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Phytochemical, Proximate, and Elemental Analysis of Chia Seed (*Salvia hispanica* L.) from Dawanau Grain Market, Kano State, Nigeria

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ABSTRACT
Plants have shown a lots of importance in the field of phytomedicine, phamacognosy and herbal sciences, the seed of chia seed (*Salvia hispanica*) was collected from local farmers at Dawanau grain market Kano State with the aim of determination of phytochemicals, proximate and elemental Analysis and were identified at the herbarium of plant science department of Bayero University Kano with assertion number (BUKHAN 0539), 250g of the processed seed were macerated in 500 ml of methanol, water, ethyl acetate and hexane for 72 hrs. Standard method was followed for qualitative and quantitative phytochemical analysis, proximate and elemental analysis, furthermore thin layer chromatography and Fourier transform infrared microscopic analysis was conducted. The result of the analysis shows that flavonoid and carbohydrate was present throughout the solvents used, while all the proximate and heavy metal analysis were found to be within the limit of EP/WHO 2011 and WHO 1984 with the exception of Manganese. The result of the thin layer Chromatography result has the retention factor and number of spot. The FTIR analysis of the extract reveal that the I.R spectra indicated the present of different functional group such as amine, ketones, carboxylic acid and phenol. Conclusively the plant sample has shown the present of phytochemical substances that are remarkably useful in the field of ethonomedicine.

Keywords: *Salvia hispanica;* Phytochemical; Proximate Analysis

INTRODUCTION
Plants have proven to be a suitable alternative for the synthetic and semi-synthetic based drug for the cure of several disease conditions. Buba *et al.* (2016) reported that plants are major source of novel drug compounds, and as such it has contributed immensely to human health and well-being. Several plants have demonstrated their usefulness in the field of phytomedicine, pharmacognosy, herbal science, pharmaceutical chemistry among others (Franco *et al.*, 2014).

Plants produce a great diversity of phytochemical substances that could be active in many fields of medicine. Natural products from plant are proven template for new drug development**.** The plants have played a remarkable role to cure and avert different diseases from ancient times (Kong *et. al.,* 2013). The use of medicinal plants as an alternative form of treatment is well documented and practiced worldwide (Ezeja *et. al.,* 2011; Kareem *et. al.,* 2015). The medicinal value of plants depends on their chemical constituents which are called phytochemicals. These phytochemicals provide a definite physiological action on the human body (Kumar *et. al.,* 2019). Studies have shown that plant contain numerous phytochemicals which have both medicinal and pharmacological values (Ajayi *et. al.,* 2011; Kareem *et. al.,* 2015). Mineral elements are considered to be of great importance in the treatment and prevention of disease, and in the general well-being of individuals as they perform a critical function in the physiological and biochemical processes (Nelson, 2010). Also, knowledge of chemical constituents of plant is necessary for the discovery of therapeutic agents of importance and their precursors, Chia is an edible seed that comes from the desert plant "*Salvia hispanica*" belonging to the mint family. *Salvia hispanica*, or chia, is a species of a flowering plant in the mint family. Chia means strength and used the tiny black and white seeds as an energy booster. Chia seeds are an unprocessed whole grain food that can be absorbed by the body as seeds (Illian *et al.,* 2011). The crop is increasingly earning popularity in East Africa because of its economic and human-health benefits (Kibui *et al.,* 2018). It is referred to as the seed of the 21st century, new gold, super nutrient and super food (Dinçoğlu and Yeşildemir, 2019). The shape of the chia seed is flat and oval. It is 2.0 - 2.5 mm long, 1 to 2 mm wide, and 0.8 - 1.0 mm thick (Ixtaina *et al.,* 2008). The color of the coat of the seed varies from grey, black and white-spotted, which are slightly different from each other. However, black seeds are more common, and white seeds are slightly larger than black seeds (Ixtaina *et al.,* 2008, Ayerza, 2013). Increased consumption of chia seeds and a greater variety of plant foods provides most of the lost minerals and vitamins, in addition to phytochemicals. The capacity and value of these safe, healthy, and high-quality foods, as well as other alternative sources of rich foods such as plants, are required. Various research studies have been conducted to identify inexpensive and sustainable solutions for supplementing the needed nutrients from different plant sources such as soybeans (SB), *Moringa oleifera* (Rweyemamu *et. al.,* 2015), and chia seeds (Otondi *et. al.,* 2020). Such sources of nutrients have shown positive results, especially in fighting micro-nutrient deficiency in developing countries (Rweyemamu *et. al.,* 2015). Chia seeds have great nutritional potential because of their composition.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The seeds of *Salvia hispanica* were collected from local sellers in May, 2021 at Dawanau market Kano state. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano and was compared with a voucher specimen number BUKHAN0539.

Preparation of Plant extracts

Seeds of *S. hispanica* were crushed into powder form, (250 g) was soaked in 500 ml of Methanol, Water, Ethyl Acetate and Hexane for 72 hrs and the concentrate was evaporated to dryness and stored in desiccator for further use (Namadina *et al.,* 2015)

Determination of Physicochemical Constants of the Powdered seeds of *S. hispanica*

Moisture Content

Moisture content of the powdered sample will be determined by drying method. 4.0 g each of the powdered sample was accurately weighed and placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105 ºC for 1 hour, then cooled in a desiccator and re-weighed. Heating and weighing were repeated until a constant weight was obtained. The weight loss on drying was computed following the formula below:

$$\% \text{ of moisture} = \frac{(1 - Weight\ of\ Dry\ Sample)}{Weight\ of\ Wet\ Sample} \times 100$$

Total Ash Value

3 g of powdered plant materials was accurately weighed and placed separately in a crucible of known weight. It was heated gently and the heat gradually increased until it is white indicating the absence of carbon. It was allowed to cool in a desiccator and weighed; this was repeated until a constant weight was obtained. The total ash value was determined as a percentage with the formula below

$$\% \text{ Ash Value} = \frac{(Weight\ of\ Residual\ Ash)}{Original\ Weight\ of\ Powder} \times 100$$

Determination of Cellulose Fibre

The Cellulose fibre of the samples was determined using the (Van Soest *et. al.*, 1991) fibre analysis. The pulverized, air dried sample (1 g) was weighed in an empty crucible (W_1). At room temperature, 100 ml of Sodium Dodecyl Benzene Sulphonate ($C_{18}H_{29}NaO_3S$) and 0.5 g of Sodium Sulfite (Na_2SO_3) was added to the crucible, followed by few drops of octanol (octilic alcohol). The mixture was boiled for 60 minutes, filtered and the residue was washed twice in both boiling water and then in cold acetone. It was then dried in an oven at 105 ºC for 8 hr, cooled off in a desiccator and weighed (W_2). The percentage of the Cellulose fibre of the sample was calculated thus as:

$$\% \text{ CF} = \frac{(W1 + W2) - W1}{weight\ of\ the\ sample} \times 100$$

Where CF is percentage of cellulose fibre

Lipid

Determination of lipid content was performed following Soxtec method previously described by Noureddini and Byun (Noureddini *et. al.*, 2010), using a Soxtec TM 2050 automated analyser (FOSS Analytical, Hillerod, Molecules 2012, 17 11143 Denmark). Petroleum ether was used for the extraction, whereas percentage of lipid was obtained following the equation below:

$$\% \text{ of lipid} = \frac{(weight\ of\ extraction\ cup + residue - weight\ of\ extraction\ cup)}{weight\ of\ sample} \times 100$$

Protein

The total nitrogen amount in the sample was determined according to the method described by Chenault (Chenault 2008) using Kjeltec TM 2200 Auto Distillation Unit (FOSS Tecator, Höganäs, Sweden). A nitrogen-to-protein conversion factor of 4.4 was use for the determination of protein present in the samples.

Elemental analysis of the Powdered Seeds

The elemental analyses of the plant materials were carried out in Bayero University Kano, Central Laboratory located at 11.9796^{o} N and 8.4779^{o} E Powdered plant material was digested using 2.5 ml of hydrochloric acid (HCl) and 7.5 ml Nitric Acid (HNO_3). The concentration of Ca, Fe, Mg, Zn, Cu, Mn, P, K, and Na were detected

Qualitative Phytochemical screening of the *S. hispanica* seeds extract

The plant extracts (aqueous, methanol ethyl acetate and hexane) were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the standard method of (Evans, 1996; Raaman, 2006; Tiwari *et. al.,* 2011; Trease and Evans, 2012; Raaman 2016; Silva *et. al.,* 2018).

Quantitative Phytochemical screening of the methanol extract of *S. hispanica*

Flavonoid Determination

10 g of the plant sample was extracted repeatedly with 100 ml of 80 % Methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipal – Abyazan, 1994).

Saponin Determination

Out of the grinded samples 10 g was weighed and put into a conical flask, 100 ml of 20 % Ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{o}C the saponin content was calculated as described by Obadoni and Ochuko (2001).

Tannin Determination

500 mg was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up of the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measure at 120 mm within 10 min (Van-Burden and Robinson, 1981).

Thin Layer Chromatography (TLC)

Thin layer chromatography was conducted at Department of phamacognocy, Bayero University Kano, TLC aluminum sheet of 20 x 20cm silica gel pre-coated plate using the one way ascending techniques for the analysis. The plates were cut into sizes of 5 x 10 cm. The Methanol extract was dissolved in the initial extraction solvents and spots was applied manually on the cut plate using capillary tubes, after which the plates was dried and develop in different solvents ratios of: Ethyl acetate 90 % and Hexane 10 % that is in the ratio of (9:1), Ethyl acetate 80 % and Hexane 20 % which is (8:2) and the last solution of Ethyl acetate 70 % and Hexane 30 % which is 7:3, in the chromatographic tank. Develop plates was sprayed using general detecting reagents (*p*-anisaldehyde/ H_2SO_4, 10 % H_2SO_4 in methanol) and specific detecting reagents: Borntragers, Dragendoff, ferric chloride, Libermann-buchards and aluminum chloride, the plates were then heated at 110 ^{o}C for 2 minutes, they were viewed under Ultraviolet rays of 365 nm long wave and 254 nm short wave). Number of spots and retardation factors (Rf values) for each of the spots was determined and recorded (Gennaro and Stahl, 2015).

Fourier Infrared Spectroscopy Analysis (FTIR)

Air-dried sample of methanol fraction from selected plant extract was analyzed for identification of characteristic functional groups using Fourier Transform Infrared (FT-IR) spectrophotometer (Shimadzu) at the Laboratory in the Department of Biochemistry, Bayero University Kano, Nigeria. A small quantity (0.1g) of the extract was place on the disc and press using a mini hand presser to form a thin film and the disc was place in the FT-IR Spectrophotometer in which spectra was measured by accumulating 64 scans at 4 cm^{-1} resolution in the spectral range of 4000 to 400 cm^{-1}. The FT-IR, spectra was used to identify the functional groups of active metabolites based on the peak values in the infra-red region.

RESULTS

Proximate analysis of *S. hispanica* seed

The result of proximate analysis of *S. hispanica* was determined. Moisture content, carbohydrate content and energy, crude proteins, crude fibre and crude fat, Ash content were found in Chia Seed as shown in Table 1 below the result was compared with the standard of EP/WHO 2011.

Table 1 Proximate analysis of *S. hispanica*

Proximate Analysis	Chia seed	EP/WHO(2011)
Ash content (g)	3.67	19.00
Moisture content (%)	6.27	12-14/10-12
Crude protein (mg)	20.41	-
Crude fiber (g)	30.15	-
Carbohydrate content (g)	8.67	-
Crude fat (g)	29.83	-
Energy (kg)	45.92	-

Elemental analysis of *S. hispanica*

The results of the elemental analysis present in the sample is presented in the Table 2 below and the concentration of Ca, Na, Mg, K, Zn, P, Fe, Mn, Cu, were within the permissible limit of the FOA/WHO standard 2004 with the exception of Manganese (Mn) .

Table 2 Elemental analysis of *S. hispanica*

Elements (ppm)	Chia seed	FAO/WHO (1984) limit* (ppm)
Calcium (Ca)	2.97	52
Sodium (Na)	0.67	-
Magnesium (Mg)	2.00	35
Potassium (K)	6.44	-
Zinc (Zn)	2.70	27.40
Phosphorus (P)	4.86	-
Iron (Fe)	3.82	20.0
Manganese (Mn)	2.67	2.00
Copper (Cu)	0.81	3.00

Qualitative phytochemical screening of *S. hispanica*

From the result of the qualitative analysis done it was observed that carbohydrate and flavonoids were found to be present in all the solvent used while phenol and resins were found to be absent throughout the solvent.

Table 3 Qualitative phytochemical screening of Chia seed (*Salvia hispanica)*

Phytochemical Test	Solvent Used			
	Methanol	Aqueous	Ethyl Acetate	Hexane
Carbohydrate				
Molish test	+	+	+	+
Fehling test	+	+	+	+
Saponin				
Floathing test	+	+	_	_
Flavonoids				
Shinoda test	+	+	+	+
NAOH test	+	+	+	+
Alkaloids				
Mayers test	_	_	+	+
Dragendroffs test	_	_	+	+
Wagners test	_	_	+	+
Steroids and Triterpenes				
Salkowski test	+	+	_	+
Salkowski test	+	+	_	+
Cardiac glycoside				
Keller_killanis test	+	+	_	_
Tannins				
Ferric chloride test	+	_	_	_
Bromine H_2O test	+	+	_	_
Anthraquinones				
Bontragers test	+	+	_	_
Amino acid test	+	+	_	_
Phenol test	_	_	_	_
Resins test	_	_	_	_

Key: + Present - Absent

Quantitative Phytochemical screening of methanol extract of *S. hispanica*

Table 4 shows the results for the quantitative phytochemical content of chia seed, flavonoids, saponin and tannin were detected from the extract of the plant sample however tannins is having the highest (291.0 mg/g), followed by the saponins (8.0 mg/g), least was the flavonoids with (2.84 mg/g).

Table 4 Quantitative Phytochemical screening of methanol extract of *S. hispanica*

Metabolite Quantity (mg/g)	Chia seed (*Salvia hispanica)* ± SEM*
Flavonoids	2.84.00±0.44
Saponins	8.00±0.24
Tannins	291.00±0.49

Thin layer chromatographic analysis of *S. hispanica* extract using three solvent

The thin layer chromatographic analysis of chia seed **(***S. hispanica)* extract were carried out on the thin layer plate using three solvent ratio system of Hexane and Ethyl acetate. The chromatographic profile of methanol extract for Chia seed in the solvent ratio of 7:3 ethyl acetate and hexane shows seven (7) spot positions followed by ratio 8:2 with six (6) spot positions while ratio 9:1 also has seven (7) spot position on the TLC plate. The distance travel by the spot on the T.L.C plate of methanol extract was found to be 8.3 cm, 8.4 cm, and 8.4 cm for ratio 7:3, 8:2 and 9:1 respectively. Retention factor of each spot position was calculated and recorded accordingly. However for the ethyl acetate extract of the chia seed sample five (5), seven (7), and six (6) spots positions have been detected with the distance travel by the spot to be 8.3 cm, 8.3 cm, and 8.4 cm from the solvent ratio 7:3, 8:2 and 9:1 respectively. In the hexane extract of the plant sample six (6) spot positions was recorded in the solvent ratio of 7:3 having the distance travel by them to be 8.3 cm, in the ratio 8:2 five (5) spot positions were seen on the plate with distance travelling of 8.3 cm, the least spot positions were seen in the solvent ratio of 9:1 which was found to be four (4) with 8.4 cm distance travelling by the spot positions as seen in the Table 5 below.

Table 5. Thin layer chromatographic analysis of *S. hispanica* extract using three solvent

Plant Extract	Solvent System	Distance travel by the Spot	No of Spot	R.F Value
Methanol extract	HE : EA (7:3)	8.3	7	0.1, 0.2, 0.3, 0.4, 0.6, 0.9, 0.9
	HE : EA (8:2)	8.4	6	0.1, 0.2, 0.3, 0.4, 0.5, 0.5
	HE : EA (9:1)	8.4	7	0.1, 0.1, 0.2, 0.2, 0.3, 0.5, 0.6
Ethyl Acetate	HE : EA (7:3)	8.3	5	0.2, 0.5, 0.6, 0.8, 0.9
extract	HE : EA (8:2)	8.3	7	0.2, 0.3, 0.4, 0.5, 0.5, 0.8, 0.9
	HE : EA (9:1)	8.4	6	0.1, 0.2, 0.2, 0.3, 0.5, 0.8
Hexane	HE : EA (7:3)	8.3	6	0.1, 0.3, 0.5, 0.6, 0.6, 0.8
Extract	HE : EA (8:2)	8.3	5	0.3, 0.4, 0.7, 0.8, 0.9
	HE : EA (9:1)	8.4	4	0.1, 0.3, 0.5, 0.9

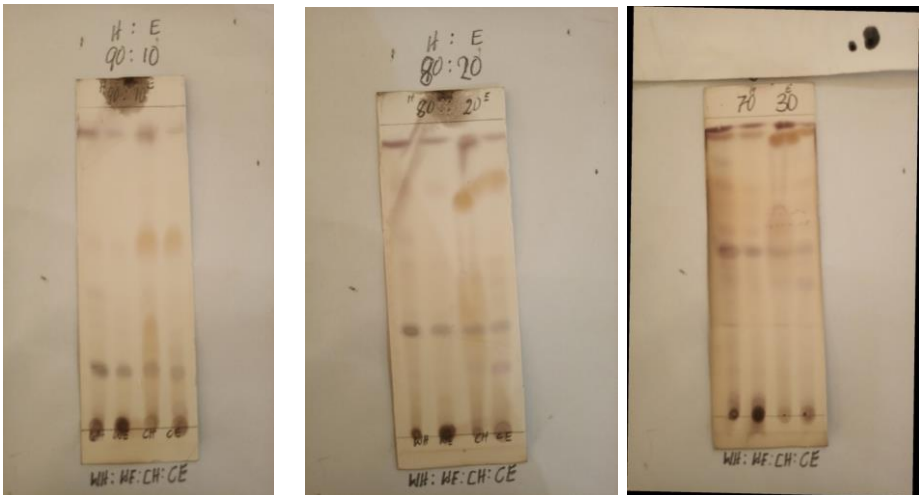

Figure 1: Thin layer chromatogram with showing separation of some compounds

Peak Position and Probable Inter-Atomic Bond of *Salvia hispanica* Methanol Extract by FTIR

The results of the FTIR analysis confirmed the presence of alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid and phenol. The absorbance band analysis in bioreduction process that were observed in the region between 400 - 4000 cm^{-1} are 1048, 1275, 1346, 1413, 1644, 2126, and 3339 cm^{-1}. Major peak was 3339 cm^{-1} that could be assigned to O_H Stretching vibration of alcohol and phenols functional group indicated the presence of different secondary metabolites (Steroid, terpenoids, saponins, phenols and carbohydrates). At 2126 cm^{-1} indicated the presence of C_H (Stretching), C=O Stretching of Carboxylic acid and ketone, Which shows the availability of Flavonoids, terpenoids, streroids, and saponins, 1644 cm^{-1} indicated the presence of C=C Stretching, N_H bending of primary amines, it shows the presence of Terpenoids, streroids, saponins and fatty acids. 1413 cm^{-1} indicated the presence of C_N stretching of aromatic amines, C_H bending, 1346 cm^{-1} shows the presence of $CH(CH_3)$ isobutyl and isopropyl which in turns indicated the presence of Alkaloid. 1275 and 1048 cm^{-1} indicated C_O stretching of aryl_ether and phenols (Anthraquinones present) and C_H stretching of aromatic hydrocarbon (Terpenoids, steroids, saponins, glycosides and carbohydrates present).

Table 6 Peak Position and Probable Inter-Atomic Bond of *Salvia hispanica* Methanol Extract by FTIR

IR spectra absorbtion (cm^{-1})	Functional group	Remark
3224	O_H Stretching vibration of alcohol and phenols	Steroids, Terpenoids Saponins Carbohydrates and Phenols
1640	C=C Stretching, N_H bending of primary amines	Terpenoids, Streroids, Saponins and Fatty Acids
1640	C=C Stretching, N_H bending of primary amines	Phenols, Terpenoids, Streroids, Saponins and Fatty Acids

Peak Position and Probable Inter Atomic Bond of *Salvia hispanica* Aqueous Extract by FTIR

The results of the FTIR analysis confirmed the presence of alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid and phenol. The absorbance band analysis in bioreduction process that was observed in the region between 400_4000cm^{-1} are 1640 and 3324cm^{-1}. 3324cm^{-1} that could be assigned to O_H Stretching vibration of alcohol and phenols functional group indicated the presence of different secondary metabolites (Steroid, terpenoids, saponins, phenols and carbohydrates). 1640cm^{-1} indicated the presence of C=C Stretching, N_H bending of primary amines, it shows the presence of Terpenoids, streroids, saponins and fatty acids. See table below.

Table 7 Peak Position and Probable Inter Atomic Bond of *S. hispanica* Aqueous Extract by FTIR

IR spectra absorbtion (cm^{-1})	Functional group	Remark
3339	O_H Stretching vibration of alcohol and phenols	Steroids, Terpenoids Saponins Carbohydrates and Phenols
2126	C_H (Stretching), C=O Stretching of Carboxylic acid and ketone	Flavonoids, Terpenoids, Streroids, and Saponins
1644	C=C Stretching, N_H bending of primary amines	Saponins Steroids, Terpenoids and Fatty Acids
1413	C_N stretching of aromatic amines, C_H bending	Alkaloids
1346	C_O stretching of aryl_ether and phenols	Alkaloids
1275	C_H stretching of aromatic hydrocarbon	Anthraquinone
1086	C_H stretching of aromatic hydrocarbon	Saponins Steroids, Terpenoids, Glycosides and Carbohydrates

Peak position and probable interatomic bond of Chia seed ethyl acetate extract by FTIR

The results of the FTIR analysis confirmed the presence of alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid and phenol. The absorbance band analysis in bioreduction process that were observed in the region between 400_4000cm^{-1} are 1093, 1275, 1324, 1462, 1652, 2355, and 3361cm^{-1}. Major peak was 3361cm^{-1} that could be assigned to
O_H Stretching vibration of alcohol and phenols functional group indicated the presence of different secondary metabolites (Steroid, terpenoids, saponins, phenols and carbohydrates). At 2355cm^{-1} indicated the presence of C_H (Stretching), C=O Stretching of Carboxylic acid and ketone, Which shows the availability of Flavonoids, terpenoids, streroids, and saponins, 1652cm^{-1} indicated the presence of C=C Stretching, N_H bending of primary amines, it shows the presence of Terpenoids, streroids, saponins and fatty acids. 1462cm^{-1} indicated the presence of C_N stretching of aromatic amines, C_H bending, it shows the presence of Alkaloids. At 1324cm^{-1} it shows the presence of CH(CH_3) isobutyl and isopropyl, CH deformation, _NH which indicated Alkaloid. 1275 and 1093cm^{-1} indicated C_O stretching of aryl_ether and phenols (Anthraquinones present) and C_H stretching of aromatic hydrocarbon (Terpenoids, steroids, saponins, glycosides and carbohydrates present).

Table 8. Peak position and probable inter atomic bond of *S. hispanica* ethyl acetate extract by FTIR

IR spectra absorbtion (cm^{-1})	Functional group	Remark
3361	O_H Stretching vibration of alcohol and phenols	Steroids, Terpenoids, Saponins, Phenols, Carbohydrates
2355	C_H (Stretching), C=O Stretching of Carboxylic acid and ketone	Flavonoids, Terpenoids, Saponins and Fatty Acids
1652	C=C Stretching, N_H bending of primary amines	Steroid Terpenoid, Saponin and Fatty Acid
1462	C_N stretching of aromatic amines, C_H bending	Alkaloids
1324	$CH(CH_3)$ isobutyl and isopropyl, CH deformation, _NH	Alkaloids
1275	C_O stretching of aryl_ether and phenols	Anthraquinones
1093	C_H stretching of aromatic hydrocarbon	Terpenoid, Steroid , Saponins, Glycosides, Carbohydrate

Peak position and probable inter atomic bond of Chia seed hexane extract by FTIR

The results of the FTIR analysis confirmed the presence of alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid and phenol. The absorbance band analysis in bioreduction process that were observed in the region between 400_4000cm^{-1} are 1123, 1246, 1465, 1648, 2925 and 3648cm^{-1}. Major peak was 3648cm^{-1} that could be assigned to O_H Stretching vibration of alcohol and phenols functional group indicated the presence of different secondary metabolites (Steroid, terpenoids, saponins, phenols and carbohydrates). At 2925cm^{-1} indicated the presence of C_H (Stretching), C=O Stretching of Carboxylic acid and ketone, Which shows the availability of Flavonoids, terpenoids, streroids, and saponins, 1648cm^{-1} indicated the presence of C=C Stretching, N_H bending of primary amines, it shows the presence of Terpenoids, streroids, saponins and fatty acids. 1465cm^{-1} indicated the presence of C_N stretching of aromatic amines, C_H bending, it shows the presence of Alkaloids. 1246 and 1123cm^{-1} indicated C_O stretching of aryl_ether and phenols (Anthraquinones present) and C_H stretching of aromatic hydrocarbon (Terpenoids, steroids, saponins, glycosides and carbohydrates present).

Table 9 Peak position and probable inter atomic bond of Chia seed hexane extract by FTIR

IR spectra absorbtion (cm^{-1})	Functional group	Remark
3648	O-H Stretching vibration of alcohol and phenols	Steroid, terpenoids, saponins, and phenols, carbohydrates
2925	C-H (Stretching), C=O Stretching of Carboxylic acid and ketone	Flavonoids, terpenoids, steroid, saponins
1648	C=C Stretching, N-H bending of primary amines	Steroid, terpenoids, saponins, and fatty acid
1465	C-N stretching of aromatic amines, C-H bending	Alkaloids
1246	C-O stretching of aryl-ether and phenols	Anthraquinones
1123	C-H stretching of aromatic hydrocarbon	Terpenoid, steroid , saponins, and glycosides, carbohydrate

DISCUSSION

Great variability in the physical and chemical characteristics of chia seed can be attributed to many factors, including the region where the plant was grown, climatic differences, fertility, soil pH and annual rainfall (Rodrigues, 2005). Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Scapin (2016) who worked on Chia seeds and found that moisture content may contribute to greater chemical and microbiological stability together with respect to ashes contents these data differ from the results found by Sargi *et al.* (2013), which found 78.6 g/kg for moisture and 36.3 g/kg to ashes. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body (Malhotra, 1998; Eruvbetine, 2003). Every form of living matter requires these inorganic elements or minerals for their normal life processes (Ozcan, 2003).

The elemental analysis revealed some of the elements that are present in the Chia seed are rich sources of elements that aid in the growth of plants, and as well in human body functions such as muscle contraction, bone formations, growth, metabolism, osmotic balance. The macro-minerals include calcium, phosphorus, sodium and chloride, while the micro-elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur (Eruvbetine, 2003). The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl (Murray *et al*., 2000).

The phytochemical screening of the chia seed **(***Silver hispanica***)** revealed the presence of tannins, carbohydrate, saponin flavonoids steroids and triterpens glycosides, anthraquinones while alkaloids phenol and resins were absent in the plant sample The ethyl acetate extract of the plant sample show the presence of carbohydrate, flavonoids and alkaloids while all other phytochemicals were absent. In the hexane extract more of the phytochemicals were seen than that of the ethyl acetate.

Resins is absent in all the solvent used, this may be as a result of small composition of nonpolar phytochemical constituents in the plant and the low polarity of hexane extracts (Harborne, 2009 and Namadina *et al*., 2019). Phenol was also absent in the plant extracts, however the result disagree with the work of Alhassan *et al*. (2014), whom both reported the presence of phenol in methanol extract. Nurul Syafiqah *et al*. (2019) found phenol in the chia seed in 2019. This is probable due to differences in weather and the ecology as well as geographical locations of the plant sources, time of collection and maturity. Michele *et al*. (2014) and Evans (2002) elaborated how geographical locations greatly affect the accumulation and diversity of the phytochemical. Glycoside was absent in ethyl acetate and hexane extracts. But Oyeleke *et al.* (2008) and Omonkhelin *et al.* (2009) reported that some members of plant family may possess some metabolites such as anthraquinones and cardiac glycosides (David 2005; Lin *et al*., 2019).

Plants that contain tannins are used as astringents, against diarrhea, diuretics, stomach and duodenal tumours (Aliyu 2006; Saxena *et al.*, 2013). Flavonoids in plants possess medicinal benefits which includes antioxidant and anti-inflammatory activities (Aliyu 2006; Saxena *et al*., 2012). They have the ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu, 2004; Okwu and Josiah, 2006). The flavonoid content of the plants samples supports its use for protection against diseases such as cancer and inflammation (Onyeka and Nwambekwe, 2007). Alkaloids is one of the important phytochemical found in both the plant sample, it possess lots of pharmacological and pharmaceutical properties and are used as medicines, as recreational drugs, or in entheogenic rituals (David 2019). The quality and quantity of the alkaloids present in the plants are varied depending on the type of plants and parts or tissue of the plants.

Tiwari and Rao (2002) reported that herbal therapies have the synergistic, potentiative, agonistic/antagonistic and pharmacological agents within themselves that work together in a dynamic way to produce therapeutic efficacy with minimum side effects. On the Chia seed extract *salvia hispanica* the methanol extract of when developed Hexane:Ethyl acetate (7:3, 8:2 and 9:1) and sprayed with *p*-Anisaldehyde/H_2SO_4 reagent for visualization, it gave seven (7) and six (6) and seven (7) spots respectively mostly purple colour when viewed under shot wave and long wave (254 nm and 365 nm respectively) alongside their Rf values indicating the presence of steroids or triterpenes. Ethyl acetate extract of the chia seed gave five (5), seven (7) and six (6) spots in the three (3) ratios with their Rf values and were purple and blue-black colours were seen under shot wave and long wave (254 nm and 365 nm respectively) when developed in Hexane: Ethyl acetate and virtualized with *p*-Anisaldehyde/H_2SO_4. This indicates that ethyl acetate extract is rich in flavonoids and alkaloids.

Hexane extract of the chia seed (*Salvia hispanica)* when developed in Ethyl acetate: hexane in the ratio (7:3, 8:2 and 9:1) and virtualized with *p*-Anisaldehyde/H_2SO_4, it gave six (6), five (5), and four (4) spots respectively with their Rf values and were purple and blue-black in colours. This indicates that hexane extract is rich in steroids/triterpenes, flavonoids and alkaloids. This has agrees with the result of the preliminary phytochemical screening since all of them were present in the study and has also agree with the work of (Da Silva *et al*., 2019) which found that steroids and triterpens as well as flavonoids to be absent.

The Thin Layer Chromatography chemical screening is usually done to target isolation

of new or very important constituent present in the chia seed plant extract which has marked pharmacological activities and also serves as an important agent in recognizing how metabolite for isolation behaved and can be purified; thereby, channeling scientific efforts in the desired compound(s) and prevent waste of resources and time as stated by (Patra *et al.*, 2012).

The Chia seed analysis of the FTIR using methanol extract has the highest number of absorbance among all the solvent used. The result is shown in Table 14, analysis confirmed the presence of alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid and phenol. The absorbance band analysis in bio reduction process that were observed in the region between 400 – 4000 cm^{-1} are 1048, 1275, 1346, 1383, 1413, 1566, 1644, 2126, and 3339 cm^{-1}. Major peak was 3339 cm^{-1} that could be assigned to ≡C-H stretching mode hydrogen bond (Alcohol), N-H stretching mode, O-H stretching mode (carboxylic acid), N-H stretching mode (primary amine gives two band and secondary amine gives one band. Presence of ≡C-H functional group indicated the presence of different secondary metabolites (Steroid, terpenoids saponins carbohydrates and fatty acids). 2126 cm^{-1} indicated the presence of C≡C stretching mode (Flavonoids saponins steroids and terpenoids were present). 1644 cm^{-1} indicated the presence of C=C stretching mode, C=O stretching mode aldehyde with two aromatic ring, C=O stretching mode Amide, it shows the presence of Fatty acid, saponins steroids and terpenoids. 1566 cm^{-1} indicated the presence of C=C stretching mode (Aromatic Ring), N-H bending in primary Amine, N-H bending mode Amide, NO_2 Asymetric stretching mode (Aliphatic Nitro) indicating the presence of steroid, saponins, terpenoids, fatty acids and coumarins. 1413 cm^{-1} C-O-H Bending mode group 1346 cm^{-1} and 1383 cm^{-1} both peak indicated C-O-H Bending mode, NO2 symmetric stretching mode Aliphatic Nitro, C-F Stretching mode (Alkaloids present in both). 1275 cm^{-1}C-O-H Bending mode, C-O stretching mode (Ether), C=O bending mode, C-O stretching mode (carboxylic acid), C-O stretching Mode C-F stretching mode indicating the presence of (Flavonoids saponins steroids and terpenoids). The last peak is 1048 cm^{-1} C-O stretching mode (Alcohol), C-O stretching mode (Ether), C-O Stretching Mode C-F stretching mode, showing Steroids, terpenoids, saponins, glycosides and carbohydrates.

The inter-atomic bond of the chia seed using the aqueous extract shows alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid and phenol. The absorbance band analysis in bioreduction process was observed in the region between 400 – 4000 cm^{-1} are 1640 and 3324 cm^{-1}. Major peak was 3324 cm^{-1} that could be assigned to ≡C-H stretching mode hydrogen bond O-H bond (alcohol), N-H stretching mode, O-H stretching mode (carboxylic acid), N-H stretching mode (primary amide gives two band and secondary amide gives one band. Presence of ≡C-H functional group indicated the presence of different secondary metabolites (Phenol, steroids, saponing, terpenoids, and carbohydrates). 1640 cm^{-1} indicated the presence of C=C Stretching mode, N-H Bending primary amine, C=O stretching mode conjugate of Aldehyde with two Aromatic rings, C-O stretching Mode Amide, N-H bending amide indicating the presence of Fatty acid, saponins steroids and terpenoids.

Ethyl acetate extract of the Chia seed show a lots of promising band in the FTIR region among the absorbance band include the following 1093, 1167, 1275, 1324, 1462, 1652, 2355, and 3361 cm^{-1}. Major peak was 3361 cm^{-1} that could be assigned to ≡C-H stretching mode hydrogen bond (alcohol), N-H stretching mode, O-H stretching mode (carboxylic acid).

Presence of ≡C-H functional group indicated the presence of different secondary metabolites (Steroid, terpenoids, saponins, phenols and carbohydrates). 2355 cm^{-1} indicated the presence of C≡N stretching mode indicating flavonoids, terpenoids, saponins and fatty acids, 1652 cm^{-1} C=C stretching mode and C=O stretching mode conjugate of aldehyde with two aromatic rings C=O stretching mode amide, this peak show the presence of Steroid terpenoid, saponin and fatty acid. 1462 cm^{-1} N-H bending mode in secondary amine, it shows the presence of alkaloids. 1324 cm^{-1} indicated the presence of C-O-H bending mode NO_2, symmetric stretching mode, (aliphatic), and NO_2 symmetric stretching mode (aromatic nitro) alkaloid present. 1275 cm^{-1} C-O-H bending mode group 1346 cm^{-1} and 1383 cm^{-1} both peak indicated C-O-H bending mode, NO_2 symmetric stretching mode aliphatic nitro, C-F Stretching mode (anthraquinones). 1167 cm^{-1} C-O stretching mode (alcohol), C-O stretching mode (ether), C=O bending mode C-O stretching mode showing the presence of anthraquinones. The last peak is 1093 cm^{-1} C-O stretching mode (Alcohol), C-O stretching mode (Ether), C-O stretching mode, Steroids, terpenoids, saponins, glycosides and carbohydrates present.

Hexane extract of the chi seed was determine on the FTIR absorbance with 3648 O-H stretching mode and it shows the presence of flavonoids, terpenoids, steroids, saponins and carbohydrate others bands include 2925 cm^{-1} indicated the presence of O-H stretching mode (carboxylic acid) flavonoids terpenoids, steroids and saponins. 1748 cm^{-1} C=O stretching mode (esters), this peak show the presence of glycosides, phenol, terpenoides, carbohydrates, and coumarines 1465 cm^{-1} N-H bending mode in secondary amine, it shows the presence of Alkaloids. 1246cm^{-1} C-O-H bending mode, C-O stretching mode (ether), C=O bending mode, C-O stretching mode (carboxylic acid), C-O stretching mode C-F stretching mode indicating the presence of (glycosides, phenol, terpenoides, carbohydrates, and fatty acid). 1167 cm^{-1} C-O stretching mode (alcohol), C-O stretching mode (ether), C=O bending mode C-O stretching mode showing the presence of anthraquinones.

CONCLUSION

Based on the above analysis Chia seed, it could be concluded that plants are a good source of natural product. The natural products will enable the discovery of drugs to treat diseases. Further work is needed to discover new natural products to replace the allopathic drugs.

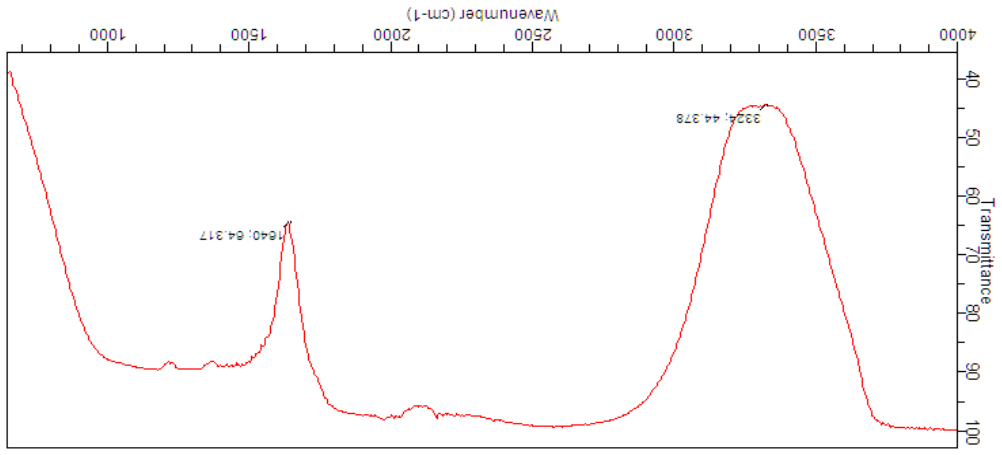

Figure 2. FTIR spectra of Chia seed aqeous extract

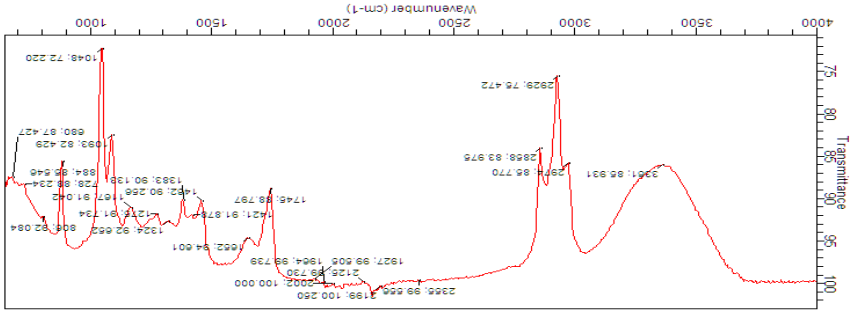

Figure 3. FTIR spectra of Chia seed ethanol extract

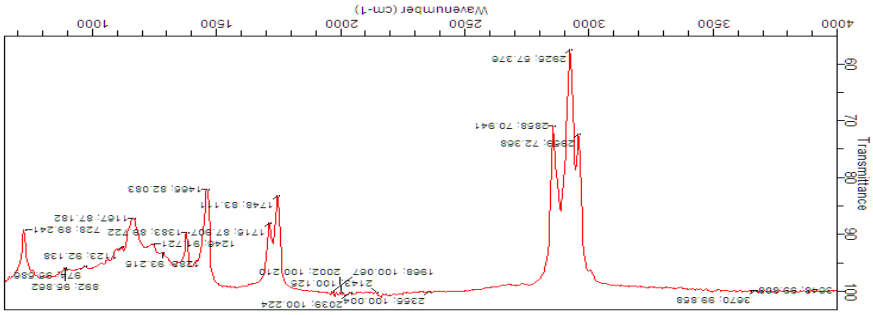

Figure 4. FTIR spectra of Chia seed hexane extract

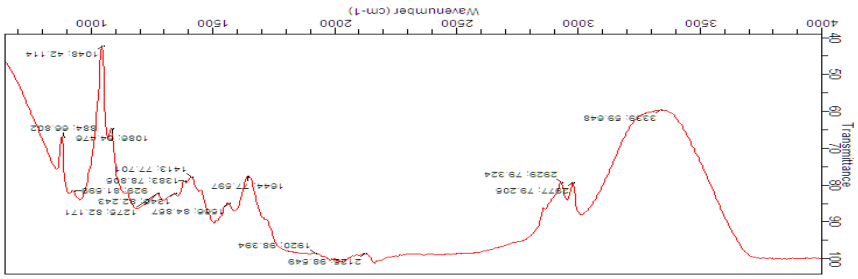

Figure 5. FTIR spectra of Chia seed methanol extract

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