IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 5 Ser. VII (Sep. –Oct. 2020), PP 14-22
www.Iosrjournals.Org

Phytochemical Profile of seeds of *Guizotia abyssinica* (L. f.) Cass (Niger or Ramtil)

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Abstract: *Botanical profile: Guizotia abyssinica (L. f.) cass. of family Asteraceae (Compositae) is an oilseed crop cultivated in Ethiopia and India. It is a moderately to well branched herb which grows up to 2 m tall. The seed contains about 40% oil with fatty acid composition of 75-80% linoleic acid, 7-8% palmitic and stearic acids, and 5-8% oleic acid.*

In the present investigation phytochemical profile of Niger seeds was assayed qualitatively and quantitatively in six solvent extracts viz. acetone, petroleum ether, ethanol, methanol, benzene and distilled water. The results revealed the presence of various phytochemicals such as tannins, phlobatannins, saponins, terpinoids, diterpinoids, emodins, flavonoids, cardiac glycosides, anthraquinones, carotenoids, reducing sugars, alkaloids, anthocyanin, coumarins, steroids, phytosterols, phenol, fatty acids, proteins and amino acids. terpenoid, diterpinoid, emodin, cardiac glycoside, anthraquinone, carotenoid, reducing sugar, alkaloids, anthocyanin, coumarin, fatty acid and protein were detected in all the six solvent extracts. Tannins, Flavonoids, Saponins, Phenols and amino acids were not detected in ethanol and methanol extracts. Phlobatannin, steroid and phytosterol were also not detected in ethanol, methanol and distilled water extracts. Emodin was detected in all extracts except petroleum ether and benzene. The seeds of Guizotia abyssinica contained a significant amount of alkaloid, flavonoids, phenolic, saponins and tannin content. The amount of flavonoids was maximum (51.75mg/gm) followed by phenols (30.85mg/gm), alkaloids (20.75mg/gm), saponins (19.65mg/gm) and tannins (17.45mg/gm).

This versatile plant is the source of many bioactive compounds. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study.

Key Words: *Guozotia abyssinica, Niger, Ramtil, Phytochemicals, acetone, petroleum ether, ethanol, methanol, benzene and distilled water.*

Date of Submission: 15-10-2020 Date of Acceptance: 31-10-2020

I. Introduction

Botanical profile: *Guizotia abyssinica* (L. f.) cass. commonly known as Niger or Ramtil belongs to family Asteraceae (Compositae). It is an oilseed crop cultivated in Ethiopia and India. It constitutes about 50% of Ethiopian and 3% of Indian oilseed production. In Ethiopia, it is cultivated on waterlogged soils where most crops and all other oilseeds fail to grow and contributes a great deal to soil conservation and land rehabilitation.

It is a moderately to well branched herb which grows up to 2 m tall. The seed contains about 40% oil with fatty acid composition of 75-80% linoleic acid, 7-8% palmitic and stearic acids, and 5-8% oleic acid (Getinet and Teklewold, 1995) [1]. The Indian types contain 25% oleic and 55% linoleic acids (Nasirullah *et al*., 1982) [2]. The meal remaining after the oil extraction is free from any toxic substance but contains more crude fiber than most oilseed meals.

Germination is epigeal and seedlings have pale green to brownish hypocotyls and cotyledons (Seegeler, 1983) [3]. The cotyledons remain on the plant for a long time. The first leaf is paired and small and successive leaves are larger. The leaves are arranged on opposite sides of the stem; at the top of the stem leaves are arranged in an alternate fashion. Leaves are 10-20 cm long and 3-5 cm wide. The leaf margin morphology varies from pointed to smooth and leaf colour varies from light green to dark green, the leaf surface is smooth. The stem of Niger is smooth to slightly rough and the plant is usually moderately to well branched. Niger stems are hollow and break easily. The number of branches per plant varies from five to twelve and in very dense plant stands fewer branches are formed. The colour of the stem varies from dark purple to light green and the stem is about 1.5 cm in diameter at the base. The plant height of Niger is an average of 1.4 m, but can vary considerably

as a result of environmental influences and heights of up to 2 m have been reported from the Birr valley of Ethiopia. The Niger flower is yellow and, rarely, slightly green. The heads are 15-50 mm in diameter with 5-20 mm long ray florets. Two to three capitulae (heads) grow together, each having ray and disk florets. The receptacle has a semi-spherical shape and is 1-2 cm in diameter and 0.5-0.8 cm high. The receptacle is surrounded by two rows of involucral bracts. The capitulum consists of six to eight fertile female ray florets with narrowly elliptic, obovate ovules. The stigma has two curled branches about 2 mm long. The hermaphrodite disk florets, usually 40-60 per capitulum, are arranged in three whorls. The disk florets are yellow to orange with yellow anthers, and a densely hairy stigma. The achene is club-shaped, obovoid and narrowly long (Seegeler, 1983) [3]. The head produces about 40 fruits. The achenes are black with white to yellow scars on the top and base and have a hard testa. The embryo is white. Niger is usually grown on light poor soils with coarse texture (Chavan, 1961) [4]. It is either grown as a sole crop or intercropped with other crops. When intercropped it receives the land preparation and cultivation of the main crop. In Ethiopia it is mainly cultivated as a sole crop on clay soils and survives on stored moisture. A more detailed description on the agronomy of Niger is presented under Agronomy.

In Africa, *G. abyssinica* is largely found in the Ethiopian highlands, particularly west of the Rift Valley. Niger is also found in some areas in Sudan, Uganda, Zaire, Tanzania, Malawi and Zimbabwe, West Indies, Nepal, Bangladesh, Bhutan and India (Weiss, 1983) [5].

India is the largest producer and exporter of Niger (Chavan, 1961) [4]. It is cultivated in Andra Pradesh, Madhya Pradesh, Orissa, Maharashtra, Bihar, Karnataka, Nagar Haveli and West Bengal states of India of which Madhya Pradesh is the largest. During 1938 to 1948 India exported up to 6968 tons of Niger annually to Western Europe, Eastern Europe and North America.

Systematic Position: Class: Dycotyledons; Sub class: Gamopetalae; Series: Inferae; Order: Asterales; Family: Compositae (Syn. Asteraceae); Genus: *Guizotia*; Species: *G. abyssinica*

Oil Content of Niger: The oil content of Niger seed varied from 30 to 50% (Seegeler, 1983) [3]. Niger meal remaining after the extraction of oil contains approximately 30% protein and 23% crude fiber. In general the Ethiopian niger meal contains less protein and more crude fiber than the Niger meal grown in India (Chavan 1961; Seegeler 1983) [4, 3]. The oil, protein and crude fiber contents of Niger are affected by the hull thickness and thick-hulled seeds tend to have less oil and protein and more crude fiber. Niger oil has a fatty acid composition typical for seed oils of the plants of Compositae family (e.g. safflower and sunflower) with linoleic acid being the dominant fatty acid. The linoleic acid content of Niger oil was approximately 55% in seeds grown in India (Nasirullah *et al.,* 1982) [2] and 75% in seeds grown in Ethiopia (Seegeler, 1983; Getinet and Teklewold, 1995) [3, 1].

Ranges of Fatty acid composition (%) of Indian and Ethiopian Niger oil as determined by Nasirullah et al., 1982; Nagaraj, 1990; Getinet and Teklewold, 1995 are as follow:

Fatty acid	India[1]	India[2]	Ethiopia[3]
Palmitic acid	8.2-8.7	6.0-9.4	7.6-8.7
Stearic acid	7.1-8.7	5.0-7.5	5.6-7.5
Oleic acid	25.1-28.9	13.4-39.3	4.8-8.3
Linoleic acid	51.6-58.4	45.4-65.8	74.8-79.1
Linolenic acid	-	-	0.0-0.9
Arachidic acid	0.4-0.6	0.2-1.0	0.4-0.8
Behenic acid	-	-	0.4-1.5

1. Nasirullah *et al*., 1982 [2]. Nagaraj, 1990 [6]. Getinet and Teklewold, 1995 [1].

Dutta *et al.,* (1994) [7] studied the lipid composition of three released and three local cultivars of Ethiopian niger. Most of the total lipid was triacylglycerides and polar lipids accounted for 0.7-0.8% of the total lipid content. The amount of total tocopherol was 720-935 µg/g oil of which approximately 90% was a-tocopherol, 3-5% was g-tocopherol and approximately 1% was ß-tocopherol. As a-tocopherol is an anti-oxidant, high levels of a-tocopherol could improve stability of niger oil. The total sterol consists of ß-sitosterol (38-43%), campesterol (~14%), stigmasterol (~14%), D5 avenasterol (5-7%) and D7 avenasterol (~4%). The amino acid composition of niger protein was deficient in tryptophan. The protein quality of Ethiopian niger was evaluated using chemical score and essential amino acid requirement score (Haile, 1972) [8]. Using chemical score and whole egg protein as a standard, methionine, lysine, cystine, isoleucine and leucine were considered as limiting amino acids. When essential amino acids were used as a reference, lysine was the limiting amino acid. A lipoprotein concentrate was isolated from niger seed using hot water/ethanol sodium chloride solution extraction (Eklund 1971a, 1971b) [9, 10]. The lipoprotein contained 4% moisture, 12% ash, 46% protein, 20% fat, 7% crude fibre and 11% soluble carbohydrate. From the amino acid composition Eklund (1971a, 1971b) [9,

10] calculated nitrogen to protein conversion ratio of 5:9. The energy content of the niger lipoprotein concentrate was 400 kcal/100 g.

Amino acid composition of whole niger flour, Niger seed lipid concentrate, high temperature soluble (HTS) fraction concentrate, Indian Niger cake, and Ethiopian Niger meal as determined by Eklund (1974) [11], Mohan *et al*., (1983) [12] and Haile (1972) [8] are as follows:

Amino acid	Whole niger seed flour[1]	Niger seed lipid-protein concentrate	HTS Fraction[1]	Niger cake[2]	Niger meal (% of protein)3
Isoleucine	**307**	**341**	**201**	**349**	**4.66**
Leucine	**388**	**505**	**308**	**589**	**6.99**
Lysine	**294**	**279**	**199**	**335**	**4.74**
Methionine	**109**	**125**	**216**	**148**	**2.06**
Cytisine	**177**	**97**	**537**	**138**	**1.40**
Phenylelanine	**327**	**385**	**130**	**378**	**4.80**
Tyrosine	**185**	**225**	**138**	**197**	**-**
Threonine	**237**	**263**	**112**	**278**	**3.73**
Tryptophan	**54**	**85**	**65**	**-**	**-**
Valine	**362**	**397**	**273**	**428**	**5.76**
Arginine	**621**	**627**	**734**	**889**	**9.36**
Histidine	**162**	**192**	**97**	**190**	**-**
Alanine	**281**	**290**	**132**	**335**	**4.06**
Aspartic acid	**619**	**673**	**427**	**823**	**9.49**
Glysine	**375**	**357**	**295**	**502**	**5.53**
Proline	**262**	**370**	**222**	**354**	**3.86**
Serine	**347**	**390**	**390**	**456**	**6.19**

1. **Eklund (1974), Samples from Ethipia (mg/g N.);**
2. **Mohan *et al*., (1983). Based on samples from India (mg/g N)**
3. **Haile (1972) based on samples from Ethiopia (% of protein).**

The Niger plant is consumed by sheep but not by cattle, to which only Niger silage can be fed (Chavan, 1961) [4]. Niger is also used as a green manure for increasing soil organic matter. Niger seed is used as a human food. The seed is warmed in a kettle over an open fire, crushed with a pestle in a mortar and then mixed with crushed pulse seeds to prepare 'wot' in Ethiopia (Seegeler, 1983) [3]. 'Chibto' and 'litlit' are prepared from crushed Niger seed mixed with roasted cereals, and is the preferred food for young boys. In Ethiopia, Niger is mainly cultivated for its edible oil. The pale yellow oil of Niger seed has a nutty taste and a pleasant odour. The traditional method for extraction of oil from Niger in Ethiopia is through a combination of warming, grinding and mixing with hot water followed by centrifugation in an 'ensera' (a container made of clay). After an hour of centrifugation by hand on a smooth soft surface the pale yellow oil settles over the meal. Niger is also crushed in small cottage expellers and large oil mills. The small, electrically powered cottage expellers are manufactured as different brands with varying capacities in Addis Abeba and Nazreth in Ethiopia. The meal remaining after extraction of the oil using Ethiopian expellers contains 6-12% oil depending on the expeller. Many expellers are found in the provinces of Arsi, Bale, Gojam, Gonder, Shoa and Wellega of Ethiopia.

In India the oil is extracted by bullock-powered local 'ghanis' and rotary mills (cottage expellers) or in mechanized expellers and hydraulic presses in large industrial areas. The Niger oil is used for cooking, lighting, anointing, painting and cleaning of machinery (Chavan 1961; Patil and Joshi 1978; Patil and Patil 1981) [4, 13, 14]. Niger oil also is a substitute for sesame oil for pharmaceutical purposes and can be used for soap-making. The meal remaining after the oil extraction contains about 24% protein and 24% crude fiber (Seegeler, 1983) [3]. Niger meal from India contains higher protein (30%) and lower crude fiber (17%) levels than meal from Ethiopia. Niger cake replacing linseed cake at levels of 0, 50 and 100% was fed as a nitrogen supplement for growing calves (Singh *et al*., 1983) [15]. No significant differences in growth rate, feed efficiency and dry matter digestibility were noticed between Niger and linseed cake and it was concluded that Niger cake can replace linseed cake in calf rations (Singh *et al.*, 1983) [15]. Similarly, four levels of Niger cake (0, 50, 75 and 100%) replacing groundnut cake were fed to large White Yorkshire pigs for 9 weeks (Roychoudhury and Mandal 1984) [16]. There was no significant difference in weight gain between rations containing either Niger or groundnut cake. Niger lipoprotein concentrate was fed to growing rats as a sole protein source for 90 days and no negative effects on growth rate were observed (Eklund, 1971b) [10]. A Niger-based agar medium can be used to distinguish *Cryptococcus neoformans* (Sant) Vaill, a fungus that causes a serious brain ailment, from other fungi (Paliwal and Randhawa 1978) [17]. There are reports that Niger oil is used for birth control and for the treatment of syphilis (Belayneh, 1991). Niger sprouts mixed with garlic and 'tej' are used to treat coughs.

Bioactive Compounds of Niger: The GC-MS analysis by Chambhare *et al*., (2017) [18] showed the presence of potential bioactive compounds; most of these are fatty acids which are known to have antimicrobial properties. These constituents include, β-ocimene, 1-Dodecene, n-hexadecanoic acid, stigmasterol, 24S 5-stigmast-7-en-3-ol; 9,12-octadecadienoic acid (Z,Z)-methyl ester; trans-13-octadecanoic acid;tetradecanoic acid, hexadecanoic acid, octadecanoic acid.

The aim of the present investigation was to assay the phytochemicals present in the seeds *of Guizotia abyssinica.*

II. Materials and Methods

In the present investigation the dried seeds of *Niger* were powdered using a mixture grinder and stored in air-tight container for future use. Six different solvents (five non polar viz. Acetone, Petroleum ether, Ethanol, Benzene and Methanol and one polar solvent, the Distilled water) were used for preparation of solvent extracts. The dried seed sample was soaked separately with acetone, petroleum ether, ethanol, benzene, methanol and distilled water under reflux condition for the solvent extract preparation. About 1 gm of the dried sample of seeds was added respectively into the test tubes containing 5 ml of solvents, and was extracted at room temperature. The important phytochemicals of seeds of *Niger* have been qualitatively and quantitatively analyzed for alkaloids, flavonoids, tannins, saponins and total phenols.

Phytochemical Analysis: Phytochemicals in seeds of *Guizotia abyssinica* were analyzed qualitatively and quantitatively in all the six solvent extracts

Qualitative Phytochemical Analysis

The extracts in all the six solvents of seeds of *Guizotia abyssinica* were tested for the presence of biological compounds by using following standard methods.

Test for Carbohydrates

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Iodine test: Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for Phenols and Tannins

Crude extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue–green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoid

Alkaline reagent test: Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins (Frothing test): Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

Test for Glycosides

Liebermann's test: Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test: Crude extracts were mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test (Cardiac Glycosides): Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the inter phase indicated the presence of cardiac glycoside.

Test for Alkaloids: The crude extract of all the six solvents was boiled in 10 ml methanol and filtered separately. 1% HCl was added followed by 6 drops of Dragendroff reagent, and the brownish-red precipitate was taken as evidence for the presence of alkaloids.
Phlobatannins: The deposition of a red precipitate denoted the presence of phlobatannins when crude extract of all the six solvent of plant material was dissolved in 10 ml of aqueous extract and few drops of 1% HCl were added in the boiling tube.

Anthraquinones: All the six solvent extracts of leaves were boiled in 10% HCl for 5 mins separately and the filtrate was allowed to cool. An equal volume of $CHCl_3$ with few drops of 10% NH_3 was added to the 2ml filtrate. The formation of rose-pink colour implies the presence of anthraquinones.

Quantitative estimation of phytochemicals
Determination of Alkaloids: Alkaloids content was measured by method suggested by Harborne (Harborne, 1973) [19]. A suspension was prepared by dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at 28^0C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^0C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

Determination of Flavonoids: The flavonoids content was also determined by Harborne (Harborne, 1973) [19] method. 5 gm of seed powder were boiled in 2M HCl for 30 min under reflux condition and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as mg/g.

Determination of Tannins: The finely powdered seeds of *Niger* were kept in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80^0C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

Determination of Saponins: 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% FeCl3 solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

Determination of total phenols: Five gms of the powdered seeds were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

For measuring alkaloids a suspension was prepared by dispersing 5 gm of the dried powdered seeds in 10% acetic acid solution in ethanol and kept at 28^0C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^0C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

For determining flavonoids 5 gm of dried seed powder were boiled in 2M HCl for 30 min under reflux and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and reported as mg/g.

For measuring tannin the finely powdered seeds of *Niger* were kept in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80^0C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of

17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

For determining saponin content 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered seed sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% $FeCl_3$ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

For determining total phenolic content five gms of the powdered leaves were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

Quantitative analysis of phytochemical constituents in six different solvent extracts

Six solvent extract of seeds of *Niger* viz. acetone, petroleum ether, ethanol, methanol, benzene and distilled water were prepared by soaking 10gm of the powdered sample in 200 ml of each of the solvent separately for 12 hrs. The extracts were then filtered using filter paper. The extracts were then concentrated to ¼ of the original extracts i.e. 50 ml.

The amount of total phenolics in extracts was determined by the Folin–Ciocalteu method. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE /gm) extract. Different aliquots of 0.1 to 1.0 ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature. Phenols react with the phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue). The absorbance of the resulting solutions was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. The concentration of total phenol in the test sample was determined from the calibration graph. The assay was carried out in triplicate and the mean values with ± SD are presented.

The aluminium chloride colorimetric method was used for flavonoids determination. Each solvent extract (0.5 ml of 1:10 gm ml^{-1}) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Quercetin (200-1000µg) plotted by using the same procedure and total flavonoids was expressed as Quircetin equivalents (QE) in mg per gm sample.

The results obtained have been presented in Table-1, 2 and 3; Figure-1 and 2.

Table- 1: Phytochemicals of seeds of *Guizotia abyssinica* analysed qualitatively in six different solvent extracts

Solvent extracts of leaves	Ta	Phl	Sap	Terp	Dter	Emd	Fla	Car	Anth	Crt	Res	Alk	Anc	Cou	Str	Pst	Phe	Fa	Prt	Aa
Acetone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Petroleum ether	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ethanol	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+
Methanol	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	-
Benzene	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Distilled water	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+

Ta= Tannin; Phl= Phlobatannin; Sap= Saponin; Ter= Terpinoid; Dtr = Diterpinoid ; Emd= Emodin ; Fla= Flavonoid; Car= Cardiac glycoside; Anth= Anthraquinones; Crt= Carotenoids; Res= Reducing sugar; Alk= Alkaloid; Anc= Anthocyanin; Cou= Coumarin; Str= Steroids; Pstr= Phytosterol; Phe= Phenol; FA= Fatty acids; Prt= Protein; Aa= Aminoacids

Table- 2: Quantitative estimation of Phytochemicals in dried seeds of *Guizotia abyssinica*

Phytochemicals	Amount in mg/gm
Alkaloids	20.75±0.23
Flavonoids	51.75±0.35
Phenols	30.85±0.61
Saponins	19.65±0.26
Tannins	17.45±0.27

Mean ± SD of five measurements

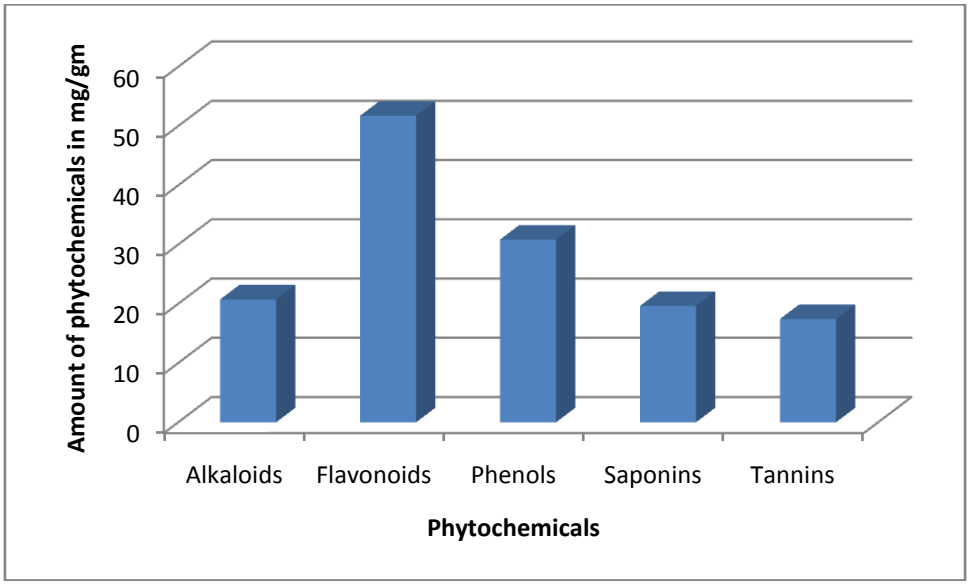

Figure-1: Phytochemicals present in the dried seeds of *Guizotia abyssinica*

Table- 3: Comparative analysis of total Alkaloids, Flavonoids, Phenol, Saponins and Tannins in six different solvent extracts of dried seeds of *Guizotia abyssinica* (amount in mg/gm)

Solvent Extracts	Total Alkaloids	Total Flavonoids	Total Phenol	Total Saponins	Total Tannin
Acetone	27.46±0.35	32.57±0.43	21.64±0.65	21.55±0.31	21.35±0.18
Petroleum ether	17.75±0.25	34.65±0.17	25.65±0.61	22.45±0.23	21.55±0.13
Ethanol	25.76±0.35	12.75±0.15	11.34±0.24	12.75±0.22	9.25±0.21
Methanol	27.65±0.17	12.45±0.16	11.41±0.21	13.38±0.31	9.25±0.15
Benzene	16.77±0.16	38.35±0.41	24.85±0.11	25.25±0.45	17.35±0.14
Distilled water	41.82±0.17	41.77±0.25	26.35±0.12	35.35±0.41	23.45±0.15

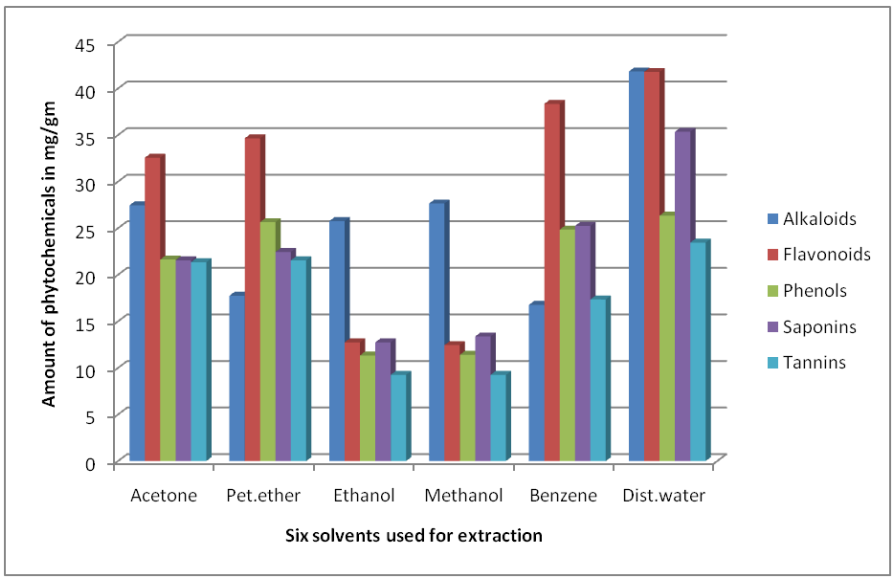

Figure-2: Comparative analysis of phytochemicals in six different solvent extracts of seeds of *Guizotia abyssinica*

Tatal phenol in mg/gm is measured as Gallic Acid Equivalent (GAE/g extract); Total flavonoids in mg/gm is measured as Quercetin Equivalent (QE)/g extract.
Mean ± SD of five measurements

III. Results

The present study was carried out on six solvent extracts of dried seeds of *Guizotia abyssinica* to investigate the presence of important phytochemicals. All the six extracts revealed the presence of various phytochemicals such as tannins, phlobatannins, saponins, terpinoids, diterpinoids, emodins, flavonoids, cardiac glycosides, anthraquinones, carotenoids, reducing sugars, alkaloids, anthocyanin, coumarins, steroids, phytosterols, phenol, fatty acids, proteins and amino acids (Table-1) Of these 20 phytochemicals terpenoid, diterpinoid, emodin, cardiac glycoside, anthraquinone, carotenoid, reducing sugar, alkaloids, anthocyanin, coumarin, fatty acid and protein were detected in all the six solvent extracts. Tannins, Flavonoids, Saponins, Phenols and amino acids were not detected in ethanol and methanol extracts. Phlobatannin, steroid and phytosterol were also not detected in ethanol, methanol and distilled water extracts. Emodin was detected in all extracts except petroleum ether and benzene (Table-1).

From the results (Table- 2; Fig- 1) it is evident that the dried seeds of *Guizotia abyssinica* contained a significant amount of alkaloid, flavonoids, phenolic, saponins and tannin content. The amount of flavonoids was maximum (51.75mg/gm) followed by phenols (30.85mg/gm), alkaloids (20.75mg/gm), saponins (19.65mg/gm) and tannins (17.45mg/gm) (Table- 2; Fig- 1).

The comparative analysis of phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts from dried seeds of Niger has been presented in Table- 3 and Fig- 2. From the results it is evident that the concentration of total alkaloids was maximum in distilled water extract (41.82mg/gm), followed by methanol extract (27.65mg/gm), acetone extract (27.46 mg/gm), ethanol extract (25.75mg/gm), petroleum ether extract (17.75mg/gm), benzene extract (16.77mg/gm). The concentration of total flavonoids was minimum in ethanol and methanol extracts (11.34mg/gm and 11.41mg/gm respectively). The distilled water extract contained maximum amount of total flavonoids (41.77mg/gm), followed by benzene extract (38.35mg/gm), petroleum ether extract (34.65mg/gm) and acetone extract (32.57mg/gm). The amount of total phenol was minimum in ethanol and methanol extracts (15.35 and 15.45mg/gm respectively), followed by distilled water extract (12.33mg/gm), benzene and petroleum ether extracts (11.34 and 11.41mg/gm respectively). The distilled water extract contained 26.35 mg/gm of total phenol, followed by petroleum ether extract (25.65 mg/gm), benzene extract (24.85 mg/gm) and acetone extract (21.64mg/gm). Saponin concentration was minimum in ethanol and methanol extract (12.75mg/gm and 13.38mg/gm respectively) and maximum in distilled water extract (35.35mg/gm), followed by benzene extract (25.25 mg/gm)petroleum ether extract (22.45 mg/gm) and acetone extract (21.55 mg/gm). The total tannin concentration was minimum in ethanol and methanol extract (9.25mg/gm), followed by petroleum ether and acetone extract (21.35 mg/gm) and benzene extract (17.35 mg/gm (Table- 3; Fig- 2).

IV. Discussion

The dried seed extracts of *Guizotia abyssinica* showed the presence of terpenoids, steroids and phytosterols, tannins, alkaloids, glycosides, saponins, reducing sugars, phenols and flavonoids. The extraction of various phytochemicals was seen to be more effectively done in polar solvents like distilled water than the non polar (Acetone, petroleum ether, benzene) solvents. Especially, the distilled water extract of seeds showed presence of most of the tested phytochemicals. Hence, it can be reported that the universal polar solvent extract was the best one for extracting the active principle than alcoholic extracts. Flavonoids are water-soluble polyphenolic compounds which are extremely common and widespread in the plant kingdom as their glycosides. The flavonoids are known to act through scavenging or chelating process. The present findings gain support from the work of Bansi Shashi Kala *et al*., (2018) [20] who have studied the phytochemicals of dried seeds of *Guizotia abyssinica* using HPTLC technique and found a more or less similar results. The present findings are also in agreement with the work of Chambhare *et al*., (2017) [19] who also have studied the phytochemical profiles of *Guizotia abyssinica*.

V. Conclusions

The present study revealed the presence of various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids in the dried seeds of *Guizotia abyssinica*. This herb has many medicinal uses and is a nontoxic traditional medicinal plant. Numerous medicinal therapies treat their patients with herbal medicines for its extraordinary influence, though relatively little knowledge about their mode of action is available. This versatile plant is the source of many bioactive compounds. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this

plant material in future prospective study. The bioactive phytochemicals of *Guizotia abyssinica* responsible for various pharmacological activities require further investigation at scientific level.

Acknowledgement: Authors are thankful to Dr Baidyanath Kumar, Academic director, Life Science Research Centre, Patliputra (Patna) for providing necessary suggestions and support for the preparation of this manuscript.

Conflict of interest: Authors declare no conflict of interest directly or indirectly.

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Dr. Baidyanath Kumar, et. al. "Phytochemical Profile of seeds of Guizotia abyssinica (L. f.) Cass (Niger or Ramtil)." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(5), (2020): pp. 14-22.