

**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*, *Xylopiya 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 *Xylopiya 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

**Comment [U1]:** the word and *Xylopiya aethiopica* don't need to be written anymore, because Aju Mbaise has a mixture of these herbs

37 nearby competing plants. Secondary plant metabolites with reported medicinal properties include  
38 alkaloids, terpenoids, saponins, polysaccharides, waxes and fatty acids, simple phenolics,  
39 flavonoids and glycosides and their derivatives. According to [3], alkaloids are group of naturally  
40 occurring chemical compounds that contain mostly basic nitrogen atoms. It also includes some  
41 related compounds with neutral and even weakly acidic properties. According to [4], about ninety-  
42 five percent (95%) of alkaloids taste bitter with high level of toxicity, and they are naturally  
43 synthesized by a large diversity of organisms including fungi, bacteria, plants, and animals. Some  
44 of the pharmacological benefits of  
45 alkaloids include; antimalarial (e.g. quinine) [4], antiasthma (e.g. ephedrine), anticancer (e.g. homo  
46 harringtonine) [5], cholinomimetic (e.g. galantamine) [6], vasodilatory(e.g. vincamine), antiarrhyth  
47 mic (e.g. quinidine, analgesic (e.g. morphine) [7], antibacterial (e.g. chelerythrine) [8], and  
48 antihyperglycemic activities (e.g. piperine) [3]. Other alkaloids possess psychotropic (e.g. psilocin)  
49 and stimulant activities (e.g. cocaine, caffeine, nicotine, theobromine) [9] and have been used in ent  
50 heogenic rituals or as recreational drugs. Also according to [9], some alkaloids can be toxic too  
51 (e.g. atropine, tubocurarine). Flavonoids are the most common group of polyphenolic compounds in  
52 the human diet and are found mainly in plants [10]. Its widespread distribution, varieties and  
53 relatively low toxicity compared to other active plant compounds (for instance alkaloids), shows  
54 that many animals, including humans, ingest significant quantities in their diet. According to [11],  
55 some foods with high flavonoid content include parsley, onions, blueberries and other berries, black  
56 tea, green tea and oolong tea, bananas, all citrus fruits. Flavonoids are classified into six major  
57 classes, which are; flavones, flavonols, flavonones, flavanols (catechins), anthocyanidins and  
58 isoflavones. The biological and pharmacological activities of flavonoids include anti-allergic [12],  
59 anti-inflammatory [13], antioxidant [13], antibacterial [14, 15], antifungal [16, 17], antiviral [16,  
60 17], anti-cancer [18] and anti-diarrheal activities [19]. According to [20], almost every group of  
61 flavonoids is capable of acting as powerful antioxidants which can protect the human body from  
62 free radicals and reactive oxygen species. Glycosides are plant secondary metabolites composed of  
63 two components, glycone (a carbohydrate component) and aglycone (a non-carbohydrate  
64 component) [2]. According to [21], the glycone component usually consists of one or more sugar  
65 moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid is linked to  
66 a sapogenin (aglycone) to form a glycoside. Therapeutic activities of glycosides include, analgesic,  
67 antipyretic, anti-inflammatory and laxative effects [22]. Saponins are group of secondary plant  
68 metabolites with foaming characteristics and a bitter taste. This phytochemical is widely found in  
69 most vegetables, beans and herbs [23]. Its foaming ability is caused by the combination of a  
70 hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Some saponins  
71 are toxic and are known as saptotoxin. According to [24], saponins have been considered to have  
72 important roles in plants defense against pathogens, pests and herbivores due to their antimicrobial,  
73 antifungal, antiparasitic, insecticidal and anti-feedant properties. According to [25], saponins have  
74 also been found to possess hypoglycemic properties, antivirals activity and used as adjuvants in  
75 development of vaccines [26], though there is no high-quality clinical evidence that they have any  
76 beneficial effect on human health. According to [27], tannins are heterogeneous group of high  
77 molecular weight polyphenolic compounds that have the capacity to form reversible and  
78 irreversible complexes with proteins, polysaccharides (especially cellulose, hemicellulose, pectin,  
79 etc), alkaloids, nucleic acids, large molecular compounds, metallic ions, and minerals. Its  
80 therapeutic properties include its use as astringents against diarrhea, as diuretics against stomach

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

123  
124

## Materials and Methods

Comment [U2]: what is the relationship between diuretics against stomach and duodenal tumors

Comment [U3]: move to the next page, the title should not be separated from the contents

125 **Collection of Plant Samples**

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

133  
134 **Preparation of Extract**

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

Comment [U4]: what is the mesh size of the powder

141  
142 **Phytochemical Screening**

143 **Qualitative phytochemical screening**

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

147  
148 **Test for Alkaloids**

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

154  
155 **Test for Cyanide**

156 A volume of 15 ml dd. H<sub>2</sub>O was added to 0.1 g of the extract in a test tube. An alkaline picrate  
157 paper was suspended over the mixture and held in place by rubber bung. The arrangement was  
158 allowed to stand for 18 hr at room temperature. Colour change from yellow to orange indicated the  
159 presence of cyanide.

160  
161  
162 **Test for Flavonoids**

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

167  
168 **Test for Glycosides**

Comment [U5]: move to the next page, the title should not be separated from the contents

169 To 0.5g of respective pulverized plant sample was added 10 ml of distilled water and boiled for 5  
170 mins. This was filtered and about 2 ml of the respective filtrate hydrolyzed with a few drops of  
171 concentrated HCl and the solution turned alkaline with a few drops of ammonia solution,  
172 Furthermore, 5 drops of the resultant solution was added to 2 ml of Benedict's qualitative reagent  
173 and boiled. The precipitation of a reddish-brown colour indicates the presence of glycosides.  
174

#### 175 **Test for Phenols**

176 The test sample weighing 0.1 g was added to 10 ml of distilled water. The solution was heated in a  
177 boiling water bath for about 3 mins and filtered. A 2 ml aliquot of the filtrate was placed in each of  
178 3 test tubes. The filtrate in one of the test tubes was diluted with distilled water in the ratio 1:4. A  
179 blue or greenish colour indicated the presence of phenols.  
180

#### 181 **Test for Saponins**

182 About 2g of the pulverized plant samples was respectively boiled with 20 ml of distilled water in a  
183 water bath and filtered after which 10 ml of the filtrates were respectively mixed with 5 ml of  
184 distilled water in a test tube and vigorously shaken to obtain a stable persistent froth, which was  
185 then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates  
186 the presence of saponins.  
187

#### 188 **Test for Steroids**

189 To 9 ml of ethanol, was added 1g of pulverised plant sample and refluxed for a few minutes. The  
190 filtrate was concentrated to 2.5 ml in a boiling water bath after which 5 ml of hot water was added.  
191 The resultant mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate  
192 was extracted with chloroform (2.5 ml) using separation funnel. Thereafter, 1ml of 0.5 ml of  
193 concentrated sulphuric acid was added to the ethanol extract in a test tube to form a lower layer. A  
194 reddish brown interface indicates the presence of steroids.  
195

#### 196 **Test for Tannins**

197 Each plant was tested for tannins by weighing the respective pulverized samples (0.5g) and boiled  
198 in 20 ml of distilled water in a test tube, then filtered with Whatman No. 1 filter paper. Then to the  
199 filtrates, was added 0.1 % FeCl<sub>3</sub> and observed for brownish green or a blue black colouration,  
200 which indicates the presence of tannins.  
201

#### 202 **Test for Terpenoids**

203 To 1g of the extract, 9 ml of ethanol was added and refluxed for a few minutes and filtered. The  
204 filtrate was concentrated down to 2.5 ml in a boiling water bath. Hot distilled water of volume 5ml  
205 was added to the concentrated solution; the mixture was allowed to stand for 1 hour and the waxy  
206 substance was filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating  
207 funnel. The chloroform extract was evaporated to dryness in a water bath and dissolved in 3 ml of  
208 concentrated sulphuric acid and then heated for 10 mins in a water bath. A grey colour indicated the  
209 presence of terpenoids.  
210

#### 211 **Quantitative Phytochemical analysis**

212

**Comment [U6]:** check this method again, extracts only given water can be colored ?  
**Test for Phenol:** Ferric Chloride Test: About 0.2 g of plant extract was weighed and treated with 5% ferric chloride and observed for the formation of deep blue color which indicates the presence of phenol.

**Comment [U7]:** move to the next page, the title should not be separated from the contents

213 **Estimation of Alkaloid content**

214 The extract (1 g) was macerated with 20 ml of ethanol and 20% H<sub>2</sub>SO<sub>4</sub> (1:1 v/v). The filtrate (1 ml)  
215 was added to 5 ml of 60% sulphuric acid. After 5 mins, 5 ml of 0.5% formaldehyde in 60%  
216 sulphuric acid was mixed with the mixture and allowed to stand for 3 hr. The absorbance was read  
217 at 565 nm. **What standards are used for calculating alkaloid content?**

218

219 **Estimation of Cyanide content**

220 The extract weighing 1 g was macerated with 50 ml of distilled water and then filtered. To 1 ml of  
221 the filtrate, 4 ml of alkaline picrate solution was added. The mixture was boiled for 5 mins, and  
222 allowed to cool. The absorbance was measured at 490 nm. **What standards are used for**  
223 **calculating cyanide content?**

224

225 **Estimation of Flavonoid content**

226 Flavonoid content was determined in accordance with the method described by [41] with minimal  
227 modifications [42]. About 100µl of plant extracts in ethanol (10 mgml<sup>-1</sup>) was mixed with 100µl of  
228 20% aluminium trichloride, with a drop of acetic acid, and then diluted with ethanol to 5ml. The  
229 absorbance was read after 40 mins at 415nm. Blank samples were prepared from 100µl of plant  
230 extracts with a drop of acetic acid, and then diluted to 5ml with ethanol. The absorption of standard  
231 rutin solution (0.5mgml<sup>-1</sup>) in ethanol was measured under the same conditions. The amount of  
232 flavonoids in the plant extracts in rutin equivalents (RE) was calculated by the following formula:

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

234

235 where A is the absorption of plant extract solution, A<sub>0</sub> is the absorption of standard rutin solution,  
236 m is the weight of plant extract, mg and m<sub>0</sub> is the weight of rutin in the solution, mg. The flavonoid  
237 content was expressed in mg rutin equivalents/mg plant extract.

238

239 **Estimation of Glycoside content**

240 The extract weighing 1 g was macerated with 50 ml of distilled water and filtered. To the filtrate (1  
241 ml), 4 ml of alkaline picrate solution was added. The mixture was boiled for 5 mins and allowed to  
242 cool. The absorbance was read at 490 nm. **What standards are used for calculating glycoside**  
243 **content?**

244

245 **Estimation of Saponin content**

246 The extract weighing 1g was macerated with 10 ml of petroleum ether and decanted into a beaker.  
247 Another 10 ml of the petroleum ether was added into the beaker and the filtrate was evaporated to  
248 dryness. The residue was dissolved in 6 ml of ethanol. The solution (2 ml) was put in a test tube and  
249 2 ml of chromagen solution added into it. It was left to stand for 30 mins and the absorbance was  
250 read at 550 nm. **What standards are used for calculating saponin content?**

251

252

253 **Estimation of Steroid content**

**Comment [U8]:** move to the next page, the title should not be separated from the contents

254 The extract weighing 1 g was macerated with 20 ml of ethanol and filtered. To the filtrate (2 ml), 2  
255 ml of chromagen solution was added and the solution was left to stand for 30 mins. The absorbance  
256 was read at 550 nm. **What standards are used for calculating steroid content?**

257

#### 258 **Estimation of total Phenolic content**

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

264

#### 265 **Estimation of Tannin content**

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

273

#### 274 **Estimation of Terpenoid content**

275 The extract weighing 1 g was macerated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml),  
276 was added 2.5 ml of 5% aqueous phosphomolybdic acid solution and 2.5 ml of concentrated  
277 sulphuric acid and mixed. The mixture was left to stand for 30 mins and then made up to 12.5 ml  
278 with ethanol. The absorbance was read at 700 nm. **What standards are used for calculating  
279 terpenoid content?**

280

#### 281 **Statistical Analysis**

282 Data were presented as Mean  $\pm$  standard deviation.

283

#### 284 **Results**

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

290 The result obtained in the quantitative analysis carried out on the plant extract is presented in Table  
291 2. Results obtained showed that Aju Mbaise plant extract contains alkaloid (348.56mg), phenols  
292 (1265.23mg), flavonoids (765.94mg), tannins (673.67mg), glycosides (274.87mg), and terpenoids  
293 (573.63mg) in 100g of the plant extract. These are relatively higher than saponins (33.20mg),  
294 steroids (37.6mg), and cyanide (36.8mg) which are also present in the plant extract.

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304

**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

Comment [U9]: The letters in a table are usually smaller than text

314 **Table 2: Quantitative phytochemical constituents of Aju Mbaise Plant Extract**  
315

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

Comment [U10]: The letters in a table are usually smaller than text

325  
326  
327

### Discussion

328 The use of plant materials including herbal or natural health products with supposed health benefits,  
329 is increasing in developed countries, and thus brings attendant risks of toxicity and other effects on  
330 human health, despite the safe image of herbal remedies [44]. According to [2], plant's medicinal  
331 properties are dependent on the plant secondary metabolites contained in them, and these  
332 metabolites that possess medicinal properties are found only in a few species of plants. Our  
333 resource plant Aju Mbaise was not an exception, as its constituent plants possess many therapeutic



334 properties which are dependent on the secondary metabolites contained in them. The present study  
335 showed that there are many plants' secondary metabolite found in our resource plant. From the  
336 qualitative phytochemical analyses, it was observed that the ethanolic extract of cocktail of Aju  
337 Mbaise herbal mixture contains alkaloids (8.69%), flavonoids (19.10%), glycosides (6.86%),  
338 hydrogen cyanide (0.92%), phenols (31.56%), saponins (0.83%), steroids (0.94%), tannins  
339 (16.80%) and terpenoids (14.31%). This is consonant with the report of [45], that phytochemical  
340 screening of Aju Mbaise contained appreciable amount of alkaloids, tannins, flavonoids,  
341 cyanogenic glycoside, and saponin. This corresponds with a previous report by [46] who stated that  
342 plants contained active components with numerous therapeutic potentials. According to [47],  
343 tannins, saponins, terpenes, and alkaloids exist in stem bark of *Sphenocentrum jollyanum* which is  
344 one the plants found in the cocktail herbal mixture of Aju Mbaise. [48], also reported the presence  
345 of cardiac glycosides, flavonoids, trihydroxyl phenol, anthraquinones, tannins and polyphenolic  
346 compounds, such as flavone glycosides in *Cnestis ferruginea*, another plant found in the herbal  
347 mixture. Alkaloids, steroids, cardiac glycosides, saponins and tannins were also seen in the  
348 preliminary phytochemical screening of *Combretum racemosum* extracts [49], which is a  
349 constituent plant of Aju Mbaise. High tannin content was seen in *Dialium guineense* [50], which is  
350 also a constituent plant of Aju Mbaise. Other constituent plants of Aju Mbaise herbal mixture  
351 includes *Heterotis rotundifolia* which has high amount of phenolic and flavonoic compounds [51];  
352 *Napoleonaea imperialis* leaves with high amount of tannins, glycosides, saponins and proteins [52];  
353 *Palisota hirsuta* leaf extract showed high presence of flavonoids, tannins, terpenoids and  
354 alkaloids [53, 54]; *Uvaria chamae* contains medically active compounds such as oleo-resin,  
355 alkaloids, and tannins [55]; and also *Xylopiya aethiopica* which contains alkaloids, glycosides,  
356 saponins, tanins, and sterols [56]. These plants metabolites are known for their various benefits,  
357 and have been found to possess a wide range of therapeutic activities, which include protection  
358 against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect  
359 against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids possess analgesic  
360 properties. According to [57], plants containing alkaloids have been known to possess antidiarrheal  
361 activities and are known to be the largest groups of secondary metabolites in plants. Pure plant  
362 isolated alkaloids can also be used as basic medicinal agents for analgesic, antispasmodic and  
363 bactericidal effects [58]. Flavonoids are known to be antioxidants and free radical scavengers which  
364 prevent oxidative cell damage, and it has strong anticancer activity and protects the cells against all  
365 stages of carcinogenesis [59]. Flavonoids in the intestinal tract lower the risk of heart disease [60].  
366 It has been discovered in various studies that flavonoids exhibited hypoglycaemic and  
367 hypolipidemic potential [61]. Tannins have been reported to possess astringent properties that  
368 hasten the healing of wound and inflamed mucus membranes [62]. According to [63], tannins form  
369 irreversible complexes with prolin-rich protein and this results in the inhibition of cell protein  
370 synthesis that helps in the treatment of inflamed/ulcerated tissues [64]. Plants that contain tannins  
371 as major constituents are used for the treatment of intestinal disorders like diarrhoea and dysentery  
372 [65]. According to [66], the steroid, phytosterols are currently used for treating symptoms of uterine  
373 cramps, abdominal colic and menstrual irregularity, while topical progesterone in pharmacological  
374 doses is used to treat a variety of conditions including premenstrual syndrome, anovulatory cycles,  
375 dysfunctional uterine bleeding, and menopausal symptoms. Phenolic compounds are synthesized in  
376 plants as secondary metabolites. They have several biological activities which include anti-oxidant,  
377 anti-inflammatory, anti-aging and inhibitory properties. These secondary metabolites play a vital

378 role in reproduction and growth. These compounds also provide protection against harmful  
379 pathogenic microbes and predators [67]. The plant derived polyphenolic compounds are promising  
380 nutraceuticals for control of various disorders such as cardiovascular, neurological and neoplastic  
381 disease [68]. According to [69], phenolic compounds have the ability to reduce risk for  
382 development or treatment of cancers, cardiovascular disorders, obesity, diabetes, aging-diseases,  
383 urinary tract infections, and periodontal disease. [67], also reported that the richness of the  
384 polyphenolic contents of green tea and red wine has made them popular choices for associated  
385 anticancer and cardiovascular health benefits. [60], reported the hypoglycaemic potentials, wound  
386 healing properties, and haemolytic activities of saponins.

387

### 388 **Conclusion**

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

391

392

### 393 **Competing Interests**

394 Authors have declared that no competing interests exist.

395

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### 397 **References**

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