*.

Introduction

Medicinal plants, also known as medicinal herbs, have been revealed and used in traditional medicine practices since ancient times. They are used to attempt to maintain good health, whether in modern medicine or in traditional medicine [1]. According to [2], plant's medicinal properties are dependent on the plant secondary metabolites contained in them. These metabolites that possess medicinal properties are found only in a few species of plants [2]. However, development of plants or extracts having potential medicinal uses is blunted by weak scientific evidence, poor practices in the process of drug development, and insufficient financing. Some other functions of these secondary metabolites include; serving as defensive compounds against herbivores and pathogens, mechanical support to the plant, absorbing harmful ultraviolet radiation and reducing the growth of

nearby competing plants. Secondary plant metabolites with reported medicinal properties include alkaloids, terpenoids, saponins, polysaccharides, waxes and fatty acids, simple phenolics, flavonoids and glycosides and their derivatives. According to [3], alkaloids are group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. It also includes some related compounds with neutral and even weakly acidic properties. According to [4], about ninety-five percent (95%) of alkaloids taste bitter with high level of toxicity, and they are naturally synthesized by a large diversity of organisms including fungi, bacteria, plants, and animals. Some of the pharmacological benefits of alkaloids include; antimalarial (e.g. quinine) [4], antiasthma (e.g. ephedrine), anticancer (e.g. homo harringtonine) [5], cholinomimetic (e.g. galantamine) [6], vasodilatory(e.g. vincamine), antiarrhyth mic (e.g. quinidine, analgesic (e.g. morphine) [7], antibacterial (e.g. chelerythrine) [8], and antihyperglycemic activities (e.g. piperine) [3]. Other alkaloids possess psychotropic (e.g. psilocin) and stimulant activities (e.g. cocaine, caffeine, nicotine, theobromine) [9] and have been used in ent heogenic rituals or as recreational drugs. Also according to [9], some alkaloids can be toxic too (e.g. atropine, tubocurarine). Flavonoids are the most common group of polyphenolic compounds in the human diet and are found mainly in plants [10]. Its widespread distribution, varieties and relatively low toxicity compared to other active plant compounds (for instance alkaloids), shows that many animals, including humans, ingest significant quantities in their diet. According to [11], some foods with high flavonoid content include parsley, onions, blueberries and other berries, black tea, green tea and oolong tea, bananas, all citrus fruits. Flavonoids are classified into six major classes, which are; flavones, flavonols, flavonones, flavanols (catechins), anthocyanidins and isoflavones. The biological and pharmacological activities of flavonoids include anti-allergic [12], anti-inflammatory [13], antioxidant [13], antibacterial [14, 15], antifungal [16, 17], antiviral [16, 17], anti-cancer [18] and anti-diarrheal activities [19]. According to [20], almost every group of flavonoids is capable of acting as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. Glycosides are plant secondary metabolites composed of two components, glycone (a carbohydrate component) and aglycone (a non-carbohydrate component) [2]. Acoording to [21], the glycone component usually consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid is linked to a sapogenin (aglycone) to form a glycoside. Therapeutic activities of glycosides include, analgesic, antipyretic, anti-inflammatory and laxative effects [22]. Saponins are group of secondary plant metabolites with foaming characteristics and a bitter taste. This phytochemical is widely found in most vegetables, beans and herbs [23]. Its foaming ability is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Some saponins are toxic and are known as sapotoxin. According to [24], saponins have been considered to have important roles in plants defense against pathogens, pests and herbivores due to their antimicrobial, antifungal, antiparasitic, insecticidal and anti-feedant properties. According to [25], saponins have also been found to possess hypoglycemic properties, antivirals activity and used as adjuvants in development of vaccines [26], though there is no high-quality clinical evidence that they have any beneficial effect on human health. According to [27], tannins are heterogeneous group of high molecular weight polyphenolic compounds that have the capacity to form reversible and irreversible complexes with proteins, polysaccharides (especially cellulose, hemicellulose, pectin, etc), alkaloids, nucleic acids, large molecular compounds, metallic ions, and minerals. Its therapeutic properties include its use as astringents against diarrhea, as diuretics against stomach

and duodenal tumours [28], as antiseptic, anti-inflammatory, antioxidant, antimicrobial, antitumor, and haemostatic pharmaceuticals. According to [29], it also possess superoxide anion scavenging and anti-plasmin inhibitory activities. Hydrogen cyanide also known as prussic acid, is a colourless, extremely poisonous and flammable chemical compound with the chemical formula HCN. It has a faint bitter almond-like odour that some people are unable to detect owing to a recessive genetic trait. It can be produced on an industrial scale and is a highly valuable precursor to many chemical compounds ranging from polymers to pharmaceuticals. The volatile compound has been used as inhalation rodenticide and human poison, as well as for killing whales [30]. HCN is obtainable from fruits that have a pit, such as cherries, apricots, apples, and bitter almonds, from which almond oil and flavoring are made. Phenols constitute probably the largest group of plant secondary metabolites, varying in size from a simple structure with an aromatic ring to complex ones such as lignins. Phenols are antioxidants in human and plants [31]. Phenolic compounds have antioxidant and antimicrobial properties [32]. Its antioxidant activity is due to the hydroxyl functional group, and other factors such as presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety which may increase or decrease the activity [33]. Steroid is a biologically active organic compound that functions as components of cell membranes which alter membrane fluidity; and as signalling molecules. Hundreds of steroids are found in plants, animals and fungi. All steroids are manufactured in cells from the sterols; lanosterol or cycloartenol, which are derived from the cyclization of the triterpene squalene. Steroids play critical roles in a number of disorders, including malignancies like prostate cancer, where steroid production inside and outside the tumour promotes cancer cell aggressiveness [34]. Terpenoids also called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from terpenes. About 60% of known natural products are terpenoids [35]. Plant terpenoids are used for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, and ginger, the yellow colour in sunflowers, and the red colour in tomatoes [36]. The resource plant Aju Mbaise is a traditional medicine, composed of combination of leaves, roots, and trunk of medicinal tree wrapped together commonly used by the people of Mbaise in Igboland, to help detoxify, cleanse and sanitize the womb after child delivery. The bioactive compounds are not known and claims associated with the use are yet to be scientifically substantiated, though aged women who deal in this herb, have tested and proven its efficacy. According to the herbalists, this decoction gets rid of the excess water, stale and bad blood in the womb, and every post-natal substance that may be left hence allowing the stomach to return to its normal size in good time. Other claimed benefits of Aju Mbaise decoction include enhancement of ovulation and fertility, prevents halitosis (mouth odour that comes out from the stomach), stops painful and scanty menstruation, and detoxification of dead particles left after miscarriage, anti-malaria, antitumor and anti-inflammatory. [37], reported that the decoction contains bioactive compounds believed to be responsible for the observed antibacterial activities. According to [37], intake of adequate amounts of the decoction can make some contributions to the macro- and micro-mineral value of lactating mothers towards achieving the Recommended Nutrient Intake (RNI) for these minerals. The ability of this plant to demonstrate such quality is dependent on the accumulated natural products, biologically active materials and ingredients found in them. Thus, the need to determine the phytochemical composition of this herbal mixture.

Materials and Methods

Collection of Plant Samples

Fresh samples of the plants that make up Aju Mbaise were collected at Obodo Ujichi, Ahiazu and Amuzi, Ahiara Towns, both in Aboh Mbaise L.G.A, of Imo State, Nigeria. The plants were identified as *Cnestis ferruginea*, *Xylopia aethiopica*, *Uvaria chamae*, *Palisota hirsuta*, *Scleria sp*., *Napoleona imperialis*, *Dialium guineense*, *Combretum racemosun*, and *Heterotis rotundifolia*, respectively by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt. The fresh plants after collection were air-dried, cut into small pieces and blended before the extraction process. The extraction was done with ethanol as the solvent.

Preparation of Extract

The whole plants parts (leaves and stem) were washed, air dried and blended to a powdered form. Powdered sample weighing 1,000g was soaked in 3,000ml of 95% ethanol for 48 hours after which it was sieved using a muslin cloth and afterwards filtered through a Whatmann filter paper No. 1. The filtrate was concentrated using a rotary evaporator at 45^{o} C and afterwards placed on a thermostatic water bath for further drying. The concentrate (paste) was collected, weighed, kept in sterile bottles and stored at 4^{o} C until usage.

Phytochemical Screening

Qualitative phytochemical screening

Phytochemical screenings were carried out on the powdered sample using standard procedures to confirm the presence of alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides, terpenoids, and total phenolic compounds, as described by [38], [39] and [40].

Test for Alkaloids

To 0.5g of pulverized plant sample was added 5 ml of 1% HC1 and boiled for 5 mins in a steam bath. This was filtered and 1 ml of the filtrate was individually treated in various test tubes with a few drops of Draggendorf's reagent, Wagner's reagent and Mayers reagent respectively. The formation of red, reddish-brown and creamy white precipitates respectively indicates the presence of alkaloids.

Test for Cyanide

A volume of 15 ml dd. H_20 was added to 0.1 g of the extract in a test tube. An alkaline picrate paper was suspended over the mixture and held in place by rubber bung. The arrangement was allowed to stand for 18 hr at room temperature. Colour change from yellow to orange indicated the presence of cyanide.

Test for Flavonoids

The pulverized plant samples weighing 0.2g were respectively heated with 10ml of ethylacetate in boiling water bath for 3 mins. The mixture was filtered, after which 4 ml of the filtrate was vigorously shaken with 1ml of 1% aluminium chloride solution. A yellowish coloration in the layer of the ethylacetate indicates the presence of flavonoids.

Test for Glycosides

To 0.5g of respective pulverized plant sample was added 10 ml of distilled water and boiled for 5 mins. This was filtered and about 2 ml of the respective filtrate hydrolyzed with a few drops of concentrated HCI and the solution turned alkaline with a few drops of ammonia solution, Furthermore, 5 drops of the resultant solution was added to 2 ml of Benedict's qualitative reagent and boiled. The precipitation of a reddish-brown colour indicates the presence of glycosides.

Test for Phenols

The test sample weighing 0.1 g was added to 10 ml of distilled water. The solution was heated in a boiling water bath for about 3 mins and filtered. A 2 ml aliquot of the filtrate was placed in each of 3 test tubes. The filtrate in one of the test tubes was diluted with distilled water in the ratio 1:4. A blue or greenish colour indicated the presence of phenols.

Test for Saponins

About 2g of the pulverized plant samples was respectively boiled with 20 ml of distilled water in a water bath and filtered after which 10 ml of the filtrates were respectively mixed with 5 ml of distilled water in a test tube and vigorously shaken to obtain a stable persistent froth, which was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for Steroids

To 9 ml of ethanol, was added 1g of pulverised plant sample and refluxed for a few minutes. The filtrate was concentrated to 2.5 ml in a boiling water bath after which 5 ml of hot water was added. The resultant mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with chloroform (2.5 ml) using separation funnel. Thereafter, 1ml of 0.5 ml of concentrated sulphuric acid was added to the ethanol extract in a test tube to form a lower layer. A reddish brown interface indicates the presence of steroids.

Test for Tannins

Each plant was tested for tannins by weighing the respective pulverized samples (0.5g) and boiled in 20 ml of distilled water in a test tube, then filtered with Whatman No. I filter paper. Then to the filtrates, was added 0.1 % $FeCl_3$ and observed for brownish green or a blue black colouration, which indicates the presence of tannins.

Test for Terpenoids

To 1g of the extract, 9 ml of ethanol was added and refluxed for a few minutes and filtered. The filtrate was concentrated down to 2.5 ml in a boiling water bath. Hot distilled water of volume 5ml was added to the concentrated solution; the mixture was allowed to stand for 1 hour and the waxy substance was filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. The chloroform extract was evaporated to dryness in a water bath and dissolved in 3 ml of concentrated sulphuric acid and then heated for 10 mins in a water bath. A grey colour indicated the presence of terpenoids.

Quantitative Phytochemical analysis

Estimation of Alkaloid content

The extract (1 g) was macerated with 20 ml of ethanol and 20% H_2SO_4 (1:1 v/v). The filtrate (1 ml) was added to 5 ml of 60% sulphuric acid. After 5 mins, 5 ml of 0.5% formaldehyde in 60% sulphuric acid was mixed with the mixture and allowed to stand for 3 hr. The absorbance was read at 565 nm.

Estimation of Cyanide content

The extract weighing 1 g was macerated with 50 ml of distilled water and then filtered. To 1 ml of the filtrate, 4 ml of alkaline picrate solution was added. The mixture was boiled for 5 mins, and allowed to cool. The absorbance was measured at 490 nm.

Estimation of Flavonoid content

Flavonoid content was determined in accordance with the method described by [41] with minimal modifications [42]. About 100µl of plant extracts in ethanol (10 $mgml^{-1}$) was mixed with 100µl of 20% aluminium trichloride, with a drop of acetic acid, and then diluted with ethanol to 5ml. The absorbance was read after 40 mins at 415nm. Blank samples were prepared from 100µl of plant extracts with a drop of acetic acid, and then diluted to 5ml with ethanol. The absorption of standard rutin solution ($0.5mgml^{-1}$) in ethanol was measured under the same conditions. The amount of flavonoids in the plant extracts in rutin equivalents (RE) was calculated by the following formula:

$$\text{Flavonoid content} = \frac{A \times m_0}{A_0 \times m}$$

where A is the absorption of plant extract solution, A_0 is the absorption of standard rutin solution, m is the weight of plant extract, mg and m_0 is the weight of rutin in the solution, mg. The flavonoid content was expressed in mg rutin equivalents/mg plant extract.

Estimation of Glycoside content

The extract weighing 1 g was macerated with 50 ml of distilled water and filtered. To the filtrate (1 ml), 4 ml of alkaline pirate solution was added. The mixture was boiled for 5 mins and allowed to cool. The absorbance was read at 490 nm.

Estimation of Saponin content

The extract weighing 1g was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10 ml of the petroleum ether was added into the beaker and the filtrate was evaporated to dryness. The residue was dissolved in 6 ml of ethanol. The solution (2 ml) was put in a test tube and 2 ml of chromagen solution added into it. It was left to stand for 30 mins and the absorbance was read at 550 nm.

Estimation of Steroid content

The extract weighing 1 g was macerated with 20 ml of ethanol and filtered. To the filtrate (2 ml), 2 ml of chromagen solution was added and the solution was left to stand for 30 mins. The absorbance was read at 550 nm.

Estimation of total Phenolic content

The total phenolic content of extract was measured using Folin-Ciocalteu reagent. The extract weighing 1 g was macerated with 20 ml of 80% ethanol and then filtered. The filtrate (5 ml) was added to 0.5 ml of Folin-Ciocalteu reagent and allowed to stand for 30 mins. Then 2 ml of 20% sodium carbonate was added and absorbance measured at 650 nm. Total phenolic content was estimated using gallic acid as standard [43].

Estimation of Tannin content

The determination of tannin content in each sample was carried out using insoluble polyviny1-polypirrolidone (PVPP), which binds tannins as described by [44]. About, 1ml of extract dissolved in ethanol ($1mgml^{-1}$), in which the total phenolics were determined, was mixed with 100mg PVPP, vortexed, allowed to stand for 15 mins at 4ºC and then centrifuged for 10mins at 3000 rpm using a Sorvall Scientific centrifuge. In the clear supernatant, the non-tannin phenolics were determined the same way as the total phenolics content was calculated as a difference between total and non-tannin phenolic content.

Estimation of Terpenoid content

The extract weighing 1 g was macerated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), was added 2.5 ml of 5% aqueous phosphomolybdic acid solution and 2.5 ml of concentrated sulphuric acid and mixed. The mixture was left to stand for 30 mins and then made up to 12.5 ml with ethanol. The absorbance was read at 700 nm.

Statistical Analysis

Data were presented as Mean ± standard deviation.

Results

The result obtained in the qualitative analysis carried out on the plant extract is presented in Table 1 below. Results obtained showed the presence of some important phytochemicals. From the results, it was observed that phenols, flavonoids, tannins, alkaloids, saponins, steroids, glycoside, and terpenoids were present in the plant extract. It also showed the presence of hydrogen cyanide (HCN).

The result obtained in the quantitative analysis carried out on the plant extract is presented in Table 2. Results obtained showed that Aju Mbaise plant extract contains alkaloid (348.56mg), phenols (1265.23mg), flavonoids (765.94mg), tannins (673.67mg), glycosides (274.87mg), and terpenoids (573.63mg) in 100g of the plant extract. These are relatively higher than saponins (33.20mg), steroids (37.6mg), and cyanide (36.8mg) which are also present in the plant extract.

Table 1: Qualitative phytochemical constituents of Aju Mbaise Plant Extract

Phytochemical Constituent	Relative amount
Alkaloids	++
Flavonoids	+++
Glycosides	++
Hydrogen Cyanide	+
Phenols	+++
Saponins	+
Steroids	+
Tannins	+++
Terpenoids	+++

Key: + = Present in trace amount
++ = Present in average amount
+++ = Present in high amount

Table 2: Quantitative phytochemical constituents of Aju Mbaise Plant Extract

Phytochemical Constituent	Relative amount (mg/100g)
Alkaloids	348.56±7.00
Flavonoids	765.94±19.82
Glycosides	274.87±28.00
Hydrogen Cyanide	36.80±7.07
Phenols	1,265.23±67.69
Saponins	33.20±33.60
Steroids	37.60±4.65
Tannins	673.67±26.40
Terpenoids	573.63±29.16

Values represent Mean ± Standard Deviation. n = 3

Discussion

The use of plant materials including herbal or natural health products with supposed health benefits, is increasing in developed countries, and thus brings attendant risks of toxicity and other effects on human health, despite the safe image of herbal remedies [44]. According to [2], plant's medicinal properties are dependent on the plant secondary metabolites contained in them, and these metabolites that possess medicinal properties are found only in a few species of plants. Our resource plant Aju Mbaise was not an exception, as its constituent plants possess many therapeutic properties which are dependent on the secondary metabolites contained in them. The present study showed that there are many plants' secondary metabolite found in our resource plant. From the qualitative phytochemical analyses, it was observed that the ethanolic extract of cocktail of Aju Mbaise herbal mixture contains alkaloids (8.69%), flavonoids (19.10%), glycosides (6.86%), hydrogen cyanide (0.92%), phenols (31.56%), saponins (0.83%), steroids (0.94%), tannins

(16.80%) and terpenoids (14.31%). This is consonant with the report of [45], that phytochemical screening of Aju Mbaise contained appreciable amount of alkaloids, tannins, flavonoids, cyanogenic glycoside, and saponin. This corresponds with a previous report by [46] who stated that plants contained active components with numerous therapeutic potentials. According to [47], tannins, saponins, terpenes, and alkaloids exist in stem bark of *Sphenocentrum jollyanum* which is one the plants found in the cocktail herbal mixture of Aju Mbaise. [48], also reported the presence of cardiac glycosides, flavonoids, trihydroxyl phenol, anthraquinones, tannins and polyphenolic compounds, such as flavone glycosides in *Cnestis ferruginea*, another plant found in the herbal mixture. Alkaloids, steroids, cardiac glycosides, saponins and tannins were also seen in the preliminary phytochemical screening of *Combretum racemosum* extracts [49], which is a constituent plant of Aju Mbaise. High tannin content was seen in *Dialium guineense* [50], which is also a constituent plant of Aju Mbaise. Other constituent plants of Aju Mbaise herbal mixture includes *Heterotis rotundifolia* which has high amount of phenolic and flavonoic compounds [51]; *Napoleonaea imperialis* leaves with high amount of tannins, glycosides, saponins and proteins [52]; *Palisota hirsuta* leaf extract showed high presence of flavonoids, tannins, terpenoids and alkaloids [53, 54]; *Uvaria chamae* contains medically active compounds such as oleo-resin, alkaloids, and tannins [55]; and also *Xylopia aethiopica* which contains alkaloids, glycosides, saponnis, tanins, and stereols [56]. These plants metabolites are known for their various benefits, and have been found to possess a wide range of therapeutic activities, which include protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids possess analgesic properties. According to [57], plants containing alkaloids have been known to possess antidiarrheal activities and are known to be the largest groups of secondary metabolites in plants. Pure plant isolated alkaloids can also be used as basic medicinal agents for analgesic, antispasmodic and bactericidal effects [58]. Flavonoids are known to be antioxidants and free radical scavengers which prevent oxidative cell damage, and it has strong anticancer activity and protects the cells against all stages of carcinogenesis [59]. Flavonoids in the intestinal tract lower the risk of heart disease [60]. It has been discovered in various studies that flavonoids exhibited hypoglycaemic and hypolipidemic potential [61]. Tannins have been reported to possess astringent properties that hasten the healing of wound and inflamed mucus membranes [62]. According to [63], tannins form irreversible complexes with prolin-rich protein and this results in the inhibition of cell protein synthesis that helps in the treatment of inflamed/ulcerated tissues [64]. Plants that contain tannins as major constituents are used for the treatment of intestinal disorders like diarrhoea and dysentery [65]. According to [66], the steroid, phytosterols are currently used for treating symptoms of uterine cramps, abdominal colic and menstrual irregularity, while topical progesterone in pharmacological doses is used to treat a variety of conditions including premenstrual syndrome, anovulatory cycles, dysfunctional uterine bleeding, and menopausal symptoms. Phenolic compounds are synthesized in plants as secondary metabolites. They have several biological activities which include anti-oxidant, anti-inflammatory, anti-aging and inhibitory properties. These secondary metabolites play a vital role in reproduction and growth. These compounds also provide protection against harmful pathogenic microbes and predators [67]. The plant derived polyphenolic compounds are promising nutraceuticals for control of various disorders such as cardiovascular, neurological and neoplastic disease [68]. According to [69], phenolic compounds have the ability to reduce risk for development or treatment of cancers, cardiovascular disorders, obesity, diabetes, aging-diseases,

urinary tract infections, and periodontal disease. [67], also reported that the richness of the polyphenolic contents of green tea and red wine has made them popular choices for associated anticancer and cardiovascular health benefits. [60], reported the hypoglycaemic potentials, wound healing properties, and haemolytic activities of saponins.

Conclusion

This study has shown that the cocktail herbal mixture of Aju Mbaise contains tremendous amount of phytochemicals that possess numerous therapeutic potentials.

Competing Interests

Authors have declared that no competing interests exist.

References

1. Smith-Hall, C., Larsen, H. O. & Pouliot, M. (2012). People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption. *Journal of Ethnobiology and Ethnomedicine*, 8(1), 43.
2. Heinrich, M., Barnes, J., Gibbon, S. and Williamson, E. M. (2004). *Fundamentals of Pharmacognosy and Phytotherapy*. In Kingdom A.D. (Ed.). Churchil Livingstone, (2nd Ed., pp. 211-219). Elsevier Science Ltd., UK.
3. Shi, Q. I. U., Hui, S. U. N., ZHANG, A. H., Hong-Ying, X. U., Guang-Li, Y. A. N., Ying, H. A. N., & Xi-Jun, W. A. N. G. (2014). Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chinese Journal of Natural Medicines*, *12*(6), 401-406.
4. Harbourne, J.B. (1998). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. 2nd edition London: Chapman and Hall Ltd.Pp. 282.
5. Kittakoop, P., Mahidol, C., & Ruchirawat, S. (2014). Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Current topics in medicinal chemistry*, *14*(2), 239-252.
6. Russo, P., Frustaci, A., Del Bufalo, A., Fini, M. & Cesario, A. (2013). Multitarget drugs of plants origin acting on Alzheimer's disease. *Current Medicinal Chemistry*, *20*(13), 1686–93.
7. Sinatra, R. S., Jahr, J. S., & Watkins-Pitchford, J. M. (Eds.). (2010). *The essence of analgesia and analgesics*. Cambridge University Press. pp. 82–90.
8. Cushnie, T. T., Cushnie, B. & Lamb, A. J. (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, *44*(5), 377-386.
9. Robbers, J. E., Speedie, M. K., & Tyler, V. E. (1996). *Pharmacognosy and pharmacobiotechnology*. Williams & Wilkins. pp. 143–185.
10. Spencer, J. P. E. (2008). Flavonoids: modulators of brain function. *British Journal of Nutrition*, 99, 60–77.
11. Kyle, J., Butchart, C., McNeill, G., Corley, J., Gow, A. J., Starr, J. M., & Deary, I. J. (2011). Flavonoid intake in relation to cognitive function in later life in the Lothian Birth Cohort 1936. *British Journal of Nutrition*, 106(1), 141-148.

12. Yamamoto, Y. and Gaynor, R. B. (2001). Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *Journal of Clinical Investigation*, *107*(2), 135–42.
13. Cazarolli, L. H., Zanatta, L., Alberton, E. H., Figueiredo, M. S., Folador, P., Damazio, R. G., Pizzolatti, M. G. & Silva, F. R. (2008). Flavonoids: Prospective Drug Candidates. *Mini-Reviews in Medicinal Chemistry,* 8 (13), 1429–1440.
14. Cushnie, T. P. T. & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38 (2), 99–107.
15. Manner, S., Skogman, M., Goeres, D., Vuorela, P. & Fallarero, A. (2013). Systematic exploration of natural and synthetic flavonoids for the inhibition of Staphylococcus aureus biofilms. *International Journal of Molecular Sciences*, 14 (10), 19434–19451.
16. Cushnie, T. P. & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents,* 26(5), 343 – 356.
17. Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular Nutrition and Food Research,* 51(1), 116–134.
18. Ruela de Sousa, R. R., Queiroz, K. C. S., Souza, A. C. S., Gurgueira, S. A., Augusto, A. C., Miranda, M. A. & Aoyama, H. (2007). Phosphoprotein levels, MAPK activities and NFκB expression are affected by fisetin. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *22*(4), 439-444.
19. Schuier, M., Sies, H., Billek, B. and Fischer, H. (2005). Cocoa-related flavonoids inhibit CFTR-mediated chloride transport across T84 human colon epithelia. *Journal of Nutrition*, *35*(10), 2320-2325.
20. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, *79*(5), 727-747.
21. Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine,* 27, 1 – 93.
22. Brito-Arias, M. (2007). Hydrolysis of glycosides. *Synthesis and Characterization of Glycosides*, 304-313.
23. Riguera, R. (1997). Isolating bioactive compounds from marine organisms. *Journal of Marine Biotechnology*, 5(4), 187–193.
24. Lacaille-Dubois, M. A., & Wagner, H. (2000). Bioactive saponins from plants: an update. In *Studies in natural products chemistry* (Vol. 21, pp. 633-687). Elsevier.
25. Morrissey, J. P. & Osbourn, A. E. (1999). Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiological and Molecular Biological Reviews,* 63(3), 708-724.
26. Sun, H. X., Wang, H., Xu, H. S., & Ni, Y. (2009). Novel polysaccharide adjuvant from the roots of Actinidia eriantha with dual Th1 and Th2 potentiating activity. *Vaccine*, *27*(30), 3984-3991.
27. Schofield, P., Mbugua, D. M. & Pell, A. N. (2001). Analysis of condensed tannins: A review. *Animal Feed Science Technology,* 91, 21-40.
28. De Bruyne, T., Pieters, L., Deelstra, H. & Vlietinck, A. (1999). Condensed vegetables tannins: Biodiversity in structure and biological activities. *Biochemical System Ecology*, 27, 445 – 459.
29. Okuda, T., & Ito, H. (2011). Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. *Molecules*, *16*(3), 2191-2217.

30. Gunasekar, P. G., Prabhakaran, K., Li, L., Zhang, L., Isom, G. E., & Borowitz, J. L. (2004). Receptor mechanisms mediating cyanide generation in PC12 cells and rat brain. *Neuroscience research*, *49*(1), 13-18.
31. Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture*, *80*(12), 1744-1756.
32. Batawila, K., Kokou, K., Akpagana, K., Koumaglo, K., & Bouchet, P. (2002). Activité antifongique d'une espèce en voie de disparition de la flore togolaise: Conyza aegyptiaca (L.) Ait. var. lineariloba (DC.) O. Hoffm.(Asteraceae). *Acta botanica gallica*, 149(1), 41-48.
33. Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.
34. Lubik, A. A., Nouri, M., Truong, S., Ghaffari, M., Adomat, H. H., Corey, E., Cox, M. E., Li, N., Guns, E. S., Yenki, P., Buttyan, R. & Pham, S. (2017). Paracrine sonic hedgehog signaling contributes significantly to acquired steroidogenesis in the prostate tumor microenvironment. *International journal of cancer*, *140*(2), 358-369.
35. Firn, R. (2010). *Nature's chemicals: the natural products that shaped our world*. Oxford University Press on Demand.
36. Specter, M. (2009). A life of its own. *The New Yorker*, *28*.
37. Ogueke, C. C., Owuamanam, C. I., Onyedinma, C., Iroanya, A., Bede, E. N., & Nwachukwu, I. N. (2016). Antibacterial activity, phytochemical properties and mineral Content of "Aju Mbaise" decoction: A liquid extract administered to nursing mothers. *Nigerian Journal of Nutritional Sciences*, *37*(1), 114-121.
38. Harborne, J. B. (2014). *Introduction to Ecological Biochemistry*. Academic press.
39. Trease, G.E. and Evans, W.C. (1985). *Pharmacognosy*. In: Pal, S.B. (Ed.). Pharmacognosy. (11th Ed., pp. 60-75) Tindal LTD, London
40. Sofowora, A. (1993). Medicinal plants and medicine in Africa. *John Whilley Spectrum Books, Ibadan, Nigeria*, 120-123.
41. Kumaran, A. & Karunakaran, R. (2007). Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. *Food Chemistry,* 100, 356 – 361.
42. Awah, F. M., Uzoegwu, P. N., Ifeonu, P., Oyugi, J. O., Rutherford, J., Yao, X. J., Fehrmann, F., Fowke, K. R. & Eze, M. O. (2012). Free radical scavenging activity, phenolic content and cytotoxicity of selected Nigerian medicinal plants. *Food Chemistry,* 131(4), 1279 – 1286.
43. Madaan, R., Bansal, G., Kumar, S., & Sharma, A. (2011). Estimation of total phenols and flavonoids in extracts of Actaea spicata roots and antioxidant activity studies. *Indian journal of pharmaceutical sciences*, *73*(6), 666.
44. Ekor, M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4 (3): 202–4.
45. Ezejindu, C. N. & Iro, O. K. (2017). Antibacterial activity, phytochemical properties and mineral content of "Aju Mbaise" decoction administered to nursing mothers. *Direct Research Journal of Health and Pharmacology*, **5**(3), 33-38.
46. Rakesh, D. D., Handa, S. S., & Vasisht, K. (2006). Compendium of medicinal and aromatic plants ASIA. *ICS UNIDO. Asia*, *2*, 305.

47. Nia, R., Paper, D. H. & Essien, E. E. (2004). Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum jollyanum* Pierre. *The African Journal of Biomedical Research*, 7, 129–132.
48. Adisa, A. R., Farooq, A. D. & Iqbal, M. C. (2014). Protection of CCl_4-induced liver and kidney damage by phenolic compounds in leaf extracts of *Cnestis ferruginea* (de Candolle). *Pharmacognosy Research*, 6(1), 19–28.
49. Onocha, P. A., Audu, E.O., Ekundayo, O. & Dosumu, O. O. (2005). Phytochemical and antimicrobial properties of extracts of *Combretum racemosum*. *Acta Horticulturae*, 675, 97–101.
50. Arogba, S. S., Ajiboro, A. & Odukwe, I. J. (2006). A physiochemical study Nigerian Velvet tamarind (*Dialium guineense*) fruit. *The Journal of the Science of Food and Agriculture*, 66, 533-534.
51. Etekpo, S. D., N'Gaman-Kouassi, C. C., Mamyrbekova-Békro, J. A. & Békro, Y. (2018). Antioxidant profiles of alcoholic tinctures from *Heterotis rotundifolia* (sm.) by DPPH radical trapping. *European Journal of Biomedical and Pharmaceutical sciences*, 5(10), 39-45.
52. Chah, K.F., C.A. Eze, C.E. Emuelosi & C.O. Esimone, (2006). Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. *Journal of Ethnopharmacology*, 104, 164-167.
53. Kupeli, E. & Yesilada, E. (2007). Flavonoids with anti-inflammatory and antinociceptive activity from *Cistus laurifolius* L. leaves through bioassay-guided procedures. *Journal of Ethnopharmacology*, 112(3), 524-530.
54. Clavin, M., Gorzalczany, S., Macho, A., Munoz, E., Ferraro, G., Acevedo, C., & Martino, V. (2007). Anti-inflammatory activity of flavonoids from *Eupatorium arnottianum*. *Journal of Ethnopharmacology*, *112*(3), 585-589.
55. Achigan-Dako, E. G., Pasquini, M. W., Assogba Komlan, F., N'danikou, S., Yédomonhan, H., Dansi, A., & Ambrose-Oji, B. (2010). Traditional vegetables in Benin. *Institut National des Recherches Agricoles du Bénin, Imprimeries du CENAP, Cotonou*.
56. Somova, L.I., Shode, F.O., Moodley, K. & Govender, Y. (2001). Cardiovascular and diuretic activity of kaurene derivatives of *Xylopia aethiopica* and *Alepidea amatymbica*. *Journal of Ethnopharmacology,* 77, 165–74.
57. Obasi, N. B., Igboechi, A. C. & Anuforo, D. C. (1990). Studies of the antidiarrheal potentials of some composite plants. Medicinal plants in a developing economy. Proceeding of a workshop organized by the Nigeria society of Nigeria, 108- 116.
58. Stray, F. (1998). *The national guide to medicinal herbs and Plants*. Tiger books international London. Pp. 12-16.
59. Okwu, D. E. & Iroabuchi, F, (2004). Phytochemical analysis and antimicrobial activity screening of aqueous and ethanolic root extracts of *Uvaria chamae* (Beuv) and *Cnestis ferruginea*. *The Journal of Chemical Society of Nigeria, 29*(2), 112-114.
60. Okwu, D. E. (2005). Phytochemicals, Vitamins and Mineral contents of two Nigeria Medicinal plants. *International Journal of Molecular Medicine and Advance Sciences, 1*(4), 375- 381.
61. Narender, T., Khaliq, T., & Puri, A. (2006). Antidyslipidemic activity of furano-flavonoids isolated from *Indigofera tinctoria*. *Bioorganic & Medicinal chemistry letters*, 16(13), 3411-3414.

552 62. Okwu, D. E. (1999). Flavouring properties of spices on cassava Fufu. *African Journal of Root*
553 *and Tuber Crops*, *3*(2), 19-21.
554 63. Shimada, T. (2006). Salivary proteins as a defense against dietary tannins. *Journal of Chemical*
555 *Ecology*, *32*(6), 1149-1163.
556 64. Parekh, J. & Sumitra, C. V. (2007). In vitro antimicrobial activity and phytochemical analysis
557 of some Indian medicinal plants. *Turkish Journal of Biology*, 31, 53-58.
558 65. Dharmanda, S. (2003). Gallnuts and the uses of tannins in Chinese Medicine-A paper presented
559 at the Institute for Traditional Medicine. *Portlant, Oregon*, 3, 941-945.
560 66. Hudson, T. (1996). Townsend Letter for Doctors; 156.
561 67. Gautam, B., Vadivel, V., Stuetz, W., & Biesalski, H. K. (2012). Bioactive compounds
562 extracted from Indian wild legume seeds: antioxidant and type II diabetes–related enzyme
563 inhibition properties. *International journal of food sciences and nutrition*, *63*(2), 242-245.
564 68. Ullah, M. F., & Khan, M. W. (2008). Food as medicine: potential therapeutic tendencies of
565 plant derived polyphenolic compounds. *The Asian Pacific Journal of Cancer Prevention*, *9*(2),
566 187-196.
567 69. Yuan, W., Zhou, L., Deng, G., Wang, P., Creech, D., & Li, S. (2011). Anthocyanins, phenolics,
568 and antioxidant capacity of *Vaccinium L*. in Texas, USA.

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