German J Pharm Biomaterials 2022; 1(4): 27-37
https://doi.org/10.5530/gjpb.2022.4.17

GJPB

Research Article

Preliminary investigation of Dioclea reflexa seed gum as a food and potential pharmaceutical excipient

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Received: 06 July 2022; Revised: 24 August 2022; Accepted: 31 August 2022

Abstract

The chemical quality of natural gum determines its functionality and safe use. This study was to characterize some physicochemical properties and microbial load of gum obtained from *Dioclea reflexa* seed (DR), which has a history of folkloric use as a soup thickener in eastern Nigeria. The gum was extracted by aqueous maceration of DR. The microbial load was determined using the pour plate technique. The extract was screened for phytochemical constituents and analyzed for elemental content using atomic absorption spectroscopy (AAS) and scanning electron microscopy (SEM). Also, the morphology was viewed using SEM. The phytochemical screening indicated the presence of carbohydrates, starch, and simple sugars. The total viable aerobic bacterial and fungal counts were 2.0×10^1 and 1.0×10^0 (CFU/mL), respectively. The SEM micrograph showed that the polymer microstructure had dense and smooth surfaces, a property that has been associated with polysaccharides. The AAS elemental analysis showed the presence of several metals in the sample: Fe, Pb, Zn, Cd, Mg, Ca, and Na, in amounts generally within WHO permissible limits, except for Pb and Cd, whose levels were slightly above. The SEM analysis also showed the presence of K, Ca, Mg, Al, P, S, Na, and a preponderance of C and O. The presence of heavy metals could be associated with environmental pollution. DR gum's nature and chemical constituents present it as a potential food and pharmaceutical additive. Further studies should be done to validate the findings.

Keywords: gum; *Dioclea reflexa*; microbial load; phytochemical; elemental analysis

Introduction

The quality and functionality of naturally-sourced biomaterials have been very much attributed to the chemical content of the biomass [1] and the processing conditions [2, 3]. Natural gums are carbohydrate polymers of high molecular weight, composed of monosaccharide units joined by glucosidic bonds [4], constituted of a central unit of D-galactose or D-Galacturonic acid [5]. Plants, animals, or microorganisms usually produce them for structural and physiological functions [6]. Plant gums are:

- mostly exudates of the stem,
- resulting from the cell wall breakdown due to injury or other adverse conditions [1], or
- seed gums obtained from the endosperms of seeds [7,8].

Natural gums have diverse structural compositions and a wide range of physicochemical properties, thus making them useful in dosage forms as manufacturing aids, drug delivery agents, and foods or food additives [5, 1]. They are used as disintegrants, binders, emulsifiers, suspending agents, and release extenders in drug delivery [9]. In the food industry, they are used as binding agents, emulsifiers, stabilizers, and thickeners [10].

While synthetic polymers are non-renewable and non-biodegradable, limiting their pharmaceutical and food uses, natural gums, however, are biocompatible, biodegradable, and friendlier to the environment [11]. Gums are hydrocolloids possessing variant physicochemical properties that have resulted in remarkable differences in their functionalities. This important raw material is hard enough to supply to the pharmaceutical and food industries, so it is usually imported due to the local shortfall and the lack of appropriate data on the physicochemical properties which determine the uses. Hence, there is a need for their development to exploit their potential uses.

DR is sometimes referred to as "horse eye" or "sea purse" (due to the resemblance of the latter with the beautiful seeds). The plant *Dioclea reflexa* Hook F. belongs to the family Leguminosae, sub-family Papilionaceae) [2,3,12-14]. It is also referred to as sea beans and "marble vine" [2], probably because of the nature of the seed coat. DR is used locally as food, medicine, and a source of material as an effective larvicide [2,12-14]. In the South eastern part of Nigeria, DR powder, called "ukpo" is commonly used by the Igbos as a thickener in soup preparations.

We have reported the mucoadhesive functionality of DR gum in aminophylline tablet formulation [12] and some of its physicochemical and functional properties [13]. In this study, we aimed to analyse the elemental constituents in order to ascertain the level of heavy metals present in DR gum, using atomic absorption spectroscopy (AAS) and scanning electron microscopy (SEM) techniques, and qualitatively screening the phytochemical constituents as well as determining the microbial load. The study is important because it is a step toward establishing the safety and standardization of DR gum as a food and possible pharmaceutical excipient. DR gum is cheap and easy to find in Nigeria and other parts of the world.

Materials and Methods

Materials

DR, concentrated nitric acid (HNO_3) (Merck KGA, Germany), perchloric acid (H_2O_2) (BDH, England), freshly made deionized water, Ca, Cd, Cr, K, Fe, Mg, Mn, Na, Zn, and Pb salts (Sigma), Muller Hinton agar and broth, Sabouraud dextrose agar and broth, Nutrient agar, and peptone water. Bacterial isolates: *Staphylococcus aureus* (ATCC 25923) and multi-resistant *Staphylococcus aureus* (ATCC 25923); and fungal species: *Candida tropicalis* (ATCC 19092), *Candida albicans* (ATCC 2876), *Trichophyton rubrum* (ATCC 28188), *Microsporum canis*, and *Epidermophyton floccosum* isolates sourced from the Department of Microbiology and Biotechnology, NIPRD, Abuja, Nigeria, were used as test organisms. All the chemicals used were of analytical grade.

Collection and preparation of plant material

DR seeds were sourced from a local market, authenticated (Herbarium Number: NIPRD/H/6413) and extracted as earlier reported [12,13], with slight modifications. The seeds were washed, boiled in distilled water for 3 h to soften the shells, and then cracked to remove the shells. The cotyledons were cut into small-sized pieces and heated in ethanol (95%) at 75 ºC for 1.5 h to remove lipids and denature the proteins they might contain. The treated seed leaf particles were air-dried at 29 ºC for 6 h, and macerated (20% w/v) in distilled water in a flask for 18 h under mechanical shaking at the laboratory temperature (29-30 ºC), and the mucilaginous extract was filtered through a muslin cloth and centrifuged at 4000 rpm for 1.5 h to remove any solid particles. Then, acetone was added to the filtrate to precipitate the gum, which was sieved out using a clean muslin cloth. The wet gum was washed several times in acetone, exposed to air for 3 h for evaporation of the remnant organic solvent, and then

further dried in a hot air oven at 40 ºC for 2 h. The product was then pulverized using a mortar and pestle, weighed and packaged in an airtight screw-capped container till further use.

Phytochemical screening of the extract

The qualitative determination of the presence of phytochemical constituents was done following standard procedures [15,16].

Carbohydrates

To 1.0 gm of the extract in a test, the tube was added 3-4 drops of Molisch's reagent. Then 2.0 ml of concentrated sulphuric acid was carefully added to the mixture from the side of the container and observed. The formation of a purple ring at the interface and a purple precipitate on shaking indicated the presence of carbohydrates.

Starch

To an aqueous dispersion of 1.0 gm of the extract in distilled water was added 1 drop of iodine reagent. A deep blue coloration indicated the presence of starch.

Simple sugars

Equal volumes of Fehling's solutions A and B were added to a dispersion of a 1.0 gm sample of DR gum water extract. The formation of a brick red coloration was an indication of the presence of simple sugar.

Tannins

A few drops of a 10% ferric chloride solution were added to a dispersion of 1.0 gm of DR extract in water.

Alkaloid

The methanol extract of the sample was evaporated to dryness and 1% aqueous hydrochloric acid was added.

Microbial load determination

The test bacteria and fungi were maintained on nutrient agar (NA), and Sabouraud dextrose agar (SDA) slants, respectively. The loopfuls of 48 h surface growth of culture (bacteria and yeast) and spores (dermatophytes) were transferred to 0.9% NaCl solution, vortex-mixed, and the homogenous suspension was used for inoculation. The turbidity was then adjusted to match that of a 0.5 McFarland standard [17]. The DR gum extract was assessed for microbial load using Muller Hinton agar and broth, Sabouraud dextrose agar and broth, Nutrient agar and peptone water. The pour plate technique was used to determine the microbial load. Serial dilutions of the sample were introduced into molten nutrient agar and Sabouraud dextrose agar and allowed to be set in sterile Petri dishes, then incubated at 37 ºC and 25 ºC to estimate the total aerobic bacterial and fungal counts, respectively. The presence of microorganisms was determined based on their morphology on selective media and varied biochemical tests to detect the presence of any pathogenic microorganism.

Morphology

The morphology of the native DR gum was viewed using SEM (SEM, Carl Zeiss, EVO MA/10, Germany). The sample was scanned at a maximum electron accelerating voltage (ETH) of 40 eKV, and the micrograph obtained was recorded.

Elemental analysis by the atomic absorption spectroscopy technique

The elemental content assessment of the DR gum extract was done following the method earlier reported [18].

Apparatus

Atomic Absorption Spectrometer (AAS) (GBC Avanta, Model PM Version 2.2) under standard conditions with air-acetylene gas, Vecstar furnace, oven, desiccators, and glassware. The experimental conditions of the instrument were optimized, as stated in Table 1.

Table 1. Parameters for the spectroscopic determination of metals in the DR gum.

Element	Wavelength (nm)	Sensitivity (μg/ml)	Working Range (μg/ml)	Slid Width (nm)	Gas
Ca	422.7	0.020	1.0-4.0	0.5	Air-acetylene
Cd	228.0	0.009	0.2-1.8	0.5	Air-acetylene
Cr	257.9	0.050	2.0-15.0	0.2	Air-acetylene
Mg	285.2	0.003	0.1-0.4	0.5	Air-acetylene
Na	589.0	0.020	0.18-0.70	0.5	Air-acetylene
Fe	248.3	0.050	2.0-9.0	0.2	Air-acetylene
Pb	217.0	0.060	2.5-20.0	0.2	Air-acetylene
Zn	213.9	0.008	0.4-1.5	0.5	Air-acetylene

Conditions for calibration of the AAS for the elemental analysis

Analytical technique

Standard solutions (1.000 gm/l) and dilutions of the samples and standard reagents were made with deionized water. The working standards of the selected metals were prepared by diluting the standard stock solutions, which were prepared from analytical grade salts of the metals, and the results were corrected from the blanks. The wavelength of analysis, sensitivity, slid width, gas type, and the working range chosen for the analysis were as presented in Table 1. The equipment was calibrated to follow the sensitivity and detection limits of the elements, respectively, using the method of a standard calibration curve, and the results were expressed in μg/gm [18].

Acid digestion and analysis of DR gum

For the acid digestion of the sample, 1.0 gm of powdered DR gum was weighed, placed in a crucible, and heated in a furnace at 550 ºC for 6 h. It was then cooled under desiccators, and 2.5 ml of 6.0 M HNO3 was added to aid in complete dissolution. The reaction mixture was filtered (0.45 μm filter membrane) into a 20 ml volumetric flask and diluted to volume. The solution was then subjected to spectroscopy to quantitatively determine the metal contents in the spectrophotometer [AAS-GBC Avanta: Model PM, version 2.2] using the flame method [18]. Six replicate determinations were done, and the raw data obtained from the sample analysis for Ca, Cd, Cr, Fe, Mg, Mn, Na, Zn, and Pb were processed by calculating the actual concentrations in the samples using equation 1 [18]:

Metal (μg/gm) = (C x V)/W …… (1)

Where C = the concentration obtained from the spectrometer (mg/L); V = the volume of the undiluted sample solution in ml; W = the mass of the sample in grams.

Elemental analysis by scanning electron microscopy (SEM)

This was done using SEM (Carl Zeiss, EVO MA/10, Germany). Calibration was first done using the following standards for 6 iterations: C: $CaCO_3$, O: SiO_2, Na: Albite, Mg: MgO, Al: $Al2O_3$, P: GaP, S: FeS_2, K: MAD-10 Feldspar, Ca: Wollastonite, at various proportions. The specimen chamber was allowed to stay for some minutes for the attainment of vacuum prior to the scanning. The electron accelerating voltage (ETH) was then set at 0-40 eKV, and the instrument ran. The spectrum was processed, omitting no peaks, and all the elements were analysed (normalized) with 6 iterations.

Data handling

Elemental content determinations were done in replicates (n = 6) using aliquots of samples prepared for each metal following the optimized procedure. The data obtained were processed using Excel Microsoft Office, version 2007 and differences between mean values were considered significant at $p < 0.05$.

Results and Discussion

Yield of gum from the extraction

A yield of 10.2 ± 1.5 % gum extract was obtained from the starting material.

Phytochemical screening

Carbohydrates

Results of the phytochemical screening showed the presence of a purple ring at the interface, which formed a purple-coloured precipitate on shaking the mixture. This indicated that the DR gum extract consisted of carbohydrates.

Starch

The test showed the presence of a deep blue colour, indicating the presence of starch particles.

Simple sugar

A brick-red colour was observed, indicating the presence of simple sugars in the sample.

Tannins and alkaloids

The tests indicated the absence of tannins and alkaloids in the extracted DR gum. The overall physicochemical screening tests indicated that the DR gum extract was largely a carbohydrate polymer.

Microbial load

The results of the microbial load test revealed the presence of contamination of the DR gum sample with microorganisms. The microorganisms found were *Bacillus sp., Micrococcus sp., and Mucor sp*. The total viable aerobic bacteria (TVAB) and fungal counts (FC) were 2.0×10^1 (CFU/mL) and 1.0×10^0 (CFU/mL), respectively. However, the bio-burden fell within acceptable limits for *Bacillus sp*. and *Micrococcus* for non-sterile dosage forms as specified in the United States Pharmacopoeia (USP), which stated that *Escherichia coli* and *Salmonella sp*. must not be found in pharmaceutical products, whereas total viable bacterial and yeast counts must not exceed 10^2 CFU/mL [19].

Morphology

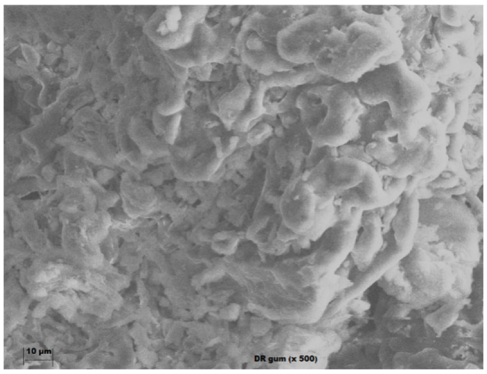
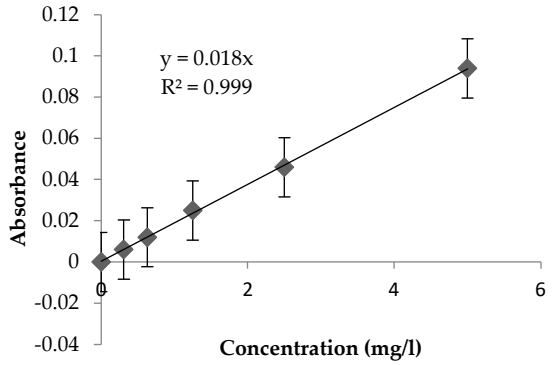


Figure 1. SEM micrographs (x 500) of the native DR gum powder. The smooth and dense surfaces of the hydrogel polymer microstructure might be attributed to polysaccharides.

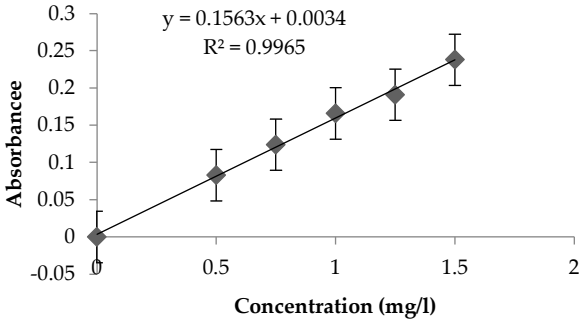
Figure 1 shows the SEM photomicrograph obtained of the native DR gum powder. The micrograph showed that the hydrogel polymer microstructure had dense and smooth surfaces, which might be attributed to polysaccharides [20]. This might predispose the gum to versatile uses as a pharmaceutical excipient. As a hydrogel, it is hydrophilic and can absorb large amounts of water molecules and swell in an aqueous environment. As such, it may function effectively as a disintegrant or drug-carrier matrix in solid dosage formulations. The amorphous nature makes it especially suited for efficient drug loading, possessing enough surface areas for drug particle adhesions and adsorptions. Moreover, the gummy nature empowers the polymer for use as a binder in solid dosage formulations and as a viscosity enhancer in liquid and semisolid formulations. We have reported its functional similarities to guar gum [13] and its mucoadhesive functionality in formulations for aminophylline tablets [12].

Elemental analysis by AAS

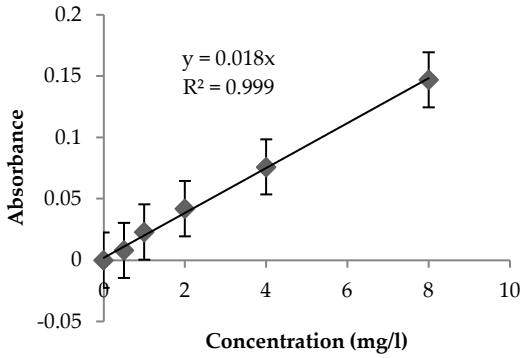
Figure 2 (a-i) shows the working curves obtained for the AAS analysis. Figure 3 shows the plots of the concentrations (mg/g) of the various elements obtained in the studied sample. The ascending order of the levels of the minerals (elements) in the sample was: Fe > Pb > Zn > Cd > Mg > Ca > Na, while Cr and Mn were below the detection limit of the instrument used. Various roles played by these elements were highlighted and discussed below.



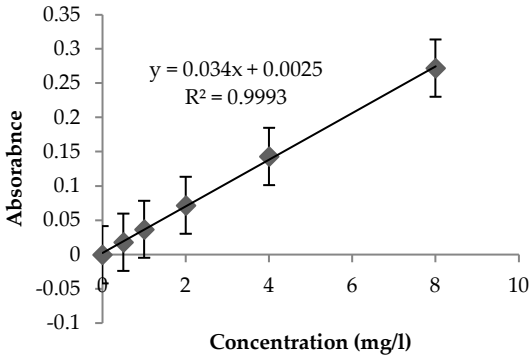
(a) Sodium (Na)



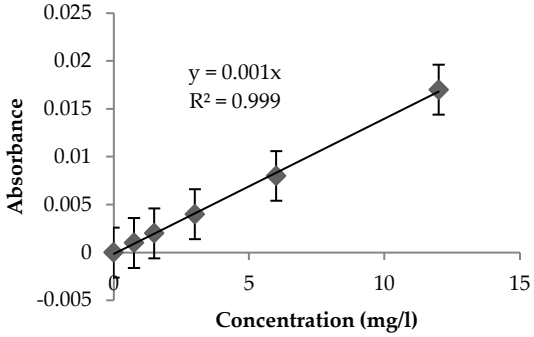
(b) Zinc (Zn)



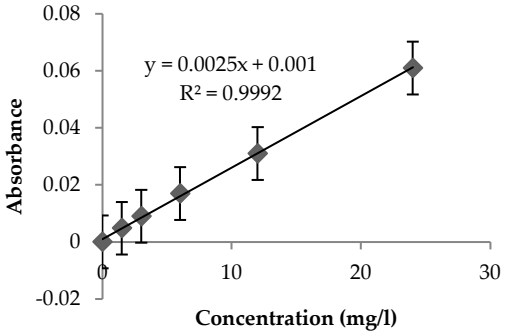
(c) Chromium (Cr)



(d) Manganese (Mn)



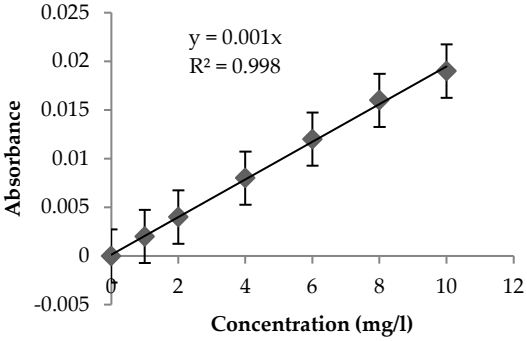
(e) Lead (Pb)



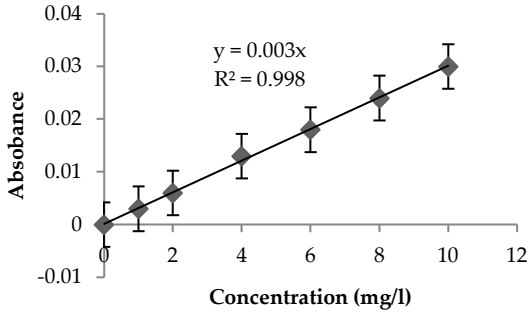
(f) Iron (Fe)

Calcium (Ca) at a specific concentration is known for its role in developing bones, teeth, muscle systems, and heart functions [21]. Its level in the seed under study was 0.70 µg/g (Figure 3), indicating a satisfactory accumulation level.

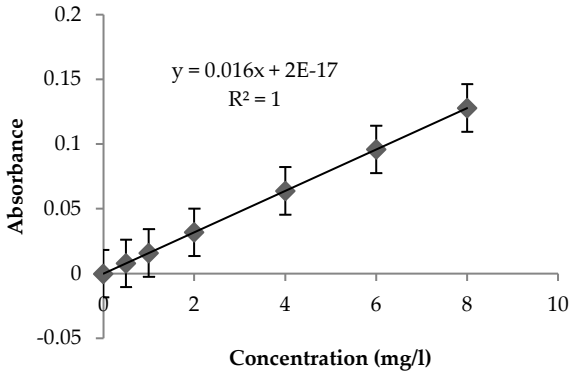
Chromium (Cr) has been recommended by the National Academy of Sciences of the US for a daily intake of 50–200 μg for adults because it is good for vision [22]. However, its critical level (5.30 ppm) could probably cause a yield reduction.



(g) Magnesium (Mg)



(h) Calcium (Ca)



(i) Cadmium (Cd)

Figure 2. Typical calibration curves obtained for some of the elements (a = Pb; b = Zn; C = Cr; d = Mn; e = Cd; f = Fe; g = Mg; h = Ca; I = Cd). All the plots showed high linearity ($R^2 > 0.99$).

When Cr is taken in large amounts, it can cause skin rashes, nasal irritation, bleeding, stomach upset, kidney and liver damage, itching in the nose, and even lung cancer. A deficiency of Cr causes disturbances in the metabolism of glucose, lipids, and proteins [23]. In this study, the Cr level was less than the instrument's detection limit. So, people who use this seed as a spice in a soup might not have to worry about health problems that can come from getting too much Cr.

Mg is an essential mineral for good health. It is required for over 300 natural biochemical processes in the body. Amongst the numerous health functions of Mg in the body is the transmission of nerve impulses, temperature regulation, energy production, detoxification, and the formation of healthy teeth and bones [24]. The Mg supplement dose associated with diarrhoea ranges from 1,000–5,000 mg, with a tolerable upper limit of 350 mg/day for individuals 9 years and older [25]. The results of the study indicated that the Mg content of the DR seed gum was lower than the upper limit, which means it is safe for consumption.

Mn sometimes referred to as the humble trace element, is essential to health, as it facilitates the utilization of vitamins C, B1, biotin, and Cl and neutralizes free radicals. Mn deficiency may cause increased problems with blood glucose levels, congenital disabilities, poor growth, decreased fertility, deafness, blindness, and paralysis. However, the symptoms of Mn deficiency have not been well-established except for limited data on demineralization and inadequate growth in children and a few others. The recommended dietary allowance is 2 mg per day [25]. The level of Mn in the studied sample fell short of the limit of detection, indicating, most probably, that the content was below its critical

concentration in the DR gum. For consumption of the seed as a spice, the sample is safe in terms of the critical level of Mn.

Fe is essential for humans and animals, a significant constituent of haemoglobin. It plays an essential role in the metabolism of major food components, such as carbohydrates, proteins, and fats, by facilitating their oxidation in the body, thereby controlling weight, which is a risk factor in diabetes [26]. The results (Figure 3) showed a high amount of Fe in the DR gum. This might be due to the absorption of traces of the element from the environment. The recommended amount of Fe in food is not more than 10-60 mg daily, and intake of less than the prescribed daily dose in diets may result in nose bleeds, myocardial infarction, and increased chances of infection in the gastrointestinal tract [26]. The results of this study have indicated that the Fe content of the DR gum sample is within the required dietary limit; therefore, its consumption in moderate amounts can provide body requirements per day.

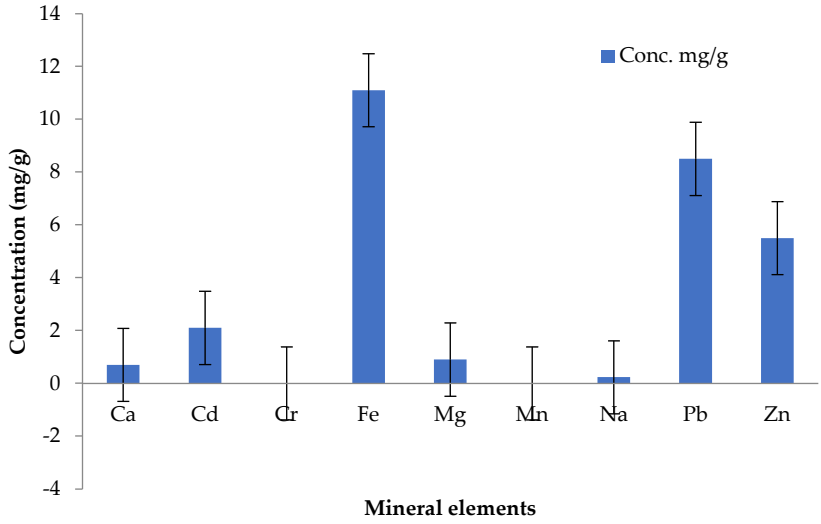


Figure 3. The Plot of the concentrations of the various elements in the sample as determined by AAS. The DR gum sample had the highest concentration of Fe compared to other elements. The concentrations of Pb and Cd in the gum material are above WHO limits of 10 ppm in herbal medicine and 1 ppm in foods, respectively, which raises some concerns.

Pb and Cd are non-essential trace elements having functions neither in the human body nor in plants. At low doses, they induce various toxic effects in humans. The typical symptoms of Pb poisoning are colic, anaemia, headache, convulsions, chronic nephritis of the kidneys, brain damage, and central nervous system disorders. Cd accumulates in the human body and damages mainly the kidneys and liver. The World Health Organization (WHO) prescribed limit for Pb content in herbal medicine is 10 ppm, while the dietary intake limit is 3 mg/week [27]. The lowest level of Cd, which can cause yield reduction, is 5-30 ppm, while the maximum acceptable concentration for foodstuffs is around 1 ppm [27]. The results of this study, as shown in Figure 3, indicated that the sample's Pb and Cd content exceeded the WHO and dietary limits for herbal products and foods. Therefore, sample consumption should be stopped or taken with caution after a rigorous confirmatory determination has been carried out to ascertain exactly where the source of the contamination is. This is because continual intake of traces of Pb and Cd can lead to long-term accumulation in the body system, which could lead to grave health implications. The presence of Pb and Cd might be due to contamination of the plant's soil. This needs further investigation.

Zn is an essential trace element for plant growth and also plays a vital role in various cellular processes, including normal growth, brain development, behavioural response, bone formation, and wound healing in man. Routine supplementation with zinc is highly recommended in managing diarrhoea, especially in infants and young children [28]. People with diabetes who are zinc deficient do not improve their perception abilities and also lose their sense of touch and smell [26]. Daily adult

dietary upper limit for zinc is 40 mg [29]. The results (Figure 3) indicated that the Zn content of the sample falls below the dietary requirement limit per day. This indicates that taking the sample only once per day will not be able to meet the zinc requirement of the body.

Elemental analysis by SEM

The results of elemental analysis by SEM showed the presence of nine elements, namely, C, K, Ca, O, Mg, Al, P, S, and Na, with C and O constituting 54.50 and 41.29 % of the atoms, respectively (Figure 4 and Table 2). The proportions of C and O were significantly ($p < 0.05$) higher than those of the other elements in the DR gum sample. The preponderance of C and O points to the likelihood of the gum being composed of mainly cellulosic polysaccharides $(C_6H_{10}O_5)_n$ [30].

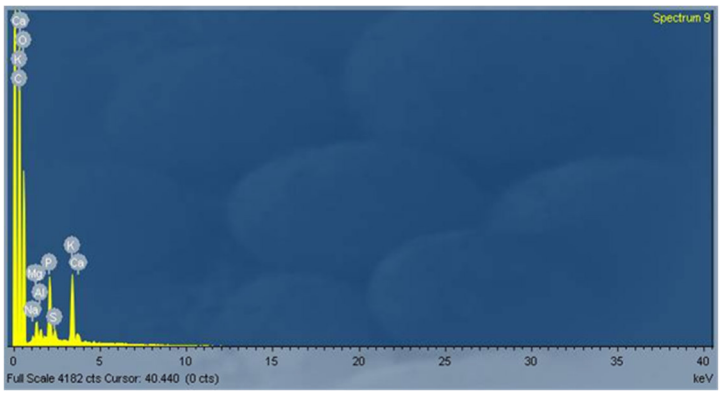


Figure 4. SEM showing the various elements present in the DR gum sample. This indicates that SEM is also useful in elemental analysis.

Table 2. Results of elemental analysis by SEM

Element	Apparent Conc	Weight (%)	Atomic (%)
C	106.20	45.04	54.50 ± 2.5*
O	67.89	45.44	41.29 ± 2.2*
Na	0.80	0.37	0.24 ± 2.3
Mg	1.74	0.86	0.51 ± 1.0
Al	0.92	0.40	0.22 ± 1.5
P	10.58	2.89	1.36 ± 1.2
S	1.20	0.46	0.21 ± 2.4
K	12.05	4.14	1.54 ± 2.2
Ca	1.06	0.40	0.14 ± 2.2
Total		100.00	

*Proportions of C and O were significantly ($p < 0.05$) more than the other elements in the DR gum sample.

The previous results of the study have provided helpful information on the chemical constituents of DR seed gum in an attempt to answer, though inconclusively, the research question about its safety as a food and potential pharmaceutical material. While the AAS analysis indicated the presence of Cd, Cr, Fe, Pb, and Zn, the SEM result did not. However, both test techniques showed the presence of Ca, Na, Mg, and Mn. The differences were attributed to the varying test conditions of the instruments. Even though AAS is usually used to do elemental analysis, the results of this study show how useful SEM is for this investigation.

The future perspective is to carry out further work on the material to establish more characteristics that will enable its informed acceptance and registration as a generally safe biomaterial for various pharmaceutical uses. The plan in our subsequent research is to expand the investigation on samples of DR seed to be collected from various locations to find out the possible influence the soil type could have on the constituents and other physicochemical qualities of the gum. We recently reported the comparable performance of DR gum to tragacanth powder as a suspending agent in metronidazole suspension [31]. In addition to the already established functionalities, we are currently exploring other possible pharmaceutical applications of the biomaterial.

Conclusion

According to the study's findings, DR gum's phytochemical contents primarily consisted of various types of polysaccharides and mineral elements. The elements found in the DR gum investigated using AAS, and SEM analysis included K, Ca, Mg, Al, P, S, Na, Mn, Zn, Fe, C, and O, with the last two constituting 54.50 ± 2.5 and 41.29 % atoms, respectively. The DR gum also had high levels of some heavy metals, such as Pb and Cd, slightly above WHO permissible limits. The sample had permissible bioburden. Notwithstanding the long-time folkloric use, it is essential to conduct quality checks on food and pharmaceutical materials to save consumers from toxicity. The study's results present DR gum as a potential food and pharmaceutical additive. We recommend improving DR gum processing for purification purposes before its use as a food or pharmaceutical additive. Further studies are necessary to establish the sources of the high Pb and Cd content in the sample and validate the findings.

Key findings

The investigation has provided preliminary information on DR gum's phytochemical constituents, morphology, elemental content, and microbial load. The new information about the biomaterial is relevant for its characterization and standardization for possible acceptance and use as a generally safe pharmaceutical and food excipient. Scientists who work on developing raw materials in the food and drug industries will be interested in the report.

Acknowledgements

We thank NIPRD, Abuja, for providing the facilities for the AAS and microbial load studies and Sheda Science and Technology Complex, Abuja, for the SEM.

Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

Financial support

None

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How to cite this article:
Mbah CC, Samali A, Aboh MI, Ogbonna JI, Builders PF, Attama AA, Ofoefule SI. Preliminary investigation of Dioclea reflexa seed gum as a food and potential pharmaceutical excipient. German J Pharm Biomaterials. 2022;1(4):27-37.