PHYTOCHEMICAL ANALYSIS AND ACUTE TOXICITY STUDIES ON *AGBO IBA PONTO* SOLD IN LAGOS METROPOLIS

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Abstract

The presence of young ladies hawking local herbal mixtures along the streets has become common in Lagos metropolis. Artisans and traders purchase these herbal mixtures popularly called *Agbo* and use as curative agent for various ailments. The present study has been carried out to determine the oral acute toxicity of the herbal mixture for typhoid fever, *Agbo iba ponto*, as well as the phytochemical analysis of samples of this mixture from three different areas in Lagos metropolis. The acute toxicity study was done in two phases. Single doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight respectively were administered to three groups of three mice each in the first phase. For the second phase, single doses of 1250 mg/kg, 2500 mg/kg and 5000 mg/kg body weight respectively administered to three groups of one mouse each and the animals were observed for 24 hours. Qualitative and quantitative phytochemical screening carried out using standard methods showed the presence of saponins, tannins, phenols, phlobatanins, terpernoids, steroids, cardiac glycosides and alkaloids. The results showed saponins as the most prominent phytoconsituent with values ranging between 35.00 ± 0.37 and 37.45 ±0.37 mg/100 g while the least values were from the terpernoids (15.93 ± 0.23 to 17.41 mg/100 g). In the acute toxicity study although the highest dose used (5000 mg/kg bwt) caused drowsiness in the experimental animals, no mortality was observed in the experimental animals. The result of this study revealed a wealth of phytoconstituents in this herbal mixture, although dosage above 5000 mg/kg body weight, it may be considered to be nontoxic for oral intake.

Key words: *Agbo iba ponto*, acute toxicity, phytochemicals, Typhoid fever

Introduction:

Typhoid fever (enteric fever) caused by the bacterium *Salmonella enteric serovar typhi* is a common disease in the tropics and subtropics (Adabara *et al*., 2012). The disease is systemic, and is often contracted by ingestion of food or water that is contaminated with the pathogen usually from a feco-oral source. Adabara *et al*. (2012) in their study reported a prevalence rate of 45.0% for typhoid fever in Minna, Nigeria similar to the 46.0% prevalence rate earlier reported in, Nepal India (Bhatta *et al.,*2005). In tropical countries including Nigeria where the disease is often encountered, they account for several cases of morbidities and mortalities (Ibekwe *et al.,* 2008). The causative organism of this disease, *Salmonella typhi,* has rapidly gained resistance to antibiotics (Butt *et al*., 2013) and some people have resorted to the use of herbal remedies.

Herbal remedies are medications prepared from plants materials (Shiel, 2018). These plant materials include seeds, berries, roots, leaves, bark or flowers (Kamatenesi *et al.,*2011). Over seventy five percent of the world population use of one herbal remedy or the other and this trend is gradual but surely on the increase (Oreagba *et al.,* 2011). Ogunsola and Egbewale (2018) attributed this increase in the use of herbal remedies to their relative affordability, acclaimed efficacy and perceived safety by users. The presence of young ladies hawking locally prepared herbal mixtures along the streets has become a common sight in Lagos metropolis. Artisans and traders purchase these herbal mixtures popularly called *Agbo* (a Yoruba word that describes a concoction of plant parts – bark, root, trunk, leaves – steeped or boiled in alcohol or water, which is used to cure various ailments). Oral interview of the hawkers revealed various forms of these locally prepared remedies. The most common of these locally prepared herbal mixtures include; *Agbo jedi-jedi* (hemorrhoids herbal medicine), *Agbo iba* (Malaria fever herbal medicine), *Agbo iba ponto* (Typhoid fever herbal medicine). Oral interview of the hawkers of these locally prepared herbal remedies show variations in the constituents and mode of preparation of the remedy for a particular ailment.

With the increased patronage of these herbal mixtures, the need to determine of the phytochemical constituents of these herbal mixtures and to carry out an acute toxicity test on these herbal mixtures becomes a necessity. It is for these reasons that phytochemical screening as well as acute toxicity studies on *Agbo iba ponto* from various localities in Lagos metropolis has been carried out in this study.

Materials and Methods

Sample Collection

In accordance with the method of Akande *et al*. (2012), this study was undertaken to identify a number of herbal remedies used in the treatment of some common diseases in Nigeria among hawkers of these remedies. They were interviewed for possible information on the constituents and mode of preparation of the typhoid remedy, *Agbo iba ponto*. Samples of the remedy were purchased from three areas, Surulere, Mushin and Ikorodu, in Lagos Metropolis.

Phytochemical screening

Qualitative tests

The methods used by Akande *et al.,* (2012) were used for all the qualitative phytochemical analysis except for the test of terpenoids where the method of Sheel *et al*., 2014 was employed.

Quantitative Tests

Determination of Total Tannins

A quantity of 0.5 g of the concentrated sample was dispersed in 50 ml of distilled water and shaken. The mixture was left undisturbed for 30 minutes at 28°C and filtered through Whatman No. 1 filter paper. A measured volume (2 ml) of the filtrate was dispersed into a 50 ml volumetric flask and 2.5 ml of 10% Na_2CO_3solution was added. The content of each flask was made up to 50 ml with distilled water and incubated at 28°C for 90 minutes. Absorbance was read at 260 nm using the reagent blank. Tannic acid was

used for the calibration curve (Salau *et al*.2013).

Total Flavonoid content estimation

An aliquot of 1 ml of sample was added to 4 ml of water which was left to stand for 5 minutes.0.3 ml of 5% Sodium nitrite and 0.3 ml of 10% $AlCl_3$ were added to the mixture and later incubated for 6 minutes at room temperature. Then, 2 ml of NaOH was added to the incubated mixture and the volume was increased to 10 ml with distilled H_2O. The absorbance was measured at 510 nm. Quercetin was used as a standard against a blank. (Aletan and Kwazo,2019)

Estimation of Saponins

A quantity of 1 g of the concentrated sample was treated with 25 ml of 20 % Ethanol and this mixture was heated in a water bath for 2 h with continuous stirring at about 55°C. The residue from the mixture was added to 50 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. Thereafter, 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 at 90 °C for 30 minutes. After evaporation the samples were dried in the oven to a constant weight (Aletan and Kwazo, 2019)

Estimation of Steroid content

The method of Aletan and Kwazo 2019 was modified for this estimation. An aliquot of 2 ml taken from the concentrated sample prepared in 50 ml of distilled water and shaken for 1 hour was transferred into a 10 ml volumetric flask. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2° C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Estimation of Total Phenolic Compound

A quantity of 0.5g of the concentrated sample was weighed and dissolved in 50 ml of water. A portion of 0.5 ml was added to 0.1ml of Folin C [Folin- Ciocalteu] reagent. It was mixed and incubated at room temperature for 15 minutes. After which 2.5 ml of sodium carbonate solution [7.5% w/v of Na_2CO_3] was added and further incubated for another 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm with the use of Gallic acid as standard. The concentration of total phenol was expressed as Gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

Determination of Alkaloids

This was determined using the method of Harborne (1973). H_2SO_4 reacts with alkaloids in the presence of formaldehyde to form a coloured complex which is read spectophotometrically at 565nm. Exactly 1ml of the sample was pipetted into a clean and dried test tube. Therafter,5ml of 60% sulphuric acid was added into the test tube, which was allowed to stand for 5mins. 5ml of 0.5% formaldehyde in 60% sulphuric acid was also added. This was mixed properly and allowed to stand for 3hrs. Absorbance was read at 565nm (Aja *et al.,* 2017)

Determination of Terpenoids

An aliquot of 1ml of each sample was pipetted into a test tube and 1ml of 5% phosphomolydic acid was added. Gradually, 1ml of sulphuric acid was also added. It was allowed to stand for 30mins and then 2mls of ethanol was added. Absorbance was read at 700nm (Aja *et al.,* 2017).

Determination of Cyanogenic Glycosides

Cyanogenic glycosides react to alkaline picrate under boiling temperature to produce a colour that is read spectrophotometerically at 490 nm. A measured quantity (1ml) of sample was pipetted into a test tube and 4ml of alkaline picrate solution was added. The mixture was boiled for 5mins and was

allowed to cool. Absorbance reading was taken at 490nm (Aja *et al*., 2017).

Determination of Phlobatannins

The concentrated sample (0.5 g) was weighed into a 50 ml beaker and 20 ml of 50% methanol was added, covered with paraffin and placed in a water bath set at 80°C for 1 hour. The mixture was properly shaken to ensure uniform mixing after which it was filtered through a Whatman No. 1 filter paper into a 50 ml volumetric flask, rinsed with aqueous methanol and then made up to the marked level with distilled water. A quantity of 1 ml of this extract was pipetted into a 50 ml volumetric flask, 20 ml of distilled water, 2.5 ml of Folin-Dennis reagent, and 10 ml of 17% sodium carbonate were added to the solution in the 50-ml flask. This mixture was homogenized thoroughly for 20 minutes and absorbance read at a wavelength of 550 nm (Salau *et al.,* 2013)

Determination of Acute Toxicity

Experimental Animals

Healthy young nulliparous and non-pregnant female albino mice with an average weight of 25g were obtained from College of Medicine, University of Lagos, Idi Araba, Lagos Nigeria. The animals were marked for individual identification, and kept under standard condition in plastic cages for 7 days prior to treatment to allow for acclimatisation. Commercially available rodent pellet was given and drinking water was always available.

Acute Toxicity Studies

This was divided into two phases. During first phase of the experiment, Single doses of 10mg/ kg, 100 mg/kg and 1000 mg/kg body weight respectively were administered orally to three groups of three mice each. A second phase of the experiment was carried out based on the result of the first phase. Single doses of 1250 mg/kg, 2500 mg/kg and 5000 mg/kg body weight respectively were administered orally to three groups of one mouse each. These were done using oral gavage after having deprived the animals of food for 12 hours. The animals were observed for behavioral changes, symptoms of toxicity and mortality after treatment in the first thirty minutes, four hours then for 24 hours and up to 14 days after administration (Gad, 2014).

Statistical analysis

All the determinations were carried out in triplicates. The results were expressed as mean ± standard deviation. The data were analyzed for statistical significance by Analysis of Variance (ANOVA)(Microsoft Office Excel 2019) using the statistical tool, post hoc test was done using the Bonferroni Procedure . Data were considered significant at $p \leq 0.05$.

Results

Phytochemical screening

The results of the phytochemical screening of samples of *Agbo iba ponto* from three localities in Lagos are presented in the Tables 1 and 2. Table 1 shows the results of the qualitative screening of *Agbo iba ponto* samples, while the results of quantitative analysis are presented in Tables 2. All the phytoconstitents screened for except flavonoids were present in all the sample. Saponins were shown to be the most prominent phytoconstituents among the phytochemicals studied with values ranging from 37.45 ± 0.37 mg/100g in samples from Ikorodu to 35.00 ± 0.37 mg/100g in samples from Mushin. Terpenoids were the least present phytoconstituents with values ranging from 17.41 ± 0.46 mg/100g in samples from Ikorodu to 15.93 ± 0.23 mg/100g in samples from Surulere. Flavonoids were present only in the samples from Ikorodu. The results of the quantitative analysis of the samples of *Agbo iba ponto* are presented in Tables 2.

Table 1: Results of the qualitative screening of *Agbo iba ponto* from three locations in Lagos Metropolis.

Phytochemicals	Surulere	Mushin	Ikorodu
Saponins	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Phlobatannins	+	+	+
Flavonoids	-	-	+
Terpernoids	+	+	+
Steroids	+	+	+
Cardiac glycosides	+	+	+
Alkaloids	+	+	+

+ indicates presence

- indicates absence

Table 2: The results of quantitative analysis of the phytoconstituents of *Agbo iba ponto* from three locations in Lagos

Phytochemicals	Surulere	Mushin	Ikorodu
Saponins (mg/100g)	36.01 ± 0.37	35.00 ± 0.37	37.45 ± 0.37
Tannins (mg/100g)	18.54 ± 0.19	19.08 ± 0.08	19.71 ± 0.16
Phenols (mg/100g)	32.04 ± 0.16	29.55 ± 0.28	30.53 ± 0.22
Phlobatannins (mg/100g)	21.71 ± 0.11	19.95 ± 0.18[b]	20.02 ± 0.29[b]
Flavonoids (mg/100g)	ND	ND	19.03 ± 0.45
Terpernoids (mg/100g)	15.93 ± 0.23[b]	16.96 ± 0.40[b]	17.41 ± 0.46[b]
Steroids (mg/100g)	20.51 ± 0.25	19.69 ± 0.25[b]	21.37 ± 0.19[b]
Cardiac glycosides (mg/100g)	27.08 ± 0.23[b]	26.51 ± 0.23[b]	27.72 ± 0.23[b]
Alkaloids(mg/100g)	18.28 ± 0.32	17.44 ± 0.12	19.83 ± 0.37

ND- Not detected

Values are means ± standard deviation

[b] indicates no significant differences between groups ($p \leq 0.05$)

Acute Toxicity studies

Oral administration of Agbo *Iba ponto* at 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight respectively did not produce any clinical signs of toxicity or mortality in the experimental animals thus the need for the second phase of the experiment. In the second phase single doses of 1250 mg/kg 2500 mg/kg body weight did not produce any clinical signs of toxicity or deaths in the experimental animals. At a dose of 5000 mg/kg body weight, however, the animals showed signs of drowsiness within the first one hour of administration but became normal after a short nap. No mortality was recorded in the experimental animal within 24hours and up to 14 days post administration.

Table 3: Results of acute toxicity studies on *Agbo iba ponto*

Phases/Groups	Dose (mg/kg bwt)	Mortality	Behaivoural Changes
Phase 1			
Group 1	10	$0/3$	Nil
Group 2	100	$0/3$	Nil
Group 3	1000	$0/3$	Nil
Phase 2			
Group 1	1250	$0/1$	Nil
Group 2	2500	$0/1$	Nil
Group 3	5000	$0/1$	Scratching, Weakness and Drowsiness

Key: $Mortality/Number\ of\ animals\ used$

Discussions

This study was undertaken to determine the phytochemical components and acute toxicity study of locally prepared herbal mixture for typhoid fever, *Agbo iba ponto.* The result of the qualitative screening revealed the presence of saponins, tannins, phenols, phlobatannins, terpernoids, steroids, cardiac glycosides and alkaloids in all the samples studied, while flavonoids was found only in the samples from Ikorodu. These were unlike the results of Akande *et al.* (2012) which had no presence of phlobatannins and cardiac glycosides. From the oral interview of some of the hawkers, this may not be unexpected as constituents and recipes of *Agbo* were seen to vary with hawkers. However, some of the hawkers were reluctant to give out their recipes due to their oath of secrecy.

In the quantitative analysis, saponins were the most prominent phytoconstituents in all the samples studied. This is similar to the observation of Akande *et al*. (2012) who were of the opinion that the high levels of saponins in the samples may act as anti-nutrients. They also noted that oral administration of haemolytic saponins to mammals in large doses is toxic and can result in death due to a massive release of erythrocyte debris and reduced oxygen-carrying capacity of the blood. However, saponins have been known to have antidiarrhoeal, anticancer and anthelmintic properties (Tiwari *et al.,* 2011). Higher levels of phenols were also found in the sample compared to the other phytoconsitituents. According to Dia and Mumper (2010) the presence of phenolic compounds indicates antimicrobial activity in plants. Furthermore, Rasool *et al.* (2010) attributed the effectiveness of the herb *Prunella vulgaris* in the treatment of typhoid fever and other bacterial infections to its high phenolic content. Appreciable amounts of cardiac glycosides, steroids, tannins, phlobatannins and alkaloids were also found in the samples and most of these are known to have activity against pathogens (Ghosh *et al*., 2010) and therefore may aid the antimicrobial activities ascribed to *Agbo iba ponto* by the hawkers. Tannins, for example, are known to exhibit antimicrobial activity against some pathogenic bacteria (Maisetta *et al.,* 2019) .Terpernoids had the least presence among the phytochemicals determined. Terpernoids have found industrial applications in the formulation of medicinal compounds such as the anticancer drug, taxol (Croteau *et al.*, 2006), the antimalarial agent, artemisin (Paddon *et al.,* 2013) and are antimicrobial in nature (Gupta and Birdi, 2017). Although the mode of microbial action of terpernoids is not clearly defined, Termentzi *et al.* (2012) ascribed it to the disruption of the microbial membranes.
According to Gupta and Birdi (2017) the major limitations in the use of plant extracts for clinical applications include their complex composition, extract instability and toxicity risks. Generally, safety studies on herbal medicines are usually done by performing acute and sub-acute toxicity tests using laboratory animals (Fennel *et al*., 2004).

In this study acute toxicity effects were observed soon after a single dose administration (within 24hours) and mice are the most frequently selected rodent species for acute toxicity testing (Gad, 2014). The choice of routes of administration depends on the intended clinical route. Since *Agbo iba ponto* is usually taken orally, oral administration was employed in this study. The administration of a single dosage of up to 2500 mg/kg body weight of herbal mixture did not reveal any signs of toxicity or mortality in the animal during the entire observation period. However, an increase to the maximum allowed dosage of 5000mg/kg body weight (OECD,2001) produced a short period of weakness and drowsiness but did not lead to mortality of the animal. No mortality was recorded in the experimental animals up to 7days after administration. Therefore the LD_{50} (the dosage which kills 50% of the animals) of this herbal mixture may be considered to be more than 5000mg/kg body weight. According to the classification of Teke and Kuete (2014) any compound with oral LD_{50} of 5000mg/kg or more should be considered as practically nontoxic. However, there is need to carry out subacute toxicity studies on this herbal mixture to ascertain the effect of repeated intake at lower dosage over a period of time.

Conclusion

In conclusion, the qualitative and quantitative analysis of *Agbo iba ponto* has shown the presence of an appreciable wealth of phytochemical constituents in this mixture. An acute toxicity study has shown that a single dose of 5000 mg/kg though led to weakness and drowsiness in mice did not

cause death. The results of this study therefore suggested that *Agbo iba ponto* is relatively nontoxic up to 5000 mg/kg body weight.

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