Physical And Phytochemicals Study Of Some Local Herbal Remedies

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Abstract: Herbal preparations have been used for an immemorial period for physiological and psychological well-being. Phytochemicals are attributable for such effects. The quality index of herbal remedies include among some parameters, phytochemicals, ash and moisture content. Analyses were carried out by ignition method to determine the moisture, ash value, acid insoluble ash and water soluble ash of herbal remedies produced in Northern Nigeria with the aim to ascertain their quality. Also, standard methods were used to determine concentrations (g/100g) of alkaloids, flavonoids, phenolics, saponins and tannins. The moisture, ash value, acid insoluble ash and water soluble ash showed range of 3.55-8.58, 3.9-43.92, 0.69-86.47 and 0.19-30.75% respectively. All the samples have favourable values for the moisture, 27.59% of the total samples have their total ash content greater than 14% which is recommended value by European Pharmacopoeia maximum acceptable limit. The remedies contain the phytochemicals in the range of (0.011-7.06, 2.76-33.47, 0.025-1.305, 0.277-8.701 and 0.037-2.07) g/100g respectively. Most of the samples may be deemed qualitative based on these indices.

Keywords: Ash, Herbal remedies, Phytochemicals, Quality, Moisture,

I. Introduction

Herbal remedies are often used for treatment of various ailments. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care need[1] (Akerele, 1993). The therapeutic effects of herbs are associated with their phytochemicals content, which are products of metabolism activities in the plants. They are also called secondary metabolites and exhibit anti-oxidation (which infer delay or inhibit the oxidation of biomolecules by regulation of oxidative chain reactions), antimicrobial, anti-mutagenic, anti-carcinogenic, anti-proliferative and vasodilatory properties [2,3] (Agbo and Mboto 2012, Okeniyi, et al 2012). However, the paradox with these phytochemicals like any other substance is that they tend to be toxic at certain concentrations. A study reported clear correlation between the herb Crotalaria and liver diseases for its high concentrations. A study reported clear correlation between the herb Crotalaria and liver diseases for its high content of pyrrolizidine alkaloid [4] (Bras et al, 1961). More worrisome is that these herbs are seldom standardized and the situation become more adverse as the knowledge of herbs is kept within family with sanctity and secrecy[5] (Bhat et al, 2014).

To ensure quality for vegetable raw material, the WHO, (1992)[6] recommends that the pharmacopoeia specifications for the plant material must include among others, determination of moisture, total ash, acid insoluble ash and phytochemicals (active constituents).

The residue remaining after incineration of plant material is the total ash content or ash value. It represents both physiological ash and non-physiological ash. Physiological ash is derived from plant tissues due to biochemical processes while non-physiological ash consists of residue of the extraneous matter (such as sand, soil, etc) deliberately or non-deliberately adhering to plant samples itself. Physiological ash gets dissolved in the dilute acid while some of the non-physiological ash remains undissolved [7] (Shellard, 1958). Total ash may compose of carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were taken up from the soil or environment[8] (Kunle, 2000).

Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth in the samples. The values also indicate the magnitude of presence of oxalates, carbonates, phosphates, oxides and silicates respectively. Thus, the values are indices of quality of herbal remedies.

In Northern Nigeria as elsewhere, most herbal remedies are not registered with the regulatory body, the National Agency for Food and Drug Administration and Control (NAFDAC) and are not regulated. Hence, the aim of this study is to determine these parameters to substantiate the quality of the herbal remedies.
II. Materials And Methods

All glassware were scrupulously cleaned with 10% HNO₃ rinsed several times with deionised water and air-dried. Reagents of analytical grades were used except otherwise stated. A total of 29 samples, 6 packaged and 23 unpackaged were purchased from wholesalers herbalists in Kano, Chiranci, Maiagatari, Kawo Kaduna and Zaria towns of Kano, Katsina, Jigawa and Kaduna States, Nigeria respectively.

2.1 Total ash

About 3 g of the ground air-dried sample was weighed (W₁), into a previously ignited and weighed (W₁) crucible. Sample was ignited gradually in an electrical muffle furnace TT-EF Techmel and Techmel USA, increasing the heat to 500–600 °C until it is white, indicating the absence of carbon. It was cooled in desiccators and reweighed (W₂).

Total ash content was calculated as in equation 1:

\[
\text{Total ash (\%)} = \frac{(W_2 - W_1)}{W_1} \times 100 \quad (\text{equation 1})
\]

2.2 Acid-insoluble ash (silica & sand content)

10 ml of 2 M HCl was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was washed with 5ml of hot water and the washings were added to the crucible. The insoluble matter was filtered on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate and ignited to constant weight (W₃). The residue was allowed to cool in desiccators for 30 minutes, and then weighed. Total ash content was calculated as in equation 2:

\[
\text{Acid insoluble ash (\%)} = \frac{(W_3 - W_2)}{(W_3 - W_1)} \times 100 \quad (\text{equation 2})
\]

2.3 Water-soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ash less filter-paper. The filter was washed with hot water and then ignited in a crucible for 15 minutes at a temperature not exceeding 450 °C. The residue was allowed to cool in desiccators for 30 minutes, and then re-weighed (W₄), calculations were done according to equations 3-5:

- Weight of residue, W₆ (g) = W₄ - W₁ (equation 3)
- Weight of ash W₅ = W₃ - W₁ (equation 4)
- Water-soluble ash (mg/g) = W₅ - W₆ (equation 5)

(WHO, 2011) 9

2.4 Moisture (loss on drying)

About 3g of the air-dried sample was weighed (Wa), into a pre-dried and weighed (Wₐ) crucible. The sample was dried in an oven at 100-105 °C until two consecutive weighings (Wc) do not differ by more than 5mg. The percent loss of weight of air-dried sample was calculated by equation 6:

\[
\text{Water and volatile matter (\%)} = \frac{(W_b - W_a)}{(W_b - W_a)} \times 100 \quad (\text{equation 6})
\]

2.5 Alkaloids

5g of the herb sample was measured in a 300ml beaker and 200ml of 10% ethanoic acid in ethanol was added. The mixture was covered and allowed to stand for 4 h. The mixture was filtered and the extract was allowed to become concentrated in a water bath until it reaches about 50ml of the original volume. Conc. ammonium hydroxide was added drop wise until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid, which was dried and weighed.[10, 11] (Harborne, 1973; Obadoni and Ochuko, 2001)

2.6 Saponins

20 g of each sample was measured into 250 ml conical flask and 100 ml of 20% aqueous ethanol was added. The sample was heated at about 55°C over a hot water bath for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was then transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added to the extract and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process repeated. 60 ml of n-butanol was added and the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was then heated in a water bath and after evaporation; the sample was dried in the oven to a constant weight.
2.7 Flavonoids

10 g of herb sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through filter paper. The filtrate was transferred into a pre weighed crucible then placed on water bath to evaporate the solution to dryness. The residue was weighed to a constant weight. [12] (Boham and Kocipai, 1994)

2.8 Total phenols

2g of sample was defatted with 100ml of diethyl ether for 2hr using a soxhlet apparatus. The fat free sample was boiled with 50ml of ether for 15 min to extract the phenolic component. 5ml of the extract was measured into 50ml volumetric flask, followed by 10ml of distilled water. 2ml of ammonium hydroxide solution and 5ml of pentanol were added. The solution was made up to mark and left to react for 30min for color development. The absorbance of the solution was read using spectrophotometer at 505nm wavelength [13] (Okwu and Josiah, 2006)

2.9 Tannin

1.0 g of air dried sample was dispersed in 10 ml distilled water and stirred. The mixture was left to stand for 30 min at room temperature being shaken every 5 min. After the 30 min, it was centrifuged and the supernatant extracted. 2.5 ml of the extract was pipetted into a 50 ml volumetric flask. Also, 2.5 ml of standard tannic acid was measured into a separate 50 ml flask. Both flasks were treated with 1.0 ml Folin-Dennis reagent followed by 2.5 ml of saturated Na₂CO₃. The mixture was diluted to the 50 ml mark and incubated for 90 min at room temperature. The absorbance was measured at 725 nm in a Jenway 6000 Electronic Spectrophotometer.

\[
\text{Tannin} \% = \frac{\text{An} \times \text{C} \times 100 \times \text{Vf}}{\text{As} \times \text{W} \times \text{Va}}
\]


III. Result And Discussion

Data on the parameters studied was computed and presented as follows;

<table>
<thead>
<tr>
<th>PROPERTY/SAMPLE</th>
<th>MOISTURE (%)</th>
<th>TOTAL ASH (%)</th>
<th>ACID INSOLUBLE (%)</th>
<th>WATER SOLUBLE (%)</th>
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<td>RANGE</td>
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<td>3.9-43.92</td>
<td>0.69-86.47</td>
<td>0.19-30.75</td>
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</table>
3.1 Moisture

Moisture is one of the major factors responsible for the deterioration of drugs and herbal formulations. The moisture promotes the degradation processes caused by enzymes, development of micro organisms, oxidation and hydrolysis reactions. This study recorded moisture range of 3.55-8.58% which is deemed to be good as water content in vegetable drugs should not be greater than 14%. (www.intechopen.com) [15]

3.2 Ash values

Total ash values for this study ranges 3.9–43.92%. However, the values are unevenly distributed with only samples 14 and 28 having values of greater than 20%. The maximum value of this study is greater than 4.18 to 14.47% reported by [16] Chandel et al, (2011). Also, samples 5, 9, 13, 14, 22, 26, 28 and 29 which represents 27.59% of the total samples have their values greater than 14% which is the maximum acceptable limit recommended by European Pharmacopoeia[17] (Vaikosen et al, 2011). A high ash value is indicative of contamination, substitution or adulteration by minerals. The high value of the three samples may be attributed to addition of minerals into the plant formulations deliberately or otherwise.

3.3 Water soluble ash

Water-soluble ash is the part of the total ash content, which is soluble in water. This study showed a range of 0.19-30.75%. However, the maximum value is due to a deviation shown by sample 28 with rest of the samples ranging 0.19-9.13%.

3.4 Acid insoluble ash

It is in the range of 0.69-86.47% even though the range was widened by samples 28 and 29 having 32.48 and 86.47% respectively. The remaining samples range 0.69-17.30%. The high values may be due to formulation containing a mineral material as the composition is undisclosed. In fact, 26 samples were not packaged while those packaged were not labeled with composition.

Phytochemicals

Data on the phytochemical content in the herbs studied are presented in figures 1-5.
The result shows that all the samples contain the five phytochemicals in varied concentrations with highest concentration occurring in samples 11, 10, 16, 29, 26 while lowest were in samples 10, 25, 13, 21, 21 for tannin, saponin, alkaloid, flavonoid and total phenol respectively. The least concentration of 0.01g/100g is recorded by alkaloid sample 13 while the highest concentration 33.47g/100g was of flavonoid at sample 29. Flavonoids were shown to be at higher concentrations as even its lowest concentration is higher than the highest of tannin, saponin and phenol.

A report revealed mean phytochemicals content as alkaloid (7.270±0.009%) and flavonoids (18.23±0.040%) in Stachytarpheta angustifolia, saponins (2.500±0.014%) and total phenols (1.250±0.009%) in Anisopus manni. [18] (Aliyu et al, 2008)

These values are all within or close to the range of this study. The presence of these important phytochemicals in all samples signals their therapeutic potential. They may function in stimulating digestion, act as anti-inflammatory reducing swelling and pain, antioxidant and venotonics, antibacterial and antifungal, diuretic property that enhance the elimination of waste products and toxins and enhancing mood to give a sense of well being [19] (Fakim, 2006)

IV. Conclusions

The physical studies for this research has shown that a greater percentage of the samples are within recommended level, therefore deemed to be good as per those indices. However, the high acid insoluble ash value for some samples is an indication of non physiological content which could be an adulteration or unintentional contamination. Also, the study has shown that all the herbal formulations contain phytochemicals which are attributable for a spectrum of cure. Therefore, further pharmacological study is needed to ascertain the actual forms and dosage of the phytochemicals responsible for the claimed therapeutic effects and safety of the herbal remedies.

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