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Biochemical Evaluation of Some Locally Prepared Herbal Remedies (*Agbo*) Currently On High Demand in Lagos Metropolis, Nigeria

Akande IS*, Adewoyin OA, Njoku UF and Awosika SO

Department of Biochemistry, College of Medicine, University of Lagos, Akoka, Yaba, Nigeria

Abstract

The use of traditional herbal remedies popularly known as "agbo" over the years especially among the local populace in the South west, Nigeria has been seen as an alternative approach to orthodox health care delivery in Nigeria for various reasons. Apparently, daily health issues of Nigerians are partly taken care of by these herbal remedies and their use are on the increase in Lagos metropolis which raises safety concerns. The study was carried out to estimate the total antioxidant activity, total reducing antioxidant power, total phenolic content, tannin, total flavonoids and % DPPH+ scavenging activity of some of these locally prepared herbal samples on sale in Lagos Metropolis using standard methods. Physicochemical parameters such as pH, total dissolved solids and odour were also determined. The phytochemical screening revealed the presence of tannin, saponin, alkaloids and flavonoids in the herbal remedy samples. The samples showed wide range of values: Tannin (380.44 ± 15.1-1538.90 ± 43.53 mgGAE/ml), flavonoids (69.50 ± 11.00- 26382.98 ± 69 mgGAE/ml) phenols (30.23 ± 4.0-104.07 ± 8.3 mgGAE/ml) and %DPPH activity (24.82 ± 4.7%- 84.68 ± 12.3%). Physicochemical parameters analysis showed that the samples contained dissolved particles with "*agbo atosi*" having the highest value of 1149.20 ± 34 mg/L, "*agbo jedi*" having the lowest value of 205.33 ± 28 mg/L at 26.2°C. The samples were acidic with pH range of 5.39 – 6.75 while the odour associated with the "*agbo atosi*" was offensive in nature. This might not be unconnected with various unnatural ingredients probably contained in these preparations which are largely marketed by young and apparently ignorant female youths unfamiliar with traditional herbal formulations. Based on these findings, it is probable that these preparations though potential sources of natural antioxidants may be harmful to human health. There is also a need for standardization of dosage regimens and close scrutiny of pedigree of the peddlers of these herbal remedies by appropriate government agencies.

Keywords: *Agbo;* Phytochemicals; Antioxidants; Total phenolic content; Physicochemical

Introduction

The use of herbal medicines among Nigerians and the tendency by patients to combine this class of medicines with allopathic drugs while on hospital admission is on the increase [1].

The combination of modern drugs and traditional herbal remedies is a fast growing practice among many Nigerians as many people believe in their efficacies. These herbal remedies, popularly known as "agbo" among the south-west locals of Nigeria, are employed in the treatment of many common diseases. They include typhoid fever, malaria, and less common diseases, of which are the sexually transmitted diseases like gonorrhoea and staphylococcus. The reasons for this include affordability of these remedies as well superstitious beliefs commonly spread by the traditional herbal medicine practitioners, and their patrons alike, contribute immensely to the increase in consumption of these herbal remedies [2-4].

This is despite the availability of modern drug formulations [5]. Traditional medicine is still the predominant means in the third world for the preservation of health of the rural majority who constitute over 70% of the total population [6]. However, adequate knowledge of the methods of preparation, the possible toxicological effects of some chemical species present in the ingredients and the side effects of the various mixtures are usually unknown to many who patronize herbal medicines (since herbs contain hundreds of plant chemicals in varied concentrations) which if not properly utilised can pose a threat to the health of the consumer and could lead to the damage of vital organs such as liver and kidney that are involved in the metabolism and evacuation of toxic chemicals in the body.

Unfortunately the belief in the efficacy of herbal remedies by an average Nigerian outweighs individual knowledge of the pharmacological effects of these medicines and this could prove fatal in many cases.

The study was therefore carried out to determine phytochemicals present in some locally produced herbal remedies in Lagos, Nigeria and to evaluate antioxidant properties of common herbal remedies locally prepared in Lagos, Nigeria and the probable toxic effects of these herbal remedies.

Material and Methods

Assays of prepared herbal samples

A pilot survey was carried out to identify a number of herbal remedies used in the treatment of some common diseases in Nigeria among traditional medicine practitioners in the vicinity of Lawanson market in Surulere local government, area of Lagos state. These were interviewed for possible information on the types of herbal remedies prepared, their modes of preparation s and diseases they potentially treat. A total of 5 samples were identified which were used in the

***Corresponding author:** : Akande IS, Department of Biochemistry, College of Medicine, University of Lagos, Akoka, Yaba, Nigeria, Tel: +234-8033187864; E-mail: iakande@unilag.edu.ng, akande_idowu@yahoo.com

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treatment of malaria, typhoid, dysentery, body pains and gonorrhea. Questions were asked on the methods of preparations of these herbal remedies, some components of the concoctions brewed were identified and samples purchased for assay in the laboratory.

Reference standards

The reference standard used for was 100 µg/ml pyrocatechol (galic acid) prepared in the Laboratory of department of Biochemistry, College of Medicine, University of Lagos (CMUL), Idi-Araba campus, Lagos, Nigeria.

In the laboratory, the purchased herbal remedies were assayed for the following physical parameters: Appearance, pH, total dissolved solids colour, odour and taste using WEDC (1980) (Water, Engineering and Development Centre) Analytical methods Manual (APHA, 1975; 1980, YWA, 1972, 1979; HMSO, 1972; WHO, 1976 and DHSS).

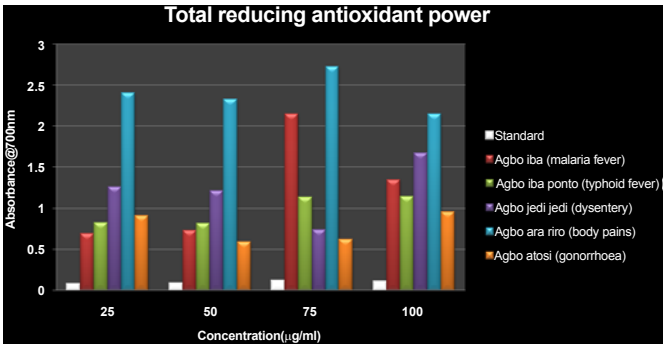

Figure 1: The total reducing anti oxidant property in agbo iba, agbo iba ponto, agbo jedi, agbo ara riro and agbo atosi.

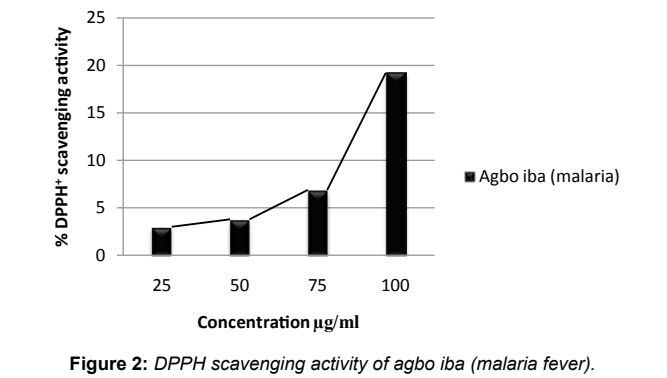

Figure 2: *DPPH scavenging activity of agbo iba (malaria fever).*

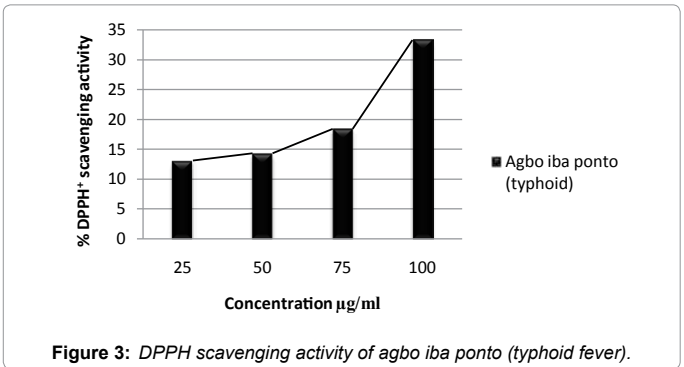

Figure 3: *DPPH scavenging activity of agbo iba ponto (typhoid fever).*

Phytochemical analysis

Assays were carried out as described in [7] with some modifications for the following phytochemicals in each sample- Tannins, phlobatannins, saponins, alkaloids, flavonoids, cardiac glycosides, and sterols. Antioxidant activity potentials were also determined.

Test for tanins

2 ml of each sample was placed in a test tube and a few drops of 0.1% ferric chloride was added and observed for brownish green or blue black coloration.

Tests for phlobatanin

4 ml of each sample was boiled with 2 ml 1% aqueous hydrochloric acid in a boiling tube. The deposition of red precipitate indicates the presence of phlobatanins.

Tests for saponin

10 ml of each sample was placed in a tube and shaken vigorously. A stable persistent forth indicates the presence of saponin

Test for alkaloid

Wagner's reagent was added to 2 ml of the sample in a test tube and allowed to stand for some time. The development of a brown, turbid solution showed the presence of Alkaloids. This procedure was repeated for each of the 5 samples

Test for flavonoid

5 ml of 10% dilute ammonium hydroxide solution was added to a portion of the samples in a test tube followed by the careful addition

S/N	Name of herbal remedy	Appearance
1.	*Agbo iba* (malaria fever)	Clear yellow solution with an offensive smell and a bitter taste
2.	*Agbo iba* ponto (typhoid fever)	Brick red solution with an offensive odour and a bitter to taste
3.	*Agbo jedi jedi* (dysentery)	Dark brown solution with no odour and a very bitter to taste
4.	*Agbo ara riro* (body pains)	Greenish white solution, very cloudy, with a sweet smell of mango and a sour taste.
5.	*Agbo atosi* (gonorrhoea)	Dirty brown solution with high amount of undissolved particles and an extremely offensive odour similar to that of faeces. Sour taste.

Table 1: Herbal remedies with the associated colour, taste and odour.

S/N	Name of Herbal Remedy	pH
1.	*Agbo iba* (malaria fever)	5.39
2.	*Agbo iba ponto* (typhoid fever)	6.22
3.	*Agbo jedi jedi* (dysentery)	5.59
4.	*Agbo ara riro* (body pains)	5.92
5.	*Agbo atosi* (gonorrhoea)	6.75

Table 2: *pH for the five sampled herbal remedies.*

S/N	Name of herbal remedy	Total dissolved solids (mg/l)
1.	Agbo iba (malaria)	914.0
2.	Agbo iba ponto (typhoid fever)	910.0
3.	Agbo jedi jedi (dysentery)	205.0
4.	Agbo ara riro (body pains)	944.0
5.	Agbo atosi (gonorrhoea)	1149.0

Table 3: Total dissolved solids in the analyzed herbal remedies samples.

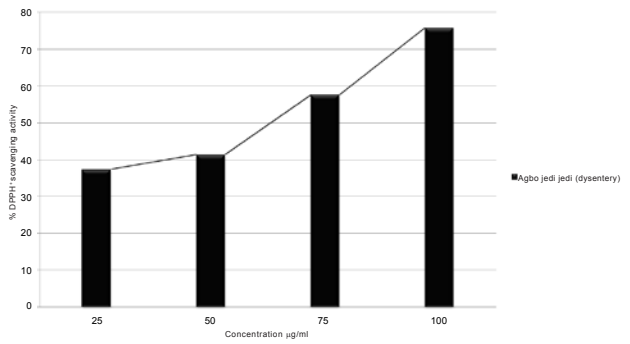

Figure 4: DPPH$^+$ scavenging activity in agbo jedi.

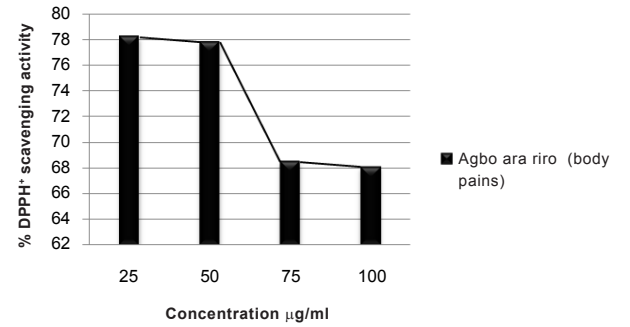

Figure 5: *DPPH scavenging activity of agbo ara riro (general body pains).*

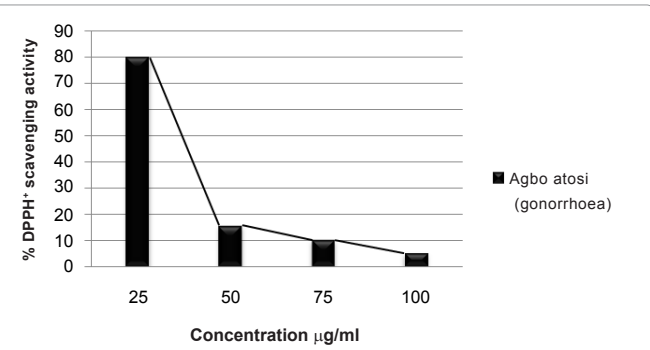

Figure 6: *DPPH scavenging activity of agbo atosi (gonorrhoea).*

of 4 drops of concentrated H_2SO_4. A yellow coloration observed in the samples indicated the presence of flavonoids.

Test for cardiac glycosides

5 ml of the samples were each treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This was underplayed with 1ml of concentrated H_2SO_4. A brown, violet or green ring of the interface indicated a deoxysugar characteristic of cardenolides.

Test for steroids

The Lieberman-burchards test was carried out. 1 ml of the sample was dissolved in 2 ml of acetic anhydride and cooled in ice. 2 drops of concentrated H_2SO_4 was then carefully added. The formation of a greenish-blue precipitation indicated the presence of steroids.

Antioxidant Activity

A stock solution of 1% sample was prepared by adding 1 ml of the concoction into a 100 ml conical flask and water was added to the sample to make it up to the 100 ml mark. The diluted samples, each of concentration 100 µg/l, were used for the following antioxidant assays—Estimation of phenolic content tannin content, flavonoid content, total antioxidant capacity (TAC), total reducing antioxidant power (TRAP) and DPPH$^+$ radical scavenging assay

Estimation of total phenolic content

Total soluble phenolic content of the extract was determined with Folin-C reagent using pyrocatechol (galic acid) 100 µg/l as a standard. An aliquot of 0.5 ml of each of the diluted samples and standard were added to 0.5 ml of water in separate test tubes. 0.2 ml of Folin-C reagent (1:2 in distilled water) was added to the test tube and, after 20 min, 2 ml of 7.5% sodium carbonate (Na_2CO_3) was added. After 30 min the absorbance was measured at 765 nm. The concentration of total phenolic component in the extract was determined as microgram of standard equivalent.

Estimation of total tannin

An aliquot of 1 ml of each of the diluted samples and standard were placed in test tubes containing 0.2 ml of $FeCl_3$. To the test tubes, 0.2 ml

Herbal remedy	Tannins	Phlobatannins	Saponins	Alkaloids	Flavonoids	Cardiac Glycosides	Sterols
Agbo iba (malaria fever)	+	-	+	+	+	-	+
Agbo iba ponto (typhoid fever)	+	-	+	+	+	-	+
Agbo jedi jedi (dysentery)	+	+	+	+	+	+	-
Agbo ara riro (body pains)	+	-	+	-	+	+	-
Agbo atosi (gonorrhoea)	+	-	+	+	+	-	+

Table 4: Phytochemicals detected in agbo *iba, agbo iba ponto, agbo jedi,agbo ara riro* and *abgo atosi*.

S/N	Herbal Remedy	Mean OD (@765nm)	Conc. (mg GAE/ml)
1.	*Agbo iba* (malaria)	0.052 ± 0.01	30.23
2.	*Agbo iba* ponto (typhoid fever)	0.157 ± 0.004	91.28
3.	*Agbo jedi jedi* (dysentery)	0.183 ± 0.004	106.40
4.	*Agbo ara riro* (body pains)	1.790 ± 0.01	104.07
5.	*Agbo atosi* (gonorrhoea)	0.113 ± 0.005	65.70

Table 5: Total phenolic content in mg galic acid equivalent (GAE) per ml of sample herbal remedies.

S/N	Herbal remedy	Mean OD (@720nm)	Conc. (mg GAE/ml)
1.	*Agbo iba* (malaria)	0.530 ± 0.02	380.44
2.	*Agbo iba ponto* (typhoid fever)	0.901 ± 0.002	643.11
3.	*Agbo jedi jedi* (dysentery)	0.870 ± 0.003	620.99
4.	*Agbo ara riro* (body pains)	0.539 ± 0.005*	1538.90
5.	*Agbo atosi* (gonorrhoea)	0.740 ± 0.01	528.19

*dilution factor for sample 4 = x4

Table 6: Total tannins content in mg galic acid equivalent (GAE) per ml of sample.

S/N	Herbal remedy	Mean OD (@720nm)	Conc. (mg GAE/ml)
1.	*Agbo iba* (malaria)	0.049 ± 0.01	69.50
2.	*Agbo iba ponto* (typhoid fever)	0.099 ± 0.004	140.43
3.	*Agbo jedi jedi* (dysentery)	0.106 ± 0.004	150.35
4.	*Agbo ara riro* (body pains)	1.550 ± 0.01*	26382.98
5.	*Agbo atosi* (gonorrhoea)	0.549 ± 0.005	778.72

*dilution factor for sample 4 = x12

Table 7: Total flavonoids content in mg galic acid equivalent (GAE) per ml of sampleherbal remedy.

S/N	Herbal remedy	Mean OD (@720nm)	Conc. (mg GAE/ml)
1.	*Agbo iba* (malaria)	0.074 ± 0.003	49.04
2.	*Agbo iba ponto* (typhoid fever)	0.279 ± 0.01	184.89
3.	*Agbo jedi jedi* (dysentery)	0.720 ± 0.01	477.14
4.	*Agbo ara riro* (body pains)	2.799 ± 0.01	1854.87
5.	*Agbo atosi* (gonorrhoea)	0.343 ± 0.002	227.30

Table 8: Total antioxidant activity in mg galic acid equivalent (GAE) per ml of sample.

of potassium ferrocyanide was added followed by 3.6 ml of water. A green colouration was produced and the absorbance was measured and at 720 nm. The concentration of tannins was measured as microgram of standard equivalent.

Total flavonoid content

The total flavanoid content was determined using $AlCl_3$ and galic acid as standard. To 0.5 ml of the already prepared stock solutions of the herbal remedies, 0.5 ml of methanol was added in test tubes. This was followed by the addition of 0.1 ml of potassium acetate (0.1 M) then $AlCl_3$ (10%) and 1.3 ml of water was added to dilute the mixture. The absorbance was read at 415 nm. The process was repeated for the blank (water). Total flavonoid content was measured as microgram equivalent of standard.

Total antioxidant capacity (TAC)

The total antioxidant capacity was measured with phosphomolybdenum using pyrocatechol as standard. An aliquot of 1 ml of each of the diluted samples and standard were placed in separate test tubes containing 1 ml of the reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in a UV spectrophotometer. The blank solution contained 1 ml of reagent solution and 1 ml of water and it was incubated under the same conditions as the rest of the samples. The total antioxidant capacity was expressed as microgram equivalents of standard

Total reducing antioxidant power (TRAP)

Reducing power ability was measured by mixing 1ml of the varying concentrations (25 µg/l, 50 µg/l, 75 µg/l and 100 µg/l) of the samples prepared from the stock to 1 ml of sodium dihydrogen phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% potassium ferricyanide and incubated at 45-50°C for 30 min. Next 1 ml aliquots of 10% trichloroacetic acid were added to the mixtures and the samples were diluted with 0.8 ml of water and shaken with 0.2 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The blank solution was prepared as above but contained 1 ml of water instead of the samples.

DPPH+ radical scavenging activity

1ml of varying concentrations (25 µg/l, 50 µg/l, 75 µg/l and 100 µg/l) of the samples, prepared from the stock, was placed in separate test tubes and 2 ml of $DPPH^+$ (2,2-diphenyl-1-picrylhydrazyl) solution was added to the tubes and left in the dark for 30 min. A control solution was also prepared using water and subjected to similar procedures. The absorbance was then measured at 515 nm. The blank solution contained only 3 mls of methanol. The % DPPH+ scavenging activity was calculated using the equation—

$$\% \ DPPH^+ \text{ scavenging activity} = [1- OD_{SAMPLE}/OD_{CTRL}] \ 100$$

Statistical Analysis

Each assay was done in triplicate. Data generated were expressed as means ± standard deviation and analyzed using one way Analysis of Variance (ANOVA)

Results and Discussion

The five samples were acidic with the pH values range of 5.30 - 6.80. All the samples were very cloudy and (*agbo atosi*) samples contained much undissolved particles hence its high total dissolved solids (TDS) values of 1149 mg/L, followed (*agbo ara riro*) at 944 mg/L. *Agbo jedi* was the clearest of the concoction which accounted for its low TDS values (205 mg/L) compared to the values of other herbal remedies. The extremely repugnant smell of "*agbo atosi*" was responsible for difficulty in the consumption of the concoction by many users interviewed. Results for phytochemistry revealed the presence tannins, phlobatannins, saponins, alkaloids, flavonoids, cardiac glycosides and sterols. However, "*Agbo ara riro*" had the highest concentration of tannins and flavonoids with values of 1538.9 mg GAE/ml and 26382.98 mg GAE/ml respectively. *Agbo ara riro* had very high phenolic contents (104.07 mg GAE/ml), following *Agbo jedi jedi* with the highest phenolic content of 106.4 mg GAE/ml.

The high concentrations of phytochemicals with antioxidant potentials in "*agbo ara riro*" account for its very high total antioxidant capacity (TAC) value of 18.549 mg GAE/ml of the sample. Although all the samples had high total reducing potentials, *agbo ara riro* showed the highest reducing potentials at all concentrations, followed by *agbo iba* and *agbo jedi jedi* in that order. *Agbo atosi* cotained the lowest reducing potential at concentration of 50 µg/ml. All the samples gave good DPPH+ scavenging activities. Although these compounds possess high antioxidant properties and are very effective in ameliorating oxidative stress and reduction of oxidation of cellular components by reactive oxygen species (ROS), however, high concentrations of some of these substances if taken in excess could have deleterious effects on the internal milieu of organs and cells such as hemolytic, membranolytic activities [8]. Most of herbal samples investigated contained high levels of saponins which could act as anti nutrients as shown by [9]. Oral administration of hemolytic 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 [10]. Unregulated use could have similar effects in man. We observed that young and middle aged ladies were the vendors for these herbal remedies in course of this study. It was clear that majority of them are not familiar with natural medicinal plants as obtained with the traditionalists of the past generation. Many of the concoction they hawk in the metropolis may therefore contain extraneous and unsafe materials. This may be partly responsible for the offensive odour and large debris noticed in these herbal products evaluated in this study.

Conclusion

Based on these findings, we conclude that, though these prepa-

rations are potential sources of natural antioxidants, but majority of those being hawked on Lagos metropolis may be harmful to human health because many of the hawkers are likely to be quacks. There is also a need for standardization of dosage regimens and close scrutiny of pedigree of the peddlers of these herbal remedies by appropriate government agencies.

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