



Preliminary Screening of Selected Phytochemicals and Analysis of Essential Trace Elements in *Boswellia serrata* Roxb, *Prosopis africana* and *Sclerocarya birrea*: Plants with Therapeutic Potentials to Treat Hemorrhoids

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Abstract The use of herbal medicines is popular worldwide with about 80% of the world population relying on herbal medicines for their primary healthcare. Plants are an important source of bioactive substances and many essential trace elements that play vital roles in general human well-being. **Objective:** The objective of this study is the preliminary screening of selected phytochemicals and analysis of essential trace elements in *Boswellia serrata* (Roxb), *Prosopis africana* (Guill & Perr) and *Sclerocarya birrea* (A.Rich) Hochst. The study also discuss the overall impact of Fe, Zn, Mn and Co and the selected phytochemicals on human health as the possible scientific basis for using the three popular medicinal plants in Zaria with therapeutic potentials to treat piles (Hemorrhoids). **Methodology:** The three most popular herbal medicines in Zaria obtained from stem barks of *Boswellia serrata* Roxb, *Prosopis africana* (Guill & Perr) and *Sclerocarya birrea* (A.Rich) Hochst were screened for alkaloids, saponins, terpenoids, phlobatannins, tannins, steroids and flavonoids using standard methods and iron, manganese, zinc and cobalt were analysed a long side reference standard material IAEA 1515 (apple leaves) using instrumental neutron activation analysis (INAA) technique. **Results:** Alkaloids, saponins, terpenoids, phlobatannins, tannins and steroids except flavonoids were detected in the three popular herbal medicines. The concentrations of Fe ranged between 87.90±11.7 and 652±21mg/kg, Mn, 15.7±0.7 and 49.70±1.40 mg/kg; Zn, 2.90±10 and 8.08±1.21 mg/kg and Co, 0.045±0.05 and 0.16±0.01 mg/kg. The concentrations of essential trace elements in the herbal medicines were in the increasing order of *Prosopis africana* > *Boswellia serrata* > *Sclerocarya birrea* and the magnitude of essential trace elements was Fe > Mn > Zn > Co. **Conclusion:** These results suggest potential efficacy of the three popular herbal medicines to be used in treating human ailments.

Key words: essential trace elements, herbal medicinal preparations, instrumental neutron activation analysis, therapeutic potential, piles.

الفحص الأولي للكيمياويات النباتية المختارة وتحليل العناصر النزرة الأساسية في

:*Prosopis africana* , *Sclerocarya birrea* & *Boswellia serrata* Roxb

النباتات ذات الإمكانيات العلاجية لعلاج البواسير

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المخلص يشيع استخدام الأدوية العشبية في جميع أنحاء العالم ، حيث يعتمد حوالي 80% من سكان العالم على الأدوية العشبية للحصول على الرعاية الصحية الأولية. تعد النباتات مصدراً مهماً للمواد النشطة بيولوجياً والعديد من العناصر الفاعلة الأساسية التي تلعب دوراً حيوياً في رفاهية الإنسان بشكل عام. وتهدف هذه الدراسة لفحص المواد الكيميائية النباتية للنباتات المختارة وتحليل العناصر النشطة الأساسية في *Sclerocarya birrea* (A.Rich) Hochst و *Prosopis africana* (Guill & Perr) و *Boswellia serrata* (Roxb). وتناقش هذه الدراسة التأثير الكلي ل Fe و Zn و Mn و Co والمواد الكيميائية النباتية وتأثيرها على صحة الإنسان كأساس علمي ممكن لاستخدام هذه النباتات الطبية الثلاثة الشائعة في Zaria وإمكانه استخدامها في علاج البواسير. الأدوية العشبية الثلاثة الأكثر شيوعاً والتي تم الحصول عليها في زاريا من لحاء ساق بوسويلياسيراتا روكسب ، وبروسوبيسافريكانا (Guill & Perr) و *Sclerocarya birrea* (A.Rich). وتم تحليل المنغنيز والزنك والكوبالت مع مواد قياسية مرجعية تستخدمها الوكالة الدولية للطاقة الذرية (1515) باستخدام تقنية تحليل تنشيط النيوترونات في أوراق التفاح (INAA). وتم اكتشاف فلويدات ، سابونين ، تيربينويدس ، فلوباتانين ، تانينات وستيرويدات باستثناء الفلافونويدات في الأدوية العشبية الثلاثة. تراوحت تركيزات Fe بين 87.9 ± 11.7 و 652 ± 21 mg / kg ، Mn ، 15.7 ± 0.7 و 49.70 ± 1.40 mg / kg ، الزنك ، 2.90 ± 10 و 8.08 ± 1.21 mg / kg و Co ، 0.045 ± 0.05 و 0.16 ± 0.01 mg / kg. كانت تركيزات العناصر النشطة

الأساسية في الأدوية العشبية بالترتيب المتزايد لـ *Prosopis africana* < *Boswellia serrata* < *Sclerocarya birrea* وحجم العناصر النشطة الأساسية كانت $Co < Zn < Mn < Fe$.

الكلمات المفتاحية: العناصر المؤثرة الأساسية، المستحضرات الطبية العشبية، تحليل تنشيط النيوترونات، الإمكانيات العلاجية، البواسير.

INTRODUCTION

The information about the traditional uses of *B. serrata* Roxb, *P. africana* (Guill& Perr.) and *S. birrea* (A.Rich) Hochst with their Hausa names; Arrabi, Kirya, and Danya and English names as Indian Olibanum, African mesquite and cedar trees respectively obtained from an earlier survey [18] is shown in Table (1). The three selected plants have the treatment of piles common to all of them and their traditional uses in this work corroborated the findings in literature as cited in Table 1. The administration of the herbal medicinal preparations obtained from medicinal plants of *B. serrata*, *P. africana* and *S. birrea* used in Zaria to treat different ailments is indicated in Table 1 in which half-cup of powdered decoction of the stem bark of *B.serrata* is usually administered two times daily for the treatment of both piles and cancer.

One spoonful of powdered *P. africana* in one cup of water taken daily is indicated for treating pile. While one spoonful of powdered *S. birrea* powder taken with food once daily is indicated for treating piles [18].

The use of herbal medicine has increased worldwide and the market is rapidly expanding at about \$60 thousand million annually [1]. It has been estimated that approximately 80% of the world population relies on herbs for their primary health care especially countries in Africa and Asia [2]. Presently, scientists seek evidence to substantiate the use of herbal medicines in primary healthcare through the evaluation of phytochemical and trace elements contents of medicinal plants. Phytochemicals include primary metabolites consisting of carbohydrates, amino acids, proteins and chlorophyll in addition to secondary metabolites that are a source of bioactive substances [3]. Plant secondary metabolites are grouped by their chemical nature into; alkaloids, saponins, terpenoids, tannins, flavonoids, polyphenols and the like that are synthesized by the plant in response to environment challenges from herbivores, insects, bacteria and fungi, while having important functions in human health [4, 5].

Essential trace elements are numbered amongst heavy metals; they occur naturally as components of the earth's crust and can be redistributed throughout the environment. However, the distribution of heavy metals in the environment is not even; some soils have higher amounts of such elements. The differences in amounts found in soils maybe due to natural processes or due to anthropogenic activities such as mining, power generation, vehicular exhaust, use of insecticides, steel manufacturing and production and use of chemical fertilizers [6]. Essential trace element uptake by plants is determined by metal mobility and bioavailability and this is the main pathway of metal transfer from sediments and water to the food chain. Biologically, essential trace elements act as potential candidates for combating metabolic disorders including diabetes, by serving as

cofactors of antioxidative enzymes that play an important role in removing reactive oxygen species (ROS) [7].

Trace element is any chemical element required by an organism for healthy growth. It may be required in large amounts (macronutrient) or in very small amounts (trace element) for example Fe, Co, Zn and Mn [8]. For healthy life, humans require varying amounts of essential elements [9]. Essential elements are commonly found in foodstuff, in fruits and in vegetables and in commercially available multivitamins [10]. It has been established that many essential trace elements play vital roles in general human well-being as well as curative roles in human diseases [11, 12]. Essential elements are present in small amounts in herbal medicines; hence a sensitive and reliable technique such as neutron activation analysis (NAA) is needed for their quantification. Instrumental Neutron Activation Analysis is a technique used to determine non-destructively large number of elements in different matrices. The principle involves the n-gamma reaction, which is the fundamental reaction for neutron activation analysis. The gamma rays emitted during the decay of the nucleus have specific energies that are characteristic for the nuclide in question [13].

MATERIALS AND METHODS

Sample collection

The stem barks of *B. serrata*, *P. africana* and *S. birrea* were randomly collected from the wild between the months of January and April, 2018 and plants from which samples were collected were taken to the herbarium in the Department of Botany, Faculty of Life Sciences Ahmadu Bello University Zaria for authentication and voucher numbers recorded.

Sample preparation for phytochemical analysis

The stem backs were dried in room-shade for four days and ground into powder using wooden pestle and mortar.

Aqueous extracts of each powdered herbal medicinal preparation were obtained by soaking 10g of the powder in 200 ml of distilled deionised water for 12 hours with shaking at 150 rpm on a Burrell Scientific Linear orbital shaker SN KS501. The solutions were filtered using Whatman filter paper No1 and evaporated to dryness on Clifton water bath set at 100° C. One (1g) of each dried crude extract was re-dissolved in 5 ml distilled deionized water for the phytochemical screening using standard methods [14, 15]. Standard deviation of standard analysed and its certified values were calculated to ascertain the accuracy of the method.

Test for Tannins

Lead acetate Test

Reagent: Lead acetate (25% w/v).

Preparation: Weigh 25g of lead acetate and dissolve in sufficient distilled de-ionised water in a 100 ml volumetric flask add few drops of 4% NaOH (4g NaOH in 100ml water) make the volume up to mark with distilled de-ionised water. Few drops of the basic lead acetate were added to 2 ml of the plant extracts; the presence of tannins was indicated by a precipitate

FeCl₃ Test

Reagent, FeCl₃ (1%)

Preparation: Fe Cl₃(1%) weigh and dissolve 1g of FeCl₃ in sufficient water contained in 100ml volumetric flask, make volume up to mark with distilled de-ionised water.

Few drops of FeCl₃ (1%) were added to 2 ml of aqueous solution of plant extracts; the presence of a brown-green or blue -black coloration was indicative of a positive test for tannins.

Test for Saponins

Frothing Test

Reagents: water and olive oil

To 10 ml of aqueous solution of the plant extracts contained in boiling tubes were added 5 ml distilled deionised water, the tubes were vigorously shaken. The formation of stable froth and emulsion after the addition of olive oil indicated positive result for Saponins.

Heamolysis Test.

To the plant extracts were added 0.2 ml of whole mammalian blood in a test tube. This was mixed and allowed to stand for about ten minutes. The reduction in intensity of colourization due to breakdown of red blood cells was indicative of positive result for the presence of saponins when compared to control containing only blood and water.

Test for Steroids.

Liebermann-Burchard Test

Reagents: Acetic anhydride, conc. H₂SO₄ and glacial acetic acid

Preparation: Use chilled acetic anhydride and H₂SO₄.

To 500ml amber glass tube fitted with polyseal cap, add 220 ml of cold acetic anhydride and 200 ml of glacial acetic acid (room temperature). Mix and add 80 ml of concentrated sulphuric acid to make a total of 500ml.

Dissolved were (2g) of sample in 10 ml chloroform. The solution was filtered into a test tube. The development of a greenish colour when 2 ml of the chloroform extract were treated with acetic anhydride, concentrated sulphuric acid and acetic acid mixture was indicative of a positive for test for steroids.

Salkowski's test

Reagent: 0.5M FeCl₃ (27 g FeCl₃ dissolved into sufficient water in 100ml volumetric flask make up volume to the mark with distilled deionised water), Sulphuric acid and perchloric acid.

Preparation: In a fume hood mix 2 ml of 0.5 M FeCl₃ and 49 ml of water and 49 ml of 70% v/v perchloric acid (70 ml of perchloric in 100 ml volumetric flask, make up volume to the mark with distilled water). A red colour produced in the lower chloroform layer when 2 ml of organic extract

dissolved in 2 ml of chloroform to which 2 ml concentrated sulphuric acid were added indicated the presence of steroids.

Test for Flavonoids

NaOH Test

Reagents: NaOH (5%w/v) and HCl (10%v/v)

Preparation: Take 5 g of sodium hydroxide pellets dissolve in sufficient distilled deionised water contained in 100ml volumetric flask make up volume to mark with water. For 100 ml (10% v/v) HCl, take 10 ml of conc HCl and add 90 ml of distilled water. Into a 10 ml test tube were placed 2 ml of the plant extracts to which were added 2 ml of 5% NaOH followed by 2 ml of 10% HCl. A yellow solution that turned colorless when HCl was added was indicative of the presence of flavonoids.

Shinoda's Test

Reagents: Magnesium ribbon and conc. HCl

To 5ml portion of aqueous extracts were added 5ml of 40% dilute ammonia solution followed by the addition of concentrated sulphuric acid (H₂SO₄). A yellow coloration that disappeared on standing indicated of presence of flavonoids.

Test for Alkaloids

Mayer's Test

Reagents: Marquis Reagent:

This was prepared by the addition of 100 ml of concentrated H₂SO₄ acid (95%v/v): 100 ml were prepared by carefully adding 96.94 ml of concentrated acid to 100 ml of water) to 5 ml of formaldehyde (Formaldehyde to prepare 5 ml of formaldehyde solution in 100-ml Pyrex screw-cap tube 1.85 g of paraformaldehyde were weighed and 3.5 ml of H₂O were added into a capped tube and the cap was loosely capped and place in a boiling water bath.

Then, 90 µl of 1 N NaOH was added and mixed for ~1 min. The solution became nearly clear. This was cooled by running the bottom of the tube under a stream of tap water).

The solution was filtered through a non-sterile 0.22-µm syringe into a clean screw-cap airtight vial. Then, into a 10 ml test tube were added 3ml of plant extracts to which were added a few drops of Mayer's reagent, the appearance of buffy coloured precipitate was taken as positive test for presence of alkaloids.

Dragendoff's test

Reagents: Bismuth nitrate 1.7 g of basic bismuth nitrate, 20 g of tartaric acid, 16 g potassium iodide and (10% w/v) picric acid (dissolve 10 g of picric acid in distilled water make the volume to mark in a 100ml volumetric flask)

Preparation: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. This solution was mixed with a solution containing 16 g of potassium iodide and 40 ml of water and diluted 10 times with 10% picric acid before use.

Aqueous extract (2 ml) were added to 2 ml 95% ethanol (95 ml of ethanol made up to 100 ml v/v) and few drops of Marquis Reagent (consisting of a mixture of formaldehyde and conc. H₂ SO₄, ratio

5:20). The formation of yellow precipitate indicated a positive test.

Test for Phlobatannins

Reagents: Aqueous solution HCl (1%v/v)

Preparation: 100 ml of HCl(1%v/v) was prepared by taking 1 ml of HCl into 100 ml volumetric flask and the volume was made up to the mark with distilled water.

Aqueous extract (2 ml) were boiled in 1% aqueous solution of HCl. The presence of precipitate indicated the presence of phlobatannins.

Test for Terpenoids:

Reagents: Conc. H₂SO₄, methanol, chloroform

Preparation: In a test tube was placed 0.8g of sample then 10 ml of methanol were added, mixed and filtered into a test tube. To 5ml of the methanol extract were added 2ml of chloroform and 3ml of concentrated H₂SO₄, a reddish -brown coloration was indicative of the presence of terpenoids.

Elemental Analysis

Neutron activation analysis was carried out on samples of *B. serrata*, *P. africana* and *S. birrea* using NIRR-1(Nigeria Research Rector -1) at the Centre for Energy Research and Training, Ahmadu Bello University, Zaria. Polyethylene films and rabbit capsules with dimensions 25mm x 45 mm were cleaned by soaking in 1.1N HNO₃ for 3 days and washed with de-ionized water. Two hundred (200 mg) each of the samples and certified reference material IAEA -1515 (apple leaves) were weighed and wrapped in clean polyethylene films. The polyethylene films were heat sealed and labelled. The calibration parameters for all the elements were determined using adopted procedures [16]. For Fe, Co and Zn the samples were irradiated at 5×10^{11} n / cm for 6 hours and allowed to decay for 10-15 days and then counted for 60 minutes. For the determination of Mn the samples were irradiated at 1×10^{11} c/ cm for 2 minutes and allowed to decay for 3-4 hours then counted for 10 minutes.

Preparing samples for radioactive assay and counting

After activating the samples, they were taken out of the rabbit capsule and the polyethylene seal was wiped with wet tissue paper and mounted on flexi glass plate the resulting gamma ray energies and intensities were determined using a solid-state High Purity Germanium (HPGe) detector. The gamma activity was measured with High Purity Germanium (HPGe) detector. The HPGe detector was coupled to a multichannel analyser.

The detector has a resolution of 1.9 KeV at 13332KeV of ⁶⁰Co.

The gamma rays passing through the detector generated free-electrons. The number of electrons (current) is related to the energy of the gamma ray. Given the differences in half-lives for various nuclides, there were optimum times to count an activated sample. Gamma ray spectra were accumulated in live time mode with dead time maintained at less than five percent, this was achieved by placing the sample at either outer or inner irradiation channels for long and short irradiation times respectively.

The NIRR-1 is a Miniature Neutron Source Reactor (MNSR) that is a low- power nuclear reactor with highly enriched Uranium as fuel, light water as moderator and Beryllium as reflector. The whole system consists of a horizontal dip-stick, high Purity Germanium (HPGe) detector with a relative efficiency of 10% at 1332.5 KeV gamma ray line, MAESTRO emulation software compatible with the ADCAM ®Multi-Channel Analyser (MCA) card and the associated electronic modules all made by EG & ORTEC that is interfaced with a Personal Computer. The efficiency curves of the detector system at near and far source detector geometries were determined by standard gamma -ray sources in the range of 59.5-2254KeV and extended to 4000KeV. The data processing and the gamma ray spectral peak areas were analysed using WINSPAN 2004 software [17]. The software requires that calibration factors be predetermined by a multi-element standard reference material for elements of interest using adopted irradiation and counting regimes.

RESULTS AND DISCUSSIONS

Results

The result for the phytochemical analysis is indicated in Table 2 in which *B. serrata* Roxb, *P. africana* (Guill & Perr.) and *S. birrea* (A. Rich) Hochst contained tannins, phlobatannins, saponins, steroids, alkaloids and terpenoids except flavonoids that are absent in all the three powdered herbal medicinal preparations.

Table 1: Information on the four selected herbal medicines used in folklore medicine in Zaria [source: 18]

Name	Local name	Method of preparation	Local uses	Route of administration	Traditional Uses cited in Literature	Dose
<i>Boswellia serrata</i>	Indian frankincense	Decoction	Treating Piles and cancer	Oral	Anti-inflammatory, pain, Rheumatoid arthritis, headaches and cancer ¹⁹	½ cup 2x daily
<i>Prosopis africana</i>	African Mesquite	powder	Treating piles, general body pains, toothaches and burns	Oral for piles, topical for general body pains and burns and filling for toothaches	Condiment, headaches, toothache, Rheumatism, fevers, skin disease, eyewash, diuretic, gonorrhoea, stomach ache, sore throat, malaria and treatment of wounds ²⁰	1 cup daily for piles, fill cavity 1x daily and apply topically once daily for pains
<i>Sclerocarya birrea</i>	Marula or cider tree	powder	Piles	Oral	Treatment of fever, boils, diarrhoea, headaches, malaria, dysentery, syphilis, hepatitis, rheumatism, leprosy and snake bites ²¹	Spoonful of powder with food once daily

Table 2: Result of Phytochemical Analysis of Herbal Medicines obtained from the Barks of three Medicinal Plants

S/N	Phytochemical Test	<i>Boswellia serrata</i> herbal medicine	<i>Prosopis africana</i> herbal medicine	<i>Sclerocarya birrea</i> herbal medicine
1	Test for Tannins (i)Lead acetate Test (ii)FeCl ₂ Test	+	+	+
2	Test for Saponins (i)Frothing Test (ii)Heamolysis Test	+	+	+
3	Test for Steroids (i)Lieberman-Burchard Test (ii)Salkowski's Test	+	+	+
4	Test for flavonoids (i)NaOH Test (ii)Shinoda's Test	-	-	-
5	Test for Alkaloids (i)Mayer's Test (ii)Dragendoff's Test	+	+	+
6	Test for Phlobatannins	+	+	+
7	Test for Terpenoids	+	+	+

Key:

+ = Presence of constituents

- = Absence of constituents

The use of instrumental neutron activation analysis offers a sensitive non-destructive method of analysis. Herbal medicines have the potentials to address many human metabolic ailments through bioactive principles and essential trace elements in their preparations [22, 23]. The essential trace elements in herbal medicinal preparations obtained from *B. serrata*, *P. africana* and *S. birrea* analysed by instrumental neutron activation analysis are indicated in Table 3. The concentrations of the essential trace elements in herbal medicinal preparation obtained from stem bark of *B. serrata* were in the range of 0.056 to 87.9 mg/kg, *P. africana* 0.045 to 112.1 mg/kg and *S.*

birrea 0.16 to 652 mg/kg. The concentration of each essential trace element detected in the three herbal medicinal preparations ranged between 87.90±11.7 and 652±21mg/kg Fe, 15.7±0.7 and 49.70±1.4 mg/kg Mn, 2.90±10 and 8.08±1.21 mg/kg Zn, and 0.045±0.05 and 0.16 ±0.04 mg/kg Co.

The concentrations of essential trace element of IAEA 1515 standard determined by NIRR-I as compared to the certificate of analysis values had standard deviations of 41.72, 2.70, 1.90 and 0.02 for Fe, Mn, Zn and Co respectively

Table 3: Essential trace elements in *Boswellia serrata*, *Prosopis africana* and *Sclerocarya birrea* herbal medicinal preparations (mg/kg)

Trace element	<i>Boswellia serrata</i> (mg/kg)	<i>Prosopis africana</i> (mg/kg)	<i>Sclerocarya birrea</i> (mg/kg)	IAEA 1515 determined values (mg/kg)	IAEA 1515 certificate values (mg/kg)	SD	[22,23] Maximum permissible Limits in medicinal plants
Fe	87.9±11.7	112.1±14.0	652±21	142±0	83±5	41.72	1239
Mn	35.80±2.10	49.7±1.40	15.7±0.7	57.9±1.4	54±3	2.70	339
Zn	5.59±1.25	8.09±1.21	2.90±10	9.75±2.42	12.5±0.3	1.94	27.4
Co	0.056±0.01	0.045±0.05	0.16±0.04	0.06±0	(0.09)	0.02	NM
Total	132.346	169.935	670.76	209.71	149.59		

Key:

SD=Standard deviation

NM=Not mentioned

Discussion

Three popular herbal medicinal preparations for treating piles were screened for saponins, alkaloids, phlobatannins, steroids, terpenoids, tannins and flavonoids in which only flavonoids were absent from all the medicinal plants screened. [24] screened seeds and pods extracts of *P. africana* for the presence of secondary metabolites and found that the seed extract contained saponins,

alkaloids, phlobatannins, steroids, flavonoids and tannins, the pod extract had the other secondary metabolites but no flavonoids just as the stem bark of the *P. africana* (Guill & Perr.) in this study. Some of these phytochemicals found in the present study are saponins, which are widely distributed in the plant kingdom and occur in different plant species for the good of man [25]. They are usually stored in plant cells as inactive precursors, but

when pathogens attack the plant, plant enzymes readily convert the inactive precursors into biologically active antibiotics against pathogens and pests [26]. Saponins are differentiated from other plant secondary metabolites by their surfactant properties, their biological activities include haemolytic, hepatoprotective, antimutagenic, antiviral, and anti-inflammatory [27]. Saponins in plants play the principal role of protection against insects, bacteria and fungi [28]. They are of great benefits to humans when they are processed into drugs and medicines, foaming agents, sweeteners and cosmetics [29]. Their anti-inflammatory activity may inform their use as remedies in folklore medicines against pile.

Terpenoids were detected in all the samples analysed. They are secondary metabolites with the isoprene unit as backbone. They have a C₄₀ carbon skeleton exemplified by caroteins, carotenoids and xanthophyll. This group of plant secondary metabolites comprises of more than 40,000 compounds found in medicinal plants [30]. They find use in pharmaceutical applications where they cure ailments as exemplified by artemisinin medicine for malaria and Taxol medicine for cancer; they also act against diabetes mellitus and hyperlipidaemia, anti-inflammatory, antiviral and antibacterial agents [30, 22]. The presence of these substances in the screened herbal medicines may warrant their use as remedies against piles and cancer due to their anti-inflammatory and anti-cancer activities.

Tannins were also detected in all samples. Tannins are stringent water-soluble phenolic compounds having molecular weights in the range of 500- 3000 they have bitter taste and are present in almost all plants [31]. [31] Classified tannins into three categories made up of condensed tannins (i.e. proanthocyanidins); flavonols-based compounds that release anthocyanidins at high temperatures and hydrolysable tannins, such as Callotannins and ellagitannins.

Even though tannins are regarded as antinutrients, because they decrease the utilization of minerals and vitamins while also inhibiting digestive enzymes and precipitating proteins, divalent cations and alkaloids, they are however, considered as 'health promoters' for possessing antimutagenic and anticarcinogenic potentials. Others have antimicrobial properties while expressing antioxidant and antiradical activities [32]. Their antimutagenic, anticarcinogenic, antioxidant and antiradical potentials may be what makes the selected herbal medicines popular in traditional medicine as remedies against cancer and pile.

Steroids are important for their antimicrobial properties, insecticidal, anti-inflammatory, analgesic properties, and central nervous system activities [33]; they are substances of importance and interest in pharmacy due to their relationship to sex hormones [34]. *B. serrata* Roxb, *P. africana* (Guill & Perr.) and *S. birrea* (A. Rich) Hochst contained steroids and could be used to alleviate sexual dysfunction.

Alkaloids were present in all the herbal medicinal preparations. Many of the alkali-like substances that contain nitrogen are poisonous. Some are

addictive, while others are medically used (codeine and morphine) used as painkillers). They have bitter taste; about 100 alkaloids are known. One class of alkaloids, pyrrolizidine are toxic to mammals and are the leading plant toxins found in 3% of the world's flowering plants [19]. Alkaloids are also common in Angiosperms (mono and dicotyledons) and they are present in Agaricaceae as hallucinogens and in Apocynaceae as Altonine alkaloids and Yohimbine. Their biological activities include antimalarial, anti-inflammatory, antimicrobial, aphrodisiac and insecticidal activities. Some alkaloids such as aristolochic acid from *Aristolochia* plant could induce tumours [19]. The presence of alkaloids, terpenoids, steroids, tannins and saponins due to their mentioned biological activities could be the scientific basis for their use in traditional remedy against piles, cancer, wounds, general body pains, toothaches and burns as stated in this study.

The concentrations of all the essential trace elements in the herbal medicine obtained from *P.africana* were higher than the concentrations in the herbal medicine obtained from *B. serrata* and *S. birrea* except for the concentrations of Fe and Co that are higher in *S. birrea* than those in both *B.serrata* and *P.africana* herbal medicines. The order of the concentrations of the essential trace elements in all the herbal medicines is Fe > Mn > Zn but for Co in all the herbal medicines the order is *P. africana* < *B. serrata* < *S. birrea*. The order of Fe concentration in the three herbal medicines was *S. birrea* > *P.africana* > *B.serrata*.

Iron is an important element that is found in haemoglobin, a protein molecule that carries oxygen to all cells of the body without which metabolism can slow down to accommodate low oxygen levels which may lead to constipation and indirectly lead to haemorrhoids [35].

Fe functions in maintaining healthy immune system and in the production of energy. It is a constituent of many enzymes such as the cytochrome enzymes that are involved in oxidative metabolism in the body; its deficiency can result in anaemia [36].

The order of the concentration of Mn and Zn was *Prosopis africana* > *Boswellia serrata* > *Sclerocarya birrea*. For Co the order is *Sclerocarya birrea* > *Boswellia serrata* > *Prosopis africana*. Zinc is an essential component of about 270 enzymes. It stabilizes the molecular structure of cellular components, by maintaining the cell and organs integrity through tissue repairs and wound healing [37]. The presence of Zn in the body enables the body system to help T-cells fight HIV and diabetes and its deficiency can lead to prostate and skin disorders [38].

Other essential trace elements detected in the three herbal medicines include Mn and Co. Mn functions as an enzyme activator of many enzymatic reactions in the human body and as well as a component of metalloenzymes [39]. Mn helps in the formation and activation of enzymes. It works as an oxidant to remove oxidative stress.

Co, a component of vitamin B₁₂ is very important in protein formation and nucleic acid regulation [40].

CONCLUSION

Three popular medicinal plants in Zaria; *Boswellia serrata* Roxb, *Prosopis africana* (Guill & Perr) and *Sclerocarya birrea* (A.Rich) Hochst bearing the Hausa of Ararrabi, Kirya and Danya and English as Indian Olibanum, African mesquite and cider trees respectively were indicated for treating piles and other human ailments. The stem barks of *Boswellia serrata* Roxb, *Prosopis africana* (Guill & Perr.) and *Sclerocarya birrea* (A.Rich) Hochst contained alkaloids, tannins, steroids, flavonoids, terpenoids, phlobatannins and saponins which may be the scientific basis for their use in traditional system of medicine for the treatment of piles and cancer. The results obtained in the analysis of the Standard Reference Material IAEA 1515 had standard deviations ranging from 0.02 to 41.7 for cobalt and iron respectively. The instrumental neutron activation analysis showed that the herbal medicinal preparations contained essential trace elements whose concentrations were within permissible limits in medicinal plants. The order of the concentrations of the essential trace elements in the aforementioned herbal medicines was *Prosopis africana* (Guill & Perr.) > *Boswellia serrata* Roxb > *Sclerocarya birrea* (A.Rich) Hochst for Fe > Mn > Zn but for concentration of Co the order was *Prosopis africana* (Guill & Perr.) < *Boswellia serrata* Roxb < *Sclerocarya birrea* (A.Rich) Hochst. These results indicate that the three popular herbal medicines can be efficacious in treating human ailments.

RECOMMENDATIONS

Even though the results of this study showed the presence of secondary metabolites and essential trace elements in the aforementioned medicinal plants; it is recommended that, their consumption should always be monitored for toxic elements to avoid the accumulation of heavy metals in the body, which may lead to cancer. Further studies could be done to determine and standardize the bioactive constituents and other essential trace elements that treat piles and cancer.

ACKNOWLEDGEMENT

We acknowledge the Centre for Energy Research and Training for the use of the Nigerian Research Reactor –I facilities.

DECLARATION OF CONFLICT OF INTEREST: No conflict of interest

REFERENCES

[1]- PAXMAG, (2008). Issue 9, The Role of NAFDAC in regulating and control of herbal medicines in Nigeria. Published by Ewu Monastery, Abuja, Nigeria.

[2]- Ekor, M., (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers In Pharmacology Vol.4*;177

[3]- Kennedy D.O. & Weightman E.L., (2011). Herbal extracts and phytochemicals: Plant secondary metabolites and enhancement of human brain function. *Advances in Nutrition vol. 2*: 32-50.

[4]- Doss, A. & S.P Anand (2012), preliminary photochemical screening of *Asteracantha longifolia* and *Pergularia daenia* *World Applied science journal* **18**(2): 233 – 235.

[5]- Baker A.J.M., & Whiting, S.N. (2000). In search of the Holy Grail- a farther step in understanding metal hyper accumulation? *New Phytologist*, **155**: 1-7.

[6]- Khan, S. A., Khan L., Hussain I., Marwat K.B., & Ashtray (2007). Profile of heavy metals in selected medicinal plants. *Pakistan Journal of weed Science and Research*, **14**(1-2): 101-110.

[7]- Roland N.N., Nicoline .T. & Victor K., (2013) Antidiabetes activity of African medicinal plants, *Medicinal Plant Research in Africa*, vol. **2**:753-786.

[8]- American Heritage Dictionary of the English Language, Fifth Edition copyright @2016 by Houghton Mifflin Harcourt Publishing Company. Published by Houghton Mifflin Harcourt Publishing Company. **Trace elements-definition of trace elements by the free Dictionary.**
<https://www.thefreedictionary.com/trace+elements>

[9]- Chrzan, A. (2016).”Monitoring bio concentration of potentially toxic trace elements in soil trophic chains” *Environmental Earth Sciences*, vol **75**(160) p 786.

[10]- NIOSH (1988), National Institute of safety and health testimony to the USA department of labour statement of the national institute of safety and health presented at the public hearing on OSHA PELs crystallization of silica. July, 1988 NIOSH policy statement, Cincinnati, Ohio, US Department of Health and Human Services, Public Health Services, Centre for Disease Control. National Institute for occupational safety and health.

[11]- Prasad, A. S. (1993). Essential and Trace Elements in Human Health and Disease: an update.

[12]- Underwood, E. J. (1977). Trace elements in animal nutrition 4th Edition, Academic Press New York. 1977.

[13]- Gaur, S. & Agnihotri R., (2016). “Trace Mineral Micronutrients and chronic Periodontitis- a Review “Biological trace Elements Research, vol.**173**(397) p1-14.

[14]- Sofowora, A. (1984). Medicinal plants and Traditional medicine in Africa, John Wiley and Sons: New York, pp130-131.

[15]- Edeoga, H.O., Okwu D.E., & Mbaebie, B.O. (2005). Phytochemical constituents of some Nigeria medicinal plants. *African Journal of Biotechnology* **4**(7):685-688.

[16]- Jonah S.A., Umar, I.M., Oladipo, M.O.A., Balogun & Adeyemo D.J., (2006). Standardization of NIRRI irradiation and counting facilities for instrumental neutron activation analysis. *Applied Radiation and Isotopes* **64**:818-822.

[17]- LIYU, (2004). WINSPAN, 2004, multi-purpose Gamma Ray Spectrum analysis software, CIAE Beijing, China.

[18]- Elisha J.J., Agbaji E.B., Nuhu A.A., & Abechi, S.E., (2016). An Ethnobotanical Survey of

- Medicinal Plants or Preparations sold in the markets of Zaria, Kaduna State, Nigeria. *International Journal of Science and Engineering* Vol **7** issue 4, pp5-25.
- [19]- Woolley J. G., (2001). Plant alkaloids, Encyclopaedia of life sciences/© 2001.
- [20]- Batawila, K., (2005). Antifungal activities of five Combretaceae used in Togolese traditional medicine. *Fitoterapia*. **76** (2):264-268.
- [21]- Maikai, V. A., Abubakar, U., Saliman, A. A. & Inuwa, T. N., (2010). Preliminary survey of medicinal plants used in treatment of animal trypanosomiasis in Kaduna state Nigeria. *Ethnobotanical leaflets* **14**: 319-326
- [22]- Shirin, K., Imad S., Shafiq, S., & Fatima K., (2010). Determination of trace elements in indigenous medicinal plant *Withania somnifera* and possible correlation with therapeutic activity. *Journal of Saudi Chemical Society* **14**:97-100
- [23]- Duduku, K., Roslam, S. & Awang, B., (2007). Phytochemical antioxidant for health and medicines-A move towards nature. *Biotechnology Molecular and Biology Review* vol. **1** (4):97-104
- [24]- Ajiboye, A. A., Agboola, D.A., Fadmu, O.Y., & Afolabi, A.O., (2013). Antibacterial, phytochemical and Proximate Analyses of *Prosopis africana* (Linn) Seed and Pod Extracts *FUTA Journal of Research in Sciences* **2013**(1):101-109.
- [25]- Hostettmann. K. & Marston, A., (1995). Saponins, chemistry and pharmacology of natural products illustrated, reprint Cambridge University Press, New York, NY Xii+548pp.
- [26]- Osbourn, A. E., (1996). Preformed antimicrobial compounds and plant defense against fungal attack. *The plant cell*, vol. **8**, pp 1821-1831.
- [27]- Sparg S.G., Light M E., & Van, S., (2004). Biological activities and distribution of plant saponins. *Journal of Ethno-pharmacology* **94**(2-3):219-243.
- [28]- Rahimi, M., Farhadi, R. & Balashahri, M.S., (2012). Effects of heavy metals on the medicinal plants. *International Journal of Agronomy and Plant Production*. Vol **3**(4): 154-158.
- [29]- Morrissey, J.P., & Osbourn, A.E., (1999). Fungal resistance to plant antibiotics as a mechanism of pathogens. *Microbiology and Molecular biology Review* **63**, pg. 708-724
- [30]- Goto, T., Takahashi, N., Hirai, S., & Kawada, T., (2000). Various Terpenoids derived from herbal and dietary plants function as PPAR modulators and regulate carbohydrate and lipids metabolism. *Hindawi PPAR Research* Vol. **2010** (2010).
- [31]- Bate-Smith Swain (1962). Flavonoid compounds. In: Comparative Biochemistry. Florkin M. Mason HS (Eds) Vol III, Academic Press, New York, 75-809.
- [32]- Amarowicz, R., (2007). Tannins: The new natural antioxidant? *European Journal of Lipids Science Technology* **109**, 549-551.
- [33]- Argal A. & A. K. Pathak., (2006). CNS activity of Caloptrops *gigantean* roots, *Journal of Ethno pharmacology* **106**: 142-145.
- [34]- Okwu D.E., (2001). Evaluation of chemical composition of indigenous species and.
- [35]- Moses, A.G. M., Gatebe, E., & Gitu, L., (2012). Profile of heavy metals in selected medicinal plants for treatments of diabetes, malaria and pneumonia in Kisii Region, Southwest, Kenya. *Global Journal of Pharmacology* **6**(3):245-251.
- [36]- Martin, H.W., Young, T.R., Kaplan, D.I., Simon, L. A., (1985). Essential trace elements in Biological systems. *Plant Soil*, **182**, p199
- [37]- Hambidge M., (2000). Human Zinc deficiency. *The Journal of Nutrition*, **130**:1344s-1349s
- [38]- Shankar, A. H., & Prasad A.S. (1998). Zinc and immune function: the biological basis of altered resistance to infection. *The American Journal of Clinical nutrition*, **68**(suppl):447s-4463s. Printed in USA 1998; in *American Society for Clinical Nutrition*.
- [39]- Brandi, M., (2018). 11 Impressive Health Benefits of Manganese-Natural <http://www.naturalfoodseries.com>
- [40]- Jasper, E.E., Yakubu, M. B. & Baganjiya, Y.P., (2017). Levels of Essential and Non-Essential Elements in Commercially available Moringa Herbal Teas Sold in Nigeria. *Nigeria Journal of Chemical Research*, vol **22** no **1**:1-8