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# Phytochemical screening, antimicrobial evaluation, and detection of caffeine and aspirin in herbal remedies used to treat typhoid fever

**Nwamaka H. Igbokwe*, Adebowale O. Adeluola, Abel O. Idowu and Stephany Ugbo**

*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Idi-Araba, University of Lagos, Lagos, Nigeria.*

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**Abstract**

The use of herbal medicines among Nigerians and the tendency by patients to combine this class of medicines with allopathic drugs is on the increase. This study was carried out to evaluate the antimicrobial quality, phytochemical screening and detection of orthodox drugs (caffeine and aspirin) present in locally prepared herbal remedies "*Agbo*" indicated for typhoid fever'. Phytochemical screening of different herbal samples for typhoid was carried out. The antimicrobial activity of these samples was evaluated against enteric bacteria: *Salmonella typhi*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Investigation of the presence of aspirin and caffeine in most acidic samples was also carried out using High Performance Liquid Chromatography (HPLC). The investigated herbal remedies for typhoid fever revealed an array of potential phytochemicals: Alkaloids, Saponins, Tannins, Cardiac glycosides, Reducing sugars, Flavonoids, Steroids and Terpenoids. Only one (5%) of the 20 samples investigated possessed antimicrobial activity against the enteric organisms with minimal zone of inhibition. All the samples investigated possessed traces of caffeine while 70% contained caffeine and aspirin. Although the herbal preparations known as "*Agbo typhoid*" showed an array of phytochemicals, caution should be exercised in their consumption since they were found inactive against the causative organism for typhoid fever, *Salmonella typhi*. Introduction of orthodox drugs to herbal remedies is unacceptable since there could be unhealthy interactions. The presence of caffeine and aspirin in "*Agbo*" could be deleterious to health.

*Keywords*: Phytochemicals; "*Agbo*"; Caffeine; Aspirin

## INTRODUCTION

Infectious diseases are major causes of morbidity and mortality in the developing world [1]. They account for about 50% of all deaths [2-4]. Some 5.8 million deaths each year in infants and children below 5 years are caused by enteric diseases worldwide [5]. The major source of enteric infections is the poor quality of accessible drinking water, contaminated food, poor standard of personal hygiene and lack of appropriate sanitation [1]. Some of the bacteria implicated in causing of enteric infections include but not limited to *Escherichia coli, Salmonella spp., Proteus spp., Shigella spp., Clostridium spp., Pseudomonas spp.,* and the *Staphylococci* [6,7]. Detecting enteric pathogens is highly important, as most of these pathogens such as *Salmonella spp.* and *Shigella spp.* are widespread agents of enteric infections.

* Corresponding author. *E-mail*: nhigbokwe@ymail.com *Tel*: +234 (0)8023328313
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Globally, people develop unique indigenous healing traditions adapted and defined by their culture, beliefs and environment, which satisfied the health needs of their communities over centuries.

The use of herbal remedies is a very common phenomenon in developing countries [8-10]. Herbs although commonly used as sources of food are also used medicinally and have been used for centuries [11]. The World Health Organization estimates that about 80% of the population in Africa use traditional medicine [12]. About 85% of traditional medicine involves the use of plants extracts [13,14]. There are different forms of herbal medicinal preparations and they include: infusions, decoctions, tinctures, macerations [15], suspensions and pastes [16]. Other methods include preparing plants in hot baths (in which the patient is soaked in it or bathed with it), inhalation of powdered plants (like snuff), steam inhalation of various aromatic plants boiled in hot water, and even aromatherapy [16]. The herbal remedies, popularly known as "*Agbo*" among the south-west locals of Nigeria, are employed in the treatment of many common diseases, which include typhoid fever [17,18], malaria [19], sexually transmitted diseases like gonorrhoea and staphylococcus [20], pile, dysentery etc.

"*Agbo*" usually comes in liquid form as decoctions, suspensions or pastes. They are usually made from the crushed or ground mixtures of different plants or plant parts such as the bark, root, leaves, and seeds. Some of them are drunk, some serve as bathing medicaments. The nature and frequency of use depend on the prescription of the herbalist. The pharmacologic properties of most herbs remain uncertain for lack of extensive research in this area. A lack of standardization and incomplete regulation of herbal remedies, poor sanitation, contamination with pesticides, microorganisms, heavy metals and incorrect dosing further complicate this issue [17].

'*Agbo*' could be sold in the raw form, whereby the patients are instructed on how to prepare the herbal remedy. It could also be sold as the already prepared form which is usually dispensed in plastics, bottles or nylons, and sometimes given to patients using a specified cup size potion for dosage measurement (non standardized) as decided by the herbalist. The common use of these herbal preparations by Nigerians for various diseases, infections and ailments prompts a need for research into the properties of these prepared products. Poor sanitation and possible contamination during preparation of these herbal remedies by traditional medical practitioners alongside the lack of standardization, the uncertainty of the pharmacologic and antimicrobial properties of these herbal preparations, incomplete regulation by the appropriate authorities are pressing issues.

The aim of this study was to determine the phytochemical composition and antimicrobial potency of locally prepared herbal remedies indicated for typhoid fever ('*Agbo*' typhoid) and screen them for the presence of orthodox drugs (caffeine and aspirin) using High Performance Liquid Chromatographic method (HPLC).

## EXPERIMENTAL

**Collection of prepared herbal samples.** A total of 20 locally prepared samples of herbal preparations for typhoid fever popularly known as "*Agbo* typhoid" were bought from different locations in Lagos State, Nigeria and labeled A to T. The samples were prepared with water (claimed) as the base solvent and dispensed in transparent nylon bags. On purchase, the composition of each of the samples was inquired from the marketers and they were aseptically transferred into sterile sample bottles from the transparent nylon bags and stored in the refrigerator at 4°C for further analysis.

**Test microorganisms.** The microorganisms used for this study were clinical isolates of *Salmonella typhi, Escherichia coli, Proteus vulgaris* and *Klebsiella pneumonia* obtained from Lagos University Teaching Hospital (LUTH), Nigeria.

**Chemicals.** All chemicals and solvents were obtained from Oxoid Co. Ltd. (Cheshire, England WA14 2DT).

**Phytochemical screening of herbal samples.** The phytochemical screening for the presence of tannins, alkaloids, cardiac glycosides, saponins, reducing sugars, flavonoids, steroids and terpenoids was carried out on the 20 herbal samples using standard procedures as described by Akande *et al.* [20].

**Evaluation of antimicrobial activity of the herbal preparations.** The antimicrobial activities of the hydro based herbal preparations on clinical isolates of enteric organisms: *Salmonella typhi, Escherichia coli, Proteus vulgaris* and *Klebsiella pneumonia* were evaluated using the agar well diffusion method as described by Ogbonnia *et al.* [21]. Different concentrations (25%, 50% and 100%) of the extract were used with Ciprofloxacin as the positive control while sterile water was the negative control.

**Determination of the minimum inhibitory and bactericidal concentrations.** The minimum inhibitory concentration (MIC) of the herbal samples was determined for each of the test organisms using the agar dilution method as described by Adeniyi and Ayepola, [22]. Serial dilutions: 50.0%, 40.0%, 35.0%, 30.0%, 25.0%, 20.0%, 15.0%, 10.0%, 5.0%, 2.5% of the herbal samples were prepared. A volume of 2 mL from each of the sample dilutions was mixed with 18 mL of Mueller Hinton agar seeded with 1 mL of 3 X $10^2$ colony forming units (cfu) of the organism by swirling, allowed to set and incubated at 37°C for 24 h. Different concentrations from the least concentration at which there was no growth and the highest concentration that showed growth were sub cultured, incubated at $37^0$C and the lowest concentration at which there was no growth was recorded as the MIC. The minimum bactericidal concentration (MBC) of the herbal extracts was determined by the method of Adeleye et al. [23]. Samples were taken from the MIC test bacteria plates with no visible growth, sub-cultured on freshly prepared Mueller Hinton agar plates and incubated at 37°C for 24 h. MBC was taken as the concentration of the sample that did not show any growth on the new set of agar plate.

**Determination of relative densities and pH of the herbal samples.** A pycnometer was used for relative densities determination. The weight of the pycnometer alone was recorded. Each sample, 50 ml was transferred into the pycnometer, weighed and recorded. The weight of the sample alone was determined by subtracting the weight of the pycnometer from the weight of the sample and pycnometer. The weight of 50 ml of the sample thus determined was then divided by 50 to give the weight per ml for each sample. The pH of each of the samples was determined using a pH meter (Mettler Toledo, Chicago)

**Screening for the presence of aspirin and caffeine in the herbal preparations using High Performance Liquid Chromatography (HPLC).** The USP, 2014 method [24] was used with slight modifications as permitted.

**Preparation of working reference standard solution**: Standard stock solution of a mixture of aspirin and caffeine in methanol at the concentrations of 500µg/mL and 250µg/mL respectively was prepared. The solution was filtered through 0.45 mm membrane filter and injected by autosampler.

**Preparation of herbal samples**: Each of the ten most acidic samples of "*Agbo*" was diluted with methanol (1 in 50 mL), filtered with a 0.45 um Millipore membrane filter and transferred into a vial for analysis. The

samples including the standard and the blank (methanol) were assayed in sequence to identify aspirin and caffeine peaks.

**Setting the chromatograph.** The Chromatograph 1120 (Compact LC,- Agilent Technologies Austria) was set with Hypersil column, H5ODS C8 25 cm x 4.0 mm (H5ODS-250AF, USA) and Methanol as the mobile phase: 0.1% Glacial Acetic Acid (30:70). The wavelength was set at 275 nm, at the temperature of 35°C with the flow rate of 1.5 mL/min. Injection volume was 20 µL with the stop time set at 8 minutes. A mixture of aspirin 500 µg/mL and Caffeine 250 µg/mL was used as the standard. The blank was HPLC grade methanol

**Statistical analysis.** ANOVA test was used to determine the statistical significant difference. The difference was regarded as significant when $P < 0.05$. Descriptive analysis of mean, standard deviation and the standard error of mean (SEM) were used to summarize the diameters of zones of inhibition (mm).

## RESULTS AND DISCUSSION

**Phytochemical screening of herbal samples.** Table 1 reveals the color, claimed composition by the marketers and the phytochemical composition of each of the herbal samples (A to T). The marketers of samples C, O, Q, R and S claimed not to know the composition of their products while marketers of samples G and T claimed to know the composition of the products but clearly refused to reveal them as shown in table 1. This indicates that most consumers of these products might not know the content of what they are taking and might not even ask questions or might not get the right answers on questioning. The herbal products were rich in Phytochemicals (Table 1). A total of 8 out of 20 samples contained Saponins (Table 1). High levels of saponins in herbal samples could act as anti-nutrients [25]. 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. Unregulated use of it could have similar effects in man. [20].

**Antimicrobial activity of the herbal preparations.** No zone of inhibition was observed on any of the bacterial culture media even at 100% concentration except Q at 100% concentration containing After5®: Acetyl salicylic acid, caffeine and Paracetamol (Table 2). The zones of inhibition of the standard ciprofloxacin are shown in table 3. The MIC and MBC of the sample that had antimicrobial activity against the organisms (sample Q) is represented in table 4. The presence of phytochemicals such as: tannins, alkaloids, steroids, anthraquinones, flavonoids and saponins in herbal preparations have been attributed to antimicrobial activity [23,26]. However, 19 out of the 20 samples showed no antimicrobial activity against the enteric organisms: *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris* (Table 2). Sample Q was the only sample with minimal antimicrobial activity against the four test organisms (Table 2) when compared to the activity observed with the positive control ciprofloxacin (Table 3). Sample Q was the sample to which a full sachet of After5® was added during purchase. The minimal anti-microbial activity observed in it could be due to the constituents of After5® (Aspirin i.e. acetylsalicylic acid, Caffeine and Paracetamol). The inactivity of the herbal preparations against the enteric organisms particularly *Salmonella typhi*, the causative agent for typhoid fever could be attributed to poor preparation practices such as improper mixing of herbs during preparation, the ignorance and illiteracy of the sellers who sold the remedies, non-conformity to standard ingredients as the sellers used different ingredients in different proportions (Table 1), denatured thermolabile components

by heating, unknown interaction between the herbal preparations and orthodox remedy. Different solvents have various degrees of solubility for different phytochemicals [27].

**Table 1.** Claimed ingredients and phytochemical composition of the Herbal products (A- T)

| Samples | Colour | Claimed Ingredients | Cardiac Gly | Sap. | R. sug. | Flav. | Ster. | Terp. | H.tan. | C.tan. | M.test | H.test | D.test |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Deep brown | Mango bark & leaves, *Sacrocephalius latifolius*, *Khaya ivorensis*, 7up® | + | - | ++ | ++ | ++ | ++ | +++ | - | - | - | - |
| B | Milky green | Blended *Blighia sapida* root mixed with fermented pap water | + | + | ++ | ++ | ++ | ++ | +++ | +++ | - | - | - |
| C | Muddy brown | Marketer claimed not to know | + | + | ++ | ++ | ++ | ++ | +++ | - | - | - | - |
| D | Muddy brown | Mango bark & *Zingiber officinale* pounded together | + | + | ++ | ++ | ++ | ++ | ++ | +++ | - | - | - |
| E | Milky green | Mango bark | + | - | ++ | ++ | ++ | ++ | +++ | +++ | - | - | - |
| F | Orange | *Enantia chlorantha*, *Azadiracta indica* & Dogonyaro | + | + | + | ++ | ++ | + | - | + | - | - | + |
| G | Brown | Marketer refused to reveal | + | - | ++ | ++ | ++ | ++ | +++ | ++ | - | - | + |
| H | Brown | "Muru", *Launea taraxacifolia* | + | - | ++ | ++ | ++ | ++ | +++ | +++ | - | - | - |
| I | Brown | *Cymbopogon citratus*, Dogonyaro, lime, *Lawsonia inermis* leaf | + | - | ++ | ++ | ++ | ++ | ++ | - | - | - | - |
| J | Brown | *Cymbopogon citratus*, Dogonyaro, lime, *Lawsonia inermis* leaf | + | - | ++ | ++ | ++ | ++ | +++ | ++ | - | - | - |
| K | Brown | Mango bark, Sugar, "Muru" | + | + | ++ | ++ | ++ | ++ | +++ | +++ | - | - | - |
| L | Brown | Lemon grass, water melon bark, Dogonyaro | + | - | ++ | ++ | ++ | ++ | +++ | ++ | - | - | + |
| M | Light brown | Mango bark, “Typhoid” bark | + | - | ++ | ++ | ++ | ++ | +++ | + | - | - | - |
| N | Brown | Lemon grass & Mango bark | + | + | ++ | ++ | ++ | ++ | +++ | +++ |  | - | - |
| O | Deep brown | Marketer claimed not to know | + | - | ++ | ++ | ++ | ++ | +++ | ++ | - | - | - |
| P | Pale green | Dogonyaro | + | + | ++ | ++ | ++ | ++ | +++ | ++ | - | - | - |
| Q | Brown | Seller claimed not to know but added a sachet of after 5 (Acetyl salicylic acid, caffeine & Paracetamol on the spot | + | - | ++ | ++ | ++ | ++ | +++ | ++ | ++ | ++ | ++ |
| R | Pale green | Marketer claimed not to know | + | - | ++ | ++ | ++ | ++ | +++ | - | +++ | +++ | +++ |
| S | Brown | Marketer claimed not to know | + | - | ++ | ++ | ++ | ++ | +++ | ++ | - | - | - |
| T | Brown | Marketer refused to reveal | + | + | ++ |  | ++ | ++ | +++ | ++ | - | - | - |

C.Gly=Cardiac glycoside, Sap.=Saponin, R,sug.=Reducing sugar, Flav.=Flavonoid, Ster.-Steroid, Terp.=Terpenoid, H.tan.,=Hydrolysable tannins, C.tan.,=condensed tannins, M.test= Mayers test, H.test= Hagers test, D.test= Dragendroffs test. + = Positive, ++ = Increasing Intensity, +++ = more Increasing Intensity -= absent.

**Table 2.** Zones of inhibition (mm) of herbal preparations (A –T)

| Organisms | 100% concentration of herbal preparations / zones of inhibition | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
| *Salmonella typhi* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.85±0.80 | - | - | - |
| *Escherichia coli* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2.05±0.71 | - | - | - |
| *Klebsiella pneumoniae* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2.0±0.00 | - | - | - |
| *Proteus vulgaris* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.95±0.71 | - | - | - |

- = No zone

**Table 3.** Zones of inhibition of Ciprofloxacin on the test organisms

| Ciprofloxacin concentration (µg/ml) | *Salmonella typhi* | *Escherichia coli* | *Klebsiella pneumoniae* | *Proteus vulgaris* |
|---|---|---|---|---|
| 50 | 24.50 ± 0.71 | 35.5 ± 0.71 | 41.50 ± 0.71 | 34.50 ± 0.71 |
| 25 | 23.50 ± 0.71 | 33.50 ± 0.71 | 36.50 ± 0.71 | 30.50 ± 0.71 |
| 12.5 | 22.50 ± 0.71 | 31.50 ± 0.71 | 30.00 ± 0.00 | 30.00 ± 0.00 |
| 6.25 | 20.00 ± 0.14 | 31.00 ± 0.00 | 28.00 ± 0.00 | 28.00 ± 0.00 |

**Table 4.** MIC and MBC of sample Q

| Organisms | MIC (%v/v) | MBC (%v/v) |
|---|---|---|
| *Salmonella typhi* | 25 | 50 |
| *Escherichia coli* | 15 | 50 |
| *Klebsiella pneumoniae* | 15 | 50 |
| *Proteus vulgaris* | 20 | 50 |

**Table 5.** Relative densities and pH of the herbal samples

| Sample | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| Rel. density (g/mL) | 1.02 | 1.00 | 1.00 | 1.00 | 1.02 | 1.02 | 1.01 | 1.01 | 1.01 | 1.01 |
| pH | 4.69 | 6.10 | 5.10 | 5.40 | 3.68 | 4.18 | 4.48 | 4.69 | 4.93 | 5.48 |

| Sample | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|
| Rel- density (g/mL) | 1.01 | 1.00 | 1.00 | 1.01 | 1.02 | 1.02 | 0.98 | 1.00 | 1.00 | 1.00 |
| pH | 4.06 | 4.60 | 4.80 | 4.29 | 4.15 | 3.67 | 3.90 | 4.60 | 4.30 | 4.30 |

**Table 6.** Caffeine & aspirin detection in the herbal samples

| Analyte | Retention time (min) | Substance present |
|---|---|---|
| Mixed Standard | 4.201 | Caffeine standard |
| | 7.079 | Aspirin standard |
| Sample D | 4.250, 7.083 | Caffeine, Aspirin |
| Sample F | 4.294, 7.151 | Caffeine, Aspirin |
| Sample G | 4.262, 7.066 | Caffeine, Aspirin |
| Sample J | 4.289 | Caffeine |
| Sample L | 4.295, 7.163 | Caffeine, Aspirin |
| Sample N | 4.296, 7.155 | Caffeine, Aspirin |
| Sample P | 4.285, 7.130 | Caffeine, Aspirin |
| Sample Q | 4.298, 7.142 | Caffeine, Aspirin |
| Sample R | 4.261 | Caffeine |
| Sample T | 4.287 | Caffeine |

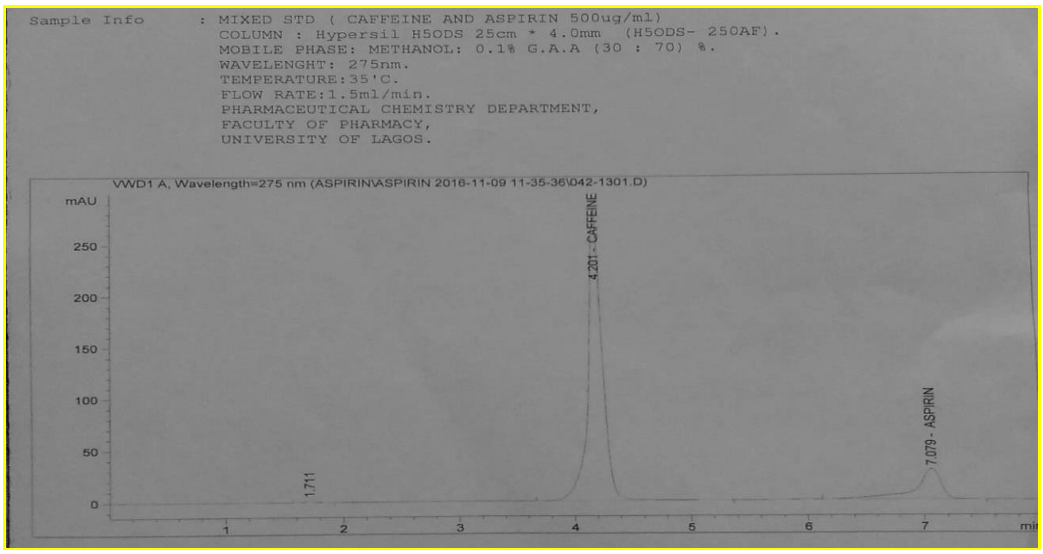


**Fig 1.** Chromatogram of mixed standard, Aspirin and Caffeine

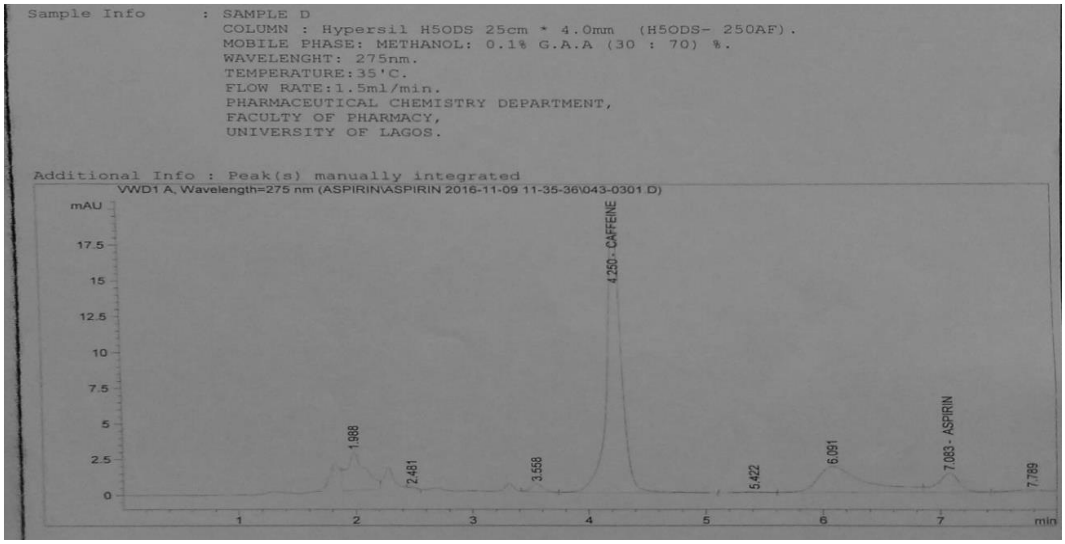


**Fig 2.** Chromatogram of sample showing presence of Aspirin and caffeine

Water was the claimed solvent of preparation by the sellers and though several phytochemicals were found present in the samples, the poor solubility of the phytochemicals in water could be one of the reasons for the poor antimicrobial activity.

**Relative densities and pH.** The relative densities and pH of the herbal samples are represented in table 5. All the samples were acidic with a pH range of 3.67 -6.07 (Table 5). Q was the most acidic sample with a pH of 3.67. Aspirin (acetylsalicylic acid) is an acidic drug, which was added to Sample Q during purchase in the form of After5®. The introduction of the orthodox drug could have influenced the lower pH value of Sample Q. The interaction between the herbal preparation and the orthodox drug might not be known. This therefore reveals potential dangers to the consumers of "*Agbo*".

**Screening for the presence of aspirin and caffeine in the ten most acidic samples using High Performance Liquid Chromatography (HPLC).** Sample Q alongside nine other samples with lower pH values were screened using HPLC for the

presence of aspirin and caffeine, which are active ingredients in the drugs: After5® and Alabukun®. These drugs (After5® and Alabukun®) are in powdered dosage forms and are dispensed in sachets; they are cheap, available and accessible, thus can easily be purchased especially from patent medicine stores and unauthorized drug sellers. They could therefore have been added to the samples due to their cheap nature, and the ignorant notion of the sellers to potentiate medicinal action of the '*Agbo*' samples as was openly exhibited during the purchase of sample Q. Table 6 represents the drug substances, aspirin and caffeine present in the analytes based on retention times. Sample chromatograms of the standard (mixed aspirin and caffeine) and one of the samples are represented in Fig 1 and 2. Caffeine was found in all the samples investigated. A total of 7 out of the 10 samples investigated contained caffeine and aspirin as presented in table 6. Aspirin detected from the samples could have been introduced through the addition of an orthodox drug. Caffeine could have been introduced from a plant used in the herbal preparation or addition of a tea bag during the preparation of the samples. Aspirin is a synthetic drug and its presence in the herbal preparation could not have originated from constituent herbs. The only closely related compound to it is salicylic acid or salicin, which can be found in plants.

The danger of adding orthodox drugs to herbal remedies is seen in the possible drug-drug interaction. Most herbs can potentiate the effect of anticoagulant drugs such as warfarin, and antiplatelet drugs such as aspirin. [28]. Aspirin has a great potential for causing effects resulting from inhibition of cyclooxygenase. However, interactions may occur with herbal supplements whose actions involve the production of prostaglandins and / or thromboxanes. In addition, aspirin is highly plasma protein-bound and this may further predispose it to possible interactions with herbs that share this property, although such interactions have not yet been documented in the literature. [29].

**Conclusion**. Although the herbal preparations "*Agbo* typhoid" showed an array of phytochemicals, cautions should be exercised in the consumption since they were found inactive against the causative organism for typhoid fever, *Salmonella typhi*. Introduction of orthodox drugs to herbal remedies is unacceptable since there could be unhealthy interactions. The presence of caffeine and aspirin in "*Agbo*" could be deleterious to health.

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