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Original Research Article

Assessment of physicochemical properties of *Globimetula braunii* (Loranthaceae) leaf extracts

Gideon O Okpanachi[1,2]*, Avosuahi R Oyi1, Hassan Musa[1], Abdulrahman Abdulsamad[1], Ifeanyi V Emenike[2] and Yahaya Z Sule[1,3]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. [2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria. [3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria.

* For correspondence: *Email: okpanachigideon@gmail.com. Tel: +2347034927739*

Abstract

Purpose: This study is aimed at assessing the physicochemical properties of the leaves, ethanolic leaf extract (GBE), petroleum ether fraction (PEF) and n-butanol fraction (NBF) of *Globimetula braunii* leaves.
Methods: Physicochemical tests such as moisture content, pH, total ash, acid insoluble ash, water/alcohol soluble extractives and heavy metal limit tests were conducted on the leaf powder. GBE was partitioned with petroleum ether, ethyl acetate and n-butanol to get PEF, EAF and NBF fractions respectively**.** GBE, PEF and NBF were assessed for solubility, maximum wave length of absorption, moisture and light stability tests. Quality control test such as microscopy, chromatographic fingerprints and infrared spectra of NBF and PEF were carried out.
Results: GBE yield was 10.22 % w/w while PEF, EAF and NBF fractions yielded 3.8 %, 6.59 % and 14.21 % w/w respectively. The pH of the leaf powder, GBE, PEF and NBF were 6.24, 6.24, 6.20 and 6.25 respectively. GBE and NBF were sticky while PEF was oily in texture. The physicochemical tests results of the leaf powder indicated low levels of inorganic matter, silica and low levels of contamination by earth and heavy metals. The light absorption exhibited a maximum only at 227 nm. There was a visible colour change of GBE, PEF and NBF on exposure to UV light. The moisture uptake test values increased from 30 % RH to 90 % RH (GBE>NBF>PEF).
Conclusion: The outcome of the physicochemical tests gives an insight into the possible formulation approach of GBE, PEF and NBF into dosage forms.

Keywords: *G. braunii*, petroleum ether, n-butanol fractions, antihyperglyceamic property

Indexing: Index Copernicus, African Index Medicus

Introduction

Physicochemical Characterization also known as preformulation is the first learning phase prior to the development of dosage form [1]. It is expedient for a preformulation scientist to provide a catalog of information and to determine certain fundamental physical and chemical properties regarding the medicinal substance and other derived properties of the material. This outcome dictates many of the subsequent events and approaches in formulation. Currently, no general official standards/methods are available for herbal medicines however many manufacturers employ some parameters which are preliminary in nature [2]. A recommended list of determinations required in preformulation includes: spectroscopy, solubility test, melting point determination, assay development such as high performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR), stability (in solution and solid state), microscopy, powder flow, compression property and excipient compatibility studies [1]. Quality control procedures should be applied to herbal

starting materials, their extracts and finished products [3]. Quality control techniques used for plants and their extracts include; preliminary physical investigation of starting material, observation of foreign matter, moisture content, extractive values, ash values, and insoluble ash values determinations. Others include microscopical analysis; tannin content, bitterness value, swelling index measurements, heavy metal contamination limit tests, etc. [4].

Globimetula braunii belongs to the family of Loranthaceae. It is a hemi parasitic plant that grows on dicotyledonous trees and attaches itself to the host by modified roots [5]. The members of the loranthaceae family are also known as mistletoes and are widely distributed in tropical countries like Malaysia, India, Cameroon, Nigeria etc. Although regarded as a threat to agricultural yield because of its parasitic characteristics [6]; reports reveal that it is an important medicinal plant which explains the use of the leaves of *G. braunii* to hasten delivery in traditional medicine practice [7]. *G. braunii* is reputed to be effective for treating many diseases ranging from headache, leg pain to pulmonary troubles [5]. There are claims that the leaves, fruits and flowers of the subject plant have been implicated in the management of high blood pressure, while the roots attaching it to the host plant are used for other therapeutic applications like ulcer and cancer treatment [5]. It has also been reported that the plant has oxytocic properties [8], laxative properties [9], antioxidative properties [10], antilipemic and hypocholesteremic properties [11]. The ethanolic extract, petroleum ether and n-butanol fractions has been reported to have hypoglycemic property [12, 13]. Thus it is expedient for the herbal medicinal material to be harnessed for the improvement of health care delivery and to produce pharmaceutical dosage forms from the active principles [14].

This study is aimed at assessing the physicochemical properties of the leaves, ethanol extract, petroleum ether and n-butanol fractions of *G. braunii*.

Materials and Methods

Plant Collection and identification

The entire plant which was hemi parasitic on *Azadirachta indica* tree was collected in the month of May at flowering stage from Gombe State University Campus, Gombe, Nigeria. The plant was identified by Namadi Sanusi as *Globimetula braunii* (Loranthaceae) at the Herbarium Unit of Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria with Voucher number 289 and was deposited for further reference. Figure 1, below is a photograph of *G. braunii* plant. The leaves were plucked and foreign materials were removed. The leaves were air dried with the aid of a rotatory ceiling fan at room temperature for 14 days then coarsely powdered using an attrition mill and thereafter stored in an air tight container until required for use.

Fig. 1: Photograph of *G. braunii* plant (a) hemiparasitic on *Azadirachta indica* and (b) after collection

Preparation of ethanolic extract, partitioning and acute pharmacological study

The powder was weighed (2.1 kg) and extraction was carried out with 7.0 L of ethanol boiled for 15 min and allowed to stay overnight in flasks for completion of extraction process. The extracts were filtered using a filter paper and then concentrated under reduced pressure in a rotary evaporator to recover the ethanolic extract. The dried extract was collected and preserved in a desiccator containing silica gel. The percentage yield of the crude extract was determined using the formula given below:

$$\text{Percentage yield} = \frac{\text{Weight of total extract}}{\text{Weight of powdered material}} \times 100$$

Partitioning of the ethanolic extract was carried out [15]. The extract was fractionated successively with three solvents in order of their increasing polarity, namely: petroleum ether, ethyl acetate and n-butanol respectively. The dried ethanol extract (214.6 g) was dissolved in 300 mL of distilled water and poured into a separating funnel. Starting with the least polar, 900 mL of petroleum ether was added to the extract in the separation funnel (water to solvent ratio is 1:3). The mixture in the funnel divided into two layers; the lower aqueous portion layer and the upper petroleum ether fraction layer. Both the petroleum ether and aqueous layers were collected into different beakers.

The aqueous portion was reloaded into the separating funnel and fresh petroleum ether solvent (900 mL) was added to it. This process was repeated three times to ensure maximum partitioning. All the petroleum ether fractions collected were pooled and labelled PEF. This same procedure was carried out using ethyl acetate to obtain ethyl acetate fraction (EAF) and n-butanol to obtain n-butanol fraction (NBF). The leftover after collecting the NBF was named aqueous fraction (AQF). All the fractions collected were concentrated using a water bath and the percentage yield of each fraction was calculated using the formula below:

$$\text{Percentage yield} = \frac{\text{Weight of fraction}}{\text{Weight of the extract}} \times 100$$

Physicochemical tests of powdered leaves and extracts of G. braunii

The physicochemical parameters such as moisture content, pH (1 % aqueous), total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive were carried out using standard methods [16].

Moisture content

A sample of powdered leaves was accurately weighed (5 g) in a dry and flat petri dish. The sample was dried in an oven (Gallenkamp oven BS size 3) at 105 ºC and weighed continuously until two consecutive weighing did not differ by more than 5 mg. The loss of weight in terms of percentage was calculated.

pH (1% w/v aqueous)

Powdered leaves weighing 1 g was dissolved in 100 mL of distilled water, then shaken frequently and allowed to stand for 18 h. The suspension was filtered and the pH determined using pH meter (Mettler-Toledo AG 8603 Schwerzenbach, Switzerland).

Total ash

A 2 g quantity of dried powdered leaves was weighed and placed in a previously weighed crucible. It was then ignited by gradually heating it to 500 – 600 ºC in a Chamber furnace (Vecstar LF3 Chesterfield, UK) until it was incinerated. It was then cooled in a desiccator and weighed. The content of total ash was calculated in terms of percentage.

Acid insoluble ash

To the crucible containing total ash, 25 mL of HCl (~70 g/L) was added and covered with watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the liquid added to the content of the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter in crucible was ignited to constant weight cooled in a desiccator and weighed. The content of acid insoluble ash was calculated in terms of percentage.

Water-soluble extractive

A sample of the dried powdered leaves was weighed (4 g) and transferred into a glass stoppered conical flask. It was macerated with 100 mL of distilled water for 6 h, shaking frequently, and then allowed to stand for 18 h. It was Filtered rapidly making sure not to lose any solvent, and then the 25 mL of the filtrate was transferred to a tarred flat bottomed petri dish

and evaporated to dryness on a water bath. It was dried at 105 ºC for 6 h, cooled in a desiccator for 30 min and weighed. The content of water-soluble matter was calculated in terms of percentage.

Alcohol soluble extractive

The powdered leaves weighing (4 g) and transferred into a glass stoppered conical flask. It was macerated with 100 mL of absolute alcohol for 6 h, shaking frequently, and then allowed to stand for 18 h. It was filtered rapidly making sure not to lose any solvent, and then 25 mL of the filtrate was transferred to a tarred flat bottomed petri dish and evaporated to dryness on a water bath. It was dried at 105 ºC for 6 h, cooled in a desiccator for 30 min and weighed. The content of alcohol-soluble matter was calculated in terms of percentage.

Heavy metals limit Test

A sample of the powdered leaves was weighed (1 g) and transferred into a 100 mL kjeldahl flask and then a 4 mL of perchloric acid and 25 mL of nitric acid was added and allowed to stand for 24 h in a Speed Digester (BUCHI K-436 Switzerland). The mixture was heated for 40 min at 70 ºC and then the heat was increased to 120 ºC and the heating continued until solution became clear with disappearance of white fumes indicating the completion of the digestion process. The digest was diluted with 10 mL of distilled water and boiled for 15 min. The resultant solution after cooling was filtered into a 100 mL volumetric flask and diluted to the mark with distilled water. This was then transferred into screw capped polyethylene bottle and stored for further analysis. The sample was then analyzed for six heavy metals namely Chromium, Copper, Lead, Nickel, Manganese and Zinc using Atomic Absorption Spectrophotometer (AA-6800 Shimadzu Kyoto, Japan) with a digital read out system.

Solubility

A 100 mg of the ethanol extract of *G. braunii* leaves was placed in a test tube, and about 5 mL of water was added in small portions repeatedly up to 100 mL. The test tube was shaken vigorously after the addition of each portion of solvent (distilled water, ethanol and acetone) until it was completely dissolved. The procedure was repeated for PEF and NBF.

Maximum wavelength of absorption (nm)

A 10 mg of the dried ethanol extract of powdered *G. braunii* leaves was weighed on an analytical balance and transferred into a 100 mL volumetric flask containing 20 mL of distilled water and shaken well to dissolve. More distilled water was then added to the 100 mL mark to get a 100 µg/mL stock solution. A 0.4 mL of the stock solution was pipetted and made up to 5 mL to get 8 µg/mL solutions. The resultant solution (8 µg/mL) was then analyzed using a UV Spectrophotometer (UV-1800 Shimadzu Kyoto-Japan) set at a light absorption range of 180 to 350 nm to determine the maximum wavelength of absorption.

Moisture uptake

Saturated solutions of MgBr, KNO_2, NaBr, NaCl and KNO_3 were prepared to yield Relative Humidity (RH) of 30, 45, 60, 75 and 90% respectively at room temperature (Wells, 2002). Two (2 g) of each of the samples (GBE, NBF and PEF) were distributed uniformly on separate Petri dishes and placed in different desiccators at 30, 45, 60, 75 and 90% RH for 5 days after which it was reweighed. The percentage increase in weight was calculated as the moisture sorption capacity.

Light Stability

The method of photo stability testing was adapted [17]. A 2 g of each of the samples (GBE, NBF and PEF) was weighed and distributed uniformly on two petri dish and then exposed to UV light (254 to 366 nm) and visible light at room temperature for 30 days. Any observable color change was recorded.

FTIR Analysis of PEF and NBF

The potassium bromide (KBr) tablet method was employed. Five milligrams of PEF was mixed with KBr to 200 mg. The powder mix was compressed using a Sigma KBr press into a tablet shape. The tablet was placed in the sample compartment of the FTIR (Perkin Elmer L1600401 Spectrum Two DT GS, UK) and scanned at a range of 4000 to 400 cm^{-1}. The procedure was repeated for NBF.

HPLC for NBF and PEF

The HPLC analysis of NBF and PEF were carried out with HPLC (Shimadzu, Japan) under the following experimental conditions: the

samples (NBF and PEF) were analyzed by running them separately through a stationary phase of Chromolith column-Pinacle DB, C18; at temperature of 40 °C with mobile phase, 70% Acetonitrile in water in 0-30 min; flow rate, 2.0 mL/min and at maximum wavelength λ max of 254 nm.

Results

Table 1 is a summary of the physicochemical properties of *G. braunii* leaf powder. The pH of the leaf powder was 6.24. The results of extractive values (water soluble extractive, ethanol extractive) are presented. The amounts of heavy metals (chromium, copper, lead, nickel, manganese and zinc) present in the leaves are below the normal concentrations.

The percentage yield of the ethanol extract was found to 10.22 %w/w. The organoleptic properties of extract revealed that the extract was greenish brown in appearance, had a characteristic smell, sticky in texture and bitter in taste.

Table 2 shows the physicochemical assessment of GBE, NBF and PEF. The PEF was insoluble in water, ethanol and acetone. The pH of the extract/fractions was within 6.20 to 6.25. The light absorption in the range 180 to 350 nm of the solution obtained exhibited a maximum only at 227 nm. The extract/fraction moisture sorption capacity increased as the relative humidity (RH) increased from 30 to 90%. A change in the physical appearance of the extract/fraction occurred at the wavelength of 254 to 366 nm.

The SEM pictures of NBF and PEF of *G. braunii* leaf extract are presented in Fig. 2 and 3 respectively. The SEM pictures did not reveal well-defined lattices structure and shape. HPLC and FTIR of NBF and PEF were carried out for qualitative control and identification purposes of *G. braunii* leaf extract.

The chromatograms for PEF and NBF are shown in Fig. 4 and Fig. 5 respectively. The eight (8) peaks with their corresponding peak area in Fig. 4 represents eight (8) compounds while the seven (7) peaks in Fig. 5 represents seven (7) compounds in each of the fractions. The FTIR spectra of the PEF and NBF shown in Fig. 6 and 7 respectively revealed the functional groups present in each of the fractions.

Table 1: Physicochemical characterization of *G. braunii* leaf powder

Parameters	Results
Colour appearance	Greenish-brown
pH (1.0 %w/v aqueous suspension)	6.24
Loss on drying at 105 °C (%w/w)	8.06
Total ash (%w/w)	15.05
Acid-insoluble ash (%w/w)	0.65
Water-soluble extractive (%w/w)	32.0
Alcohol- soluble extractive (%w/w)	7.4
Heavy Metals	
Chromium (mg/L)	<0.6563 (<0.1-1)
Copper (mg/L)	<0.0004 (3-15)
Lead (mg/L)	0.0073 (1-5)
Nickel (mg/L)	<0.2936 (0.1-5)
Manganese (mg/L)	0.0380 (15-100)
Zinc (mg/L)	0.0039 (15-150)

Normal concentration in parenthesis [18]

Table 2: Physicochemical characterization of GBE, NBF and PEF of *G. braunii* leaf powder

Parameters	GBE	NBF	PEF
Colour appearance	Brown	Brown	greenish-brown
Texture	Sticky	Sticky	Sticky and oily
Solubility:			
Water	Soluble	Insoluble	Insoluble
Ethanol	Soluble	Insoluble	Insoluble
Acetone	Soluble	Insoluble	Insoluble
pH	6.24	6.25	6.20
Maximum wavelength of absorption (nm)	227	227	227
Moisture uptake:			
30 % RH (MgBr)	23.5	22.4	21.2
45 % RH (KNO_2)	55.3	56.4	54.1
60 % RH (NaBr)	60.7	59.3	58.4
75 % RH (NaCl)	76.5	74.2	73.9
90 % RH (KNO_3)	80.6	81.0	79.8
Light Stability:			
254-366 nm	Dark brown	Dark brown	Dark greenish brown
Visible (Room temperature)	Brown	Brown	Greenish brown

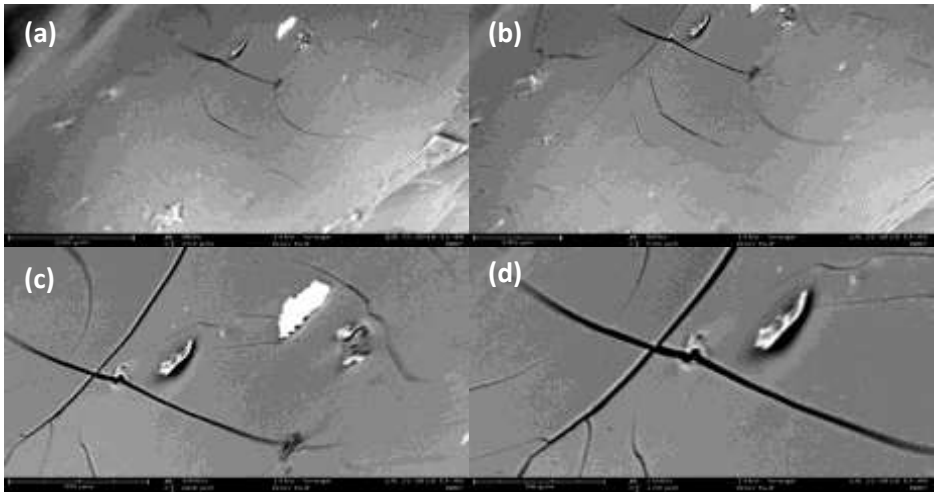

Fig 2: SEM pictures of n butanol fraction of *Globimetula braunii* leaf extract at (a) 360 x (b) 500 x (c) 1000 x and (d) 1500 x magnifications

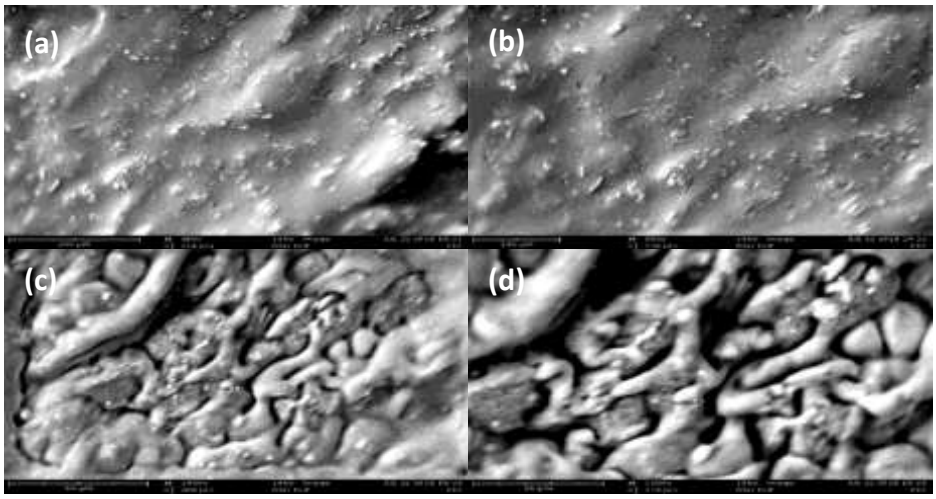

Fig. 3: SEM pictures of petroleum ether fraction of *Globimetula braunii* leaf extract at (a) 360 x (b) 500 x (c) 1000 x and (d) 1500 x magnifications

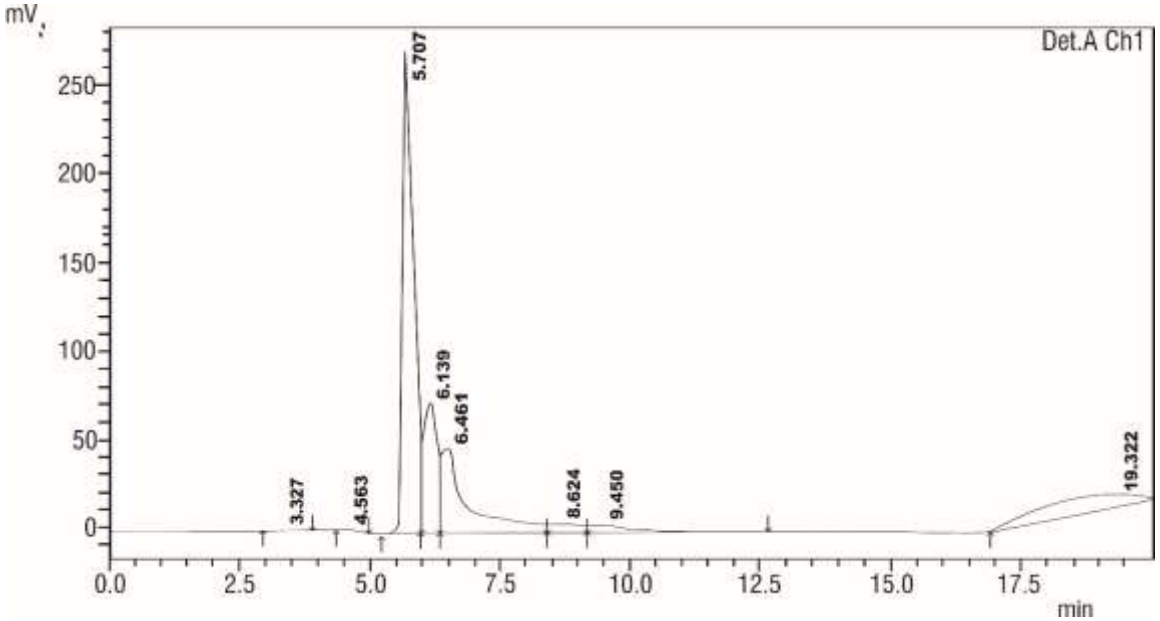

Fig. 4: HPLC Fingerprints of PEF obtained from *G. braunii* leaf extract

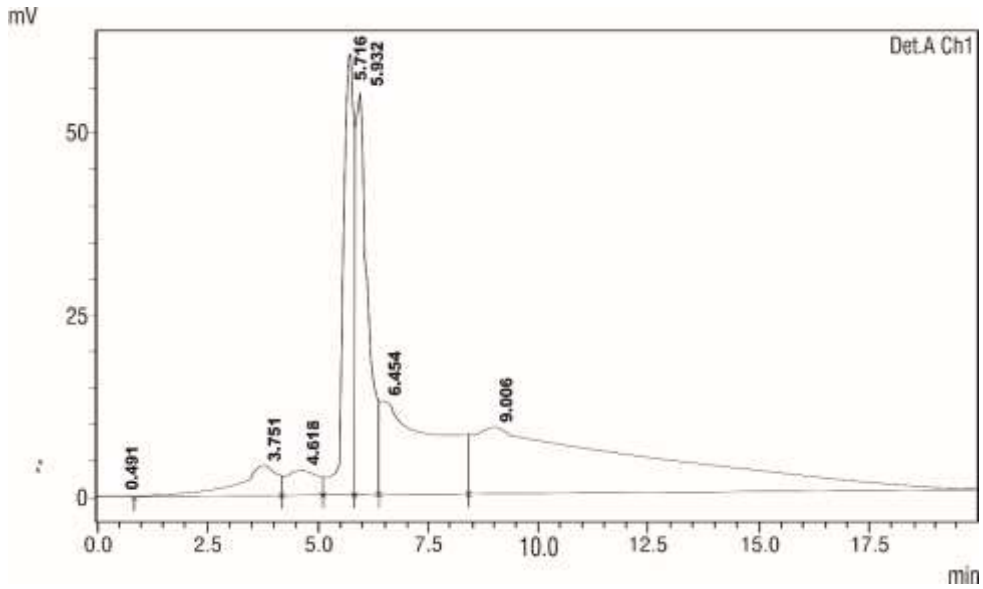

Fig. 5: HPLC Fingerprints of NBF obtained from *G. braunii* leaf extract

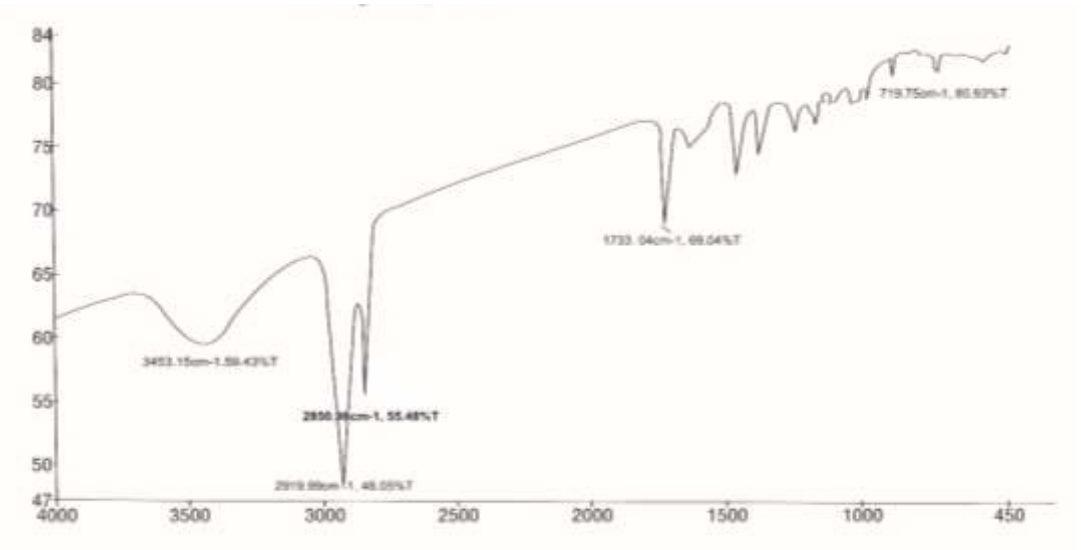

Fig. 6: FTIR Spectrum of PEF of *G. braunii* leaf extract

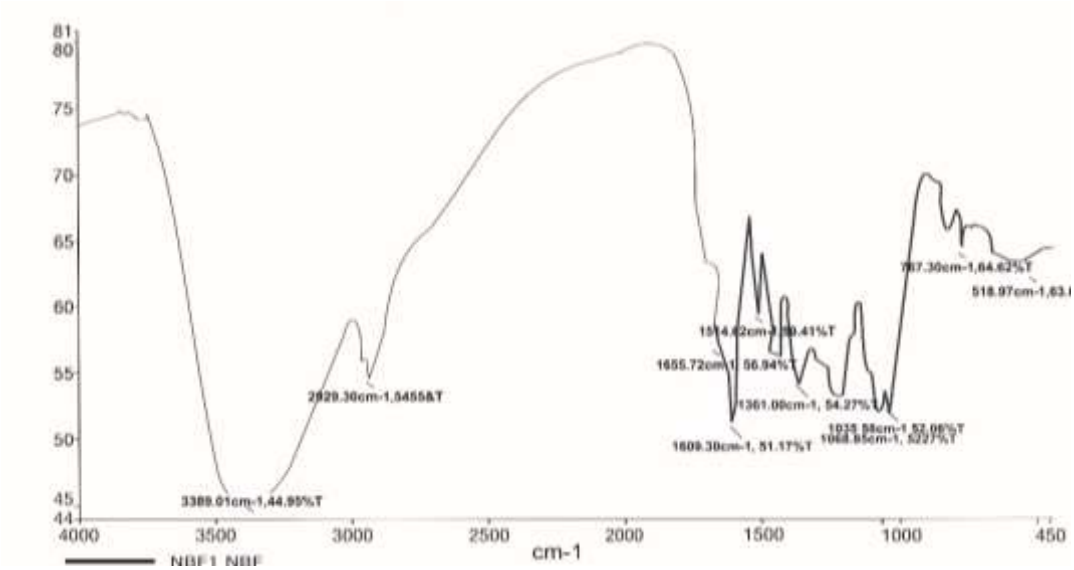

Fig. 7: FTIR Spectrum of NBF of *G. braunii* leaf extract

Discussion

Preliminary investigation of the ethanolic extract (GBE) and fractions of *G. braunii* leaf revealed that the drug candidates are soft types. The sticky nature of the ethanolic extract and NBF may be due to the presence of mucilage and high moisture content thus this gives an indication that formulation of the soft extracts into solid dosages may require vigorous processing [19]. Dry extracts are more suitable for tablet or capsule formulations while soft extracts are more widely used in liquid formulations or soft gelatin capsule formulations [3]. The sticky and oily nature of the extract/fractions will pose some difficulty in determination of their flow and compressibility properties. A plant material containing gums at high concentration may not need additional binding agent [20].

The determination of physicochemical properties is important in the standardization and quality control of herbal medicines [21]. In Table 1, the ash values and insoluble ash values are indicative of levels of inorganic matter or silica and levels of contamination with earth or silica respectively [3]. Ash values are important quantitative standards used to analyze the identity and purity of crude drugs or plant material [22]. The total ash of a plant material reflects the care taken in drug preservation and the purity of the material and the prepared drug [23]. The total Ash value which indicates the amount of minerals and earthly materials attached to the plant was 15.05 %w/w. The acid insoluble ash is a measure of the amount of silica and oxalates present in the crude drug especially as sand and siliceous earth [21]. The negligible amount of acid-insoluble siliceous matter present in the leaves was 0.65 %w/w. The significance of this value is to ensure standard limits are maintained in cases of accidental or intentional introduction of adulterants into the plant material [3].

Classical techniques of extractive values (water soluble extractive, ethanol extractive) are indicative of the presence or absence of low levels of compounds of specific polarity [4]. The extractive values obtained using water and alcohol gives an idea about the nature of chemical constituents present in the crude drug and is useful for estimation or determination of chemical constituents soluble in the solvent used for extraction [24]. The water-soluble extractive value obtained (32.0 %w/w) indicates the presence of sugar, acids and inorganic compounds while the alcohol-soluble extractive value (7.4 %w/w) signify the presence of polar constituents like phenols, alkaloids, steroids and flavonoids and other secondary metabolites present in the plant material [21]. The results of quantification of metals in plants in Table 4.9, is part of quality control, which has been established for their purity, safety and efficacy [25,26]. The heavy metal content of Chromium (<0.0656 mg/dL), Copper (<0.0004 mg/dL), Lead (0.0073 mg/dL), Nickel (<0.2936 mg/dL), Manganese (0.0380 mg/dL) and Zinc (0.0039 mg/dL) revealed that the powdered *G. braunii* leaves' heavy metal content were within the prescribed permissible limits [18,27,28].

The World Health Organization has specifications for maximum permissible levels for heavy metals in food and drug materials [29]. Heavy metal poisoning is caused by the accumulation of certain metals in the body due to exposure through food, water, industrial chemicals and in this case medicinal plant materials. While our body need small amounts of some heavy metals (zinc, copper, chromium, iron and manganese), toxic amounts are harmful. Accumulating too much of certain heavy metals in the body can lead to dangerous symptoms.

A high supplementation of copper had been implicated in liver damage. Zinc may produce adverse nutrient interactions with copper. Zinc reduces the immune functions and levels of high density lipoproteins. Lead is known to induce renal tumours, reduce cognitive development and increase blood pressure and cardiovascular disease in adults [30]. Trace amounts of trivalent chromium is required in humans for glucose and lipid metabolism but its deficiency may result to a disease called chromium deficiency whereas its hexavalent form is extremely toxic and carcinogenic [27]. Hexavalent chromium can enter the human cell because of its ability to cross the biological membrane easily and transferred into a more stable form which can damage the DNA [31]. Manganese is required in trace amounts for the normal synthesis and secretion of insulin by acting as a cofactor for a number of enzymatic systems [27]. Nickel is mostly present in the pancreas where it plays an important role in the production of insulin. Its trace quantity is required and its deficiency results in the disorder of liver, whereas at higher

concentration it shows allergic dermatitis known as nickel itch [27,32]. Iron is capable of generating reactive oxygen species which contributes to pathogenesis of diabetes and its complications such as diabetic neuropathy [27, 33,34]. Long term exposure to heavy metals may also cause the following symptoms: headache, weakness, tiredness, muscle pain, joint pain, constipation etc. [18].

The values of moisture uptake was in this order GBE > NBF > PEF. The hygroscopic and liquefying nature of the extracts can be addressed by adsorption on granulated colloidal silicon dioxide before granulation then enteric coated [20]. Preformulation studies on the herbal medicine and potential excipient combinations, good packaging, foil blisters and desiccant will accommodate moisture challenge and reduce significantly any hydrolytic instability [1]. The light absorption in the range 180 to 350 nm of the solution obtained exhibited a maximum at 227 nm. It has been reported that the UV absorption maxima of common terpenoids (largely responsible for the activity) is between 210 to 240 nm [35]. The visible colour change of the extract/fraction on exposure to UV light 254-366 nm was from brown to dark brown. This could be that UV wavelength range (290-320 nm) of higher energy causes photo degradation of herbal medicine [1].

HPLC and FTIR are widely employed for quality control and identification purposes of medicinal plants [36,37]. The number of peaks on the chromatogram corresponds to the number of compounds in the mixture as shown in Fig. 4 and 5. The height of the peak has a great role in understanding the separation efficiency of the method employed. HPLC is the most versatile and widely used technique for quality control and/or isolation of natural products [36]. HPLC has gained popularity among the numerous analytical techniques as the method of choice for finger printing study for quality control of herbal plants [38,39]. FTIR spectroscopic technique can be used for qualitative analysis of practically all compounds [37]. FTIR is a very important analytical tool for the characterization and identification of functional or chemical bonds of compounds present in an unknown mixture of plants extract [40,41].

The knowledge of FTIR as shown in Fig. 6 and 7 can be used to investigate and predict any physicochemical interactions between components of a formulation containing the active drug and therefore be applied to the selection of suitable chemically compatible excipients [1]. The characteristic peaks in the first region (4000 to 2500 cm^{-1}) corresponds to absorption caused by –NH, -CH and –OH single bonds. The functional groups absorbed in the second region (2,500 to 2,000 cm^{-1}) correspond to absorption caused by triple bonds. Characteristics peaks in the range of 2,000 to 1,500 cm^{-1} (third region) corresponds to absorption caused by double bonds such as –C=O, -C=N and –C=C. The fourth region is known as the finger print region (1,500 to 400 cm^{-1}) which contains a large number of absorption peaks that account for a large variety of single bonds. The infra-red finger prints features are important in the identification of the main components of the herbal plant extract and it can also distinguish the geographical origins of samples easily [37].

Conclusion

The outcome of the physicochemical study provides a catalog of information that will be required by the formulation scientist and serves as quality control for the powdered leaves of *G. braunii* and extracts. Also, characterization of the extract and fractions revealed that the drug candidates are soft types thus giving an indication that the formulation of the soft extracts into solid dosages may require vigorous processing.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

"We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors".
GOO (research concept, design, collection and assembly of data; writing of the article, data analysis and interpretation; critical review of the article), ARO, HM and AA (research concept, design, critical review of the article and final approval of article), IVE and YZS (review of the article and data interpretation).

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