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Phytochemical screening and quanitative analysis of active phytocontents of Guizotia abyssinica seed to knows of their therapeutic values

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Abstract*---Guizotia abyssinica Cass*. belonging to the family Asteraceae is a vegetable plant with many industrial and medicinal value. Current research describes a simple, effective and reproducible G-in-vitro propagation protocol. *G. abyssinica* and comparative phytochemical analysis of natural seeds, leaf (mature and in vitro regenerated) and *G. abyssinica* Different annotations namely. apical and axilllary buds, leaves and internode were selected for the in vitro regeneration study to assess the effect of differential concentrations on TDZ. Different parts of the plant such as seeds, natural leaf, in vitro leaf and callus were dried and extracted from different solvents and tested with various phytochemical analyzes. Of all the four annotations used, the apical shoot appeared to be the best in terms of shoot reproduction and reproduction. In vitro renewed callus has shown the presence of phenol. It may be concluded that additional suspension of hormonal compounds may be helpful in the widespread distribution and release of drugs for commercial use. The findings provide potential support for tissue tissue techniques in the production of bioactive compounds but further studies are needed as well.

***Keywords*---**phytochemicals, guizotiaabyssinica, callus, in vitro regeneration, TPC, TFC

Introduction

Niger (Guizotia abyssinica (L.f.) Cass.) Is an oil seed plant cultivated for over 5000 years. It is widely grown in Southern India and Ethiopia, two of the largest producers of Niger. [In India, it is cultivated on the slopes of hills and plains along the coast of Odisha, Chhattisgarh, in parts of Madhya Pradesh, Jharkhand, Bihar, Maharashtra, Karnataka, Andhra Pradesh and West Bengal. A dicotyledonous plant, medium to fine branches, growing up to 2 m high. [3] The plant grows very well in poorly drained, heavy clay soils. An important feature of this plant is that it provides good seed yield even under poor growing conditions. Niger is heavily cultivated for the extraction (approximately 30-50%) of oil used for soap, lighting, lubrication and used as biodiesel. Niger oil absorbs the fragrance of flowers used as a base oil in the perfume industry. The plant is used in various Indian communities for the treatment of rheumatism, rheumatoid arthritis, and infectious diseases. Plant G. abyssinica is considered an overlooked plant despite being rich in nutrients, medically and economically.

Plants have been an important source of medicine for thousands of years. Even today most people in the world still rely heavily on traditional remedies as remedies. Plants are also the source of many modern medicine. To a large extent, medicinal plant species are collected and collected in the wild and relatively few generations are grown commercially. Indian Ayurveda herb has used herbs like turmeric since the early 1900 BC. Ayurvedic medicine continues to be a health care system in India and Western Medicine.

Phytochemicals are responsible for the healing properties of plants. [16] These are unhealthy chemicals that protect people from various diseases. Phytochemicals are basically divided into two groups of primary and secondary metabolites based on the activity of plant metabolism. Basic metabolites include regular carbohydrates, amino acids, proteins and chlorophyll while secondary metabolites include alkaloids, saponins, steroids, flavonoids, tannins and more. As previously reported, niger seeds contain phytochemical compounds such as saturated fats, oils, proteins, amino acids, and flavonoids.

Materials and Methods

Collection of samples

Guizotia abyssinica from the local market of Bhopal, M.P., India were collected in the winter season. The seeds were properly cleaned with natural water and then properly removed with purified water. The seeds were then dried for 5 days at an ambient temperature for shade. Second, dried seeds were coarsely used with a mortar and pestle and then a mechanical blender was used to ground them further.

Preparation of Plant Extracts

Thirty gm. 340 ml of organic solutions of Methanol & D.W. were collected from the sample (*Guizotia abyssinica*) Extraction at Soxhlet. The extraction was completed

in 8 days at 65 °C. In order to form a paste, extracts were then evaporated at 45 °C and further transferred to sterile and refrigerated once used.

Phyto-Chemical Testing

Plant Plant Repair

Approximately 2 g of sample is measured and dissolved in solvents i.e.. Hexane, petroleum ether, methanol, chloroform, chloroform: methanol (2: 1) and water separately and were allowed to last 24 hours. After incubation for 24 hours the sample was filtered with a Whattman filter paper, filtrate was used for phytochemical testing.

Phytochemical Tests

Phytochemical analysis was performed to determine the presence of bioactive compounds using standard quality procedures.

Alkaloids Testing

Meyer test

In 1 ml of each sample solution a few drops of Meyer reagent (potassium mercuric chloride solution) are added. The formation of a cream white precipitate indicates the presence of alkaloids.

Wagner test

In a few ml of each sample solution, Wagner reagent (iodine in potassium iodide) was added, which led to the formation of reddish-brown rainfall indicating the presence of alkaloids.

Tests for Alkaloids

a. Meyer's test
   To 1 ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. Formation of cream white precipitate indicates the presence of alkaloids.
b. Wagner's test:
   To few ml of each of the sample solution, Wagner's reagent (iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.

Tests for Flavonoids

a) Jone's Test:
   To small amount of sample dissolve in 1 ml of acetone, 2 ml of 10 % aq. K2Cr2O7 and 6 ml of 6 M H2SO4. A blue green colour indicates the presence of flavonoids.
b) Test for Carbohydrates

a) Benedict's Test:
   To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic red colour precipitate indicates the presence of sugar.
b) Molisch's test:
   Treat the test solution with few drops of alcoholic alpha napthol. Add 0.2 ml of conc. H2SO4 slowly through the sides of the tube, a purple to violet ring appears at the junction.

Test for Saponins

Froath test:
To 0.05 ml of filtrate, added 5 ml of distilled water and shaken vigorously for a stable persistence froath. Froathing which persisted on warming indicates the presence of saponins.

Test for Tannins

Ferric Chloride test:
To 2 ml of extract, few drops of 5 % ferric chloride solution was added. The appearance of violet indicates the presence of tannins.

Tests for Sterols and Terpenoids

Libermann-Buchard test:
Samples were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid from the sides of the test tube was added shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids and formation of deep red colour indicates the presence of terpenoids.
Salkowski test:
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of red colour at the lower layer indicates the presence of sterols and yellow colour at the lower layer indicates the presence of terpenoids.

Test for Starch

Extracts were treated with lugol solution (1 g iodine + 2 g potassium iodide) and water, appearance of blue colour shows the presence of starch

Test for Fatty Acids

a) Spot test:
   About 0.5 ml of extract was mixed with 5 ml of ether. The extract was allowed to evaporate, on filter paper and dried. The appearance of transperence on filter paper indicates the presence of fatty acids.

Test for Reducing Sugars

Fehling's test

Equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartarate and sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and boiled, a brick red precipitate of cuprous oxide forms, indicates the presence of sugars. Test for Proteins a) Xanthoprotein test: The extracts were treated with few drops of conc. nitric acid. Yellow colour indicates the presence of proteins. Test for Amino Acids a) Ninhydrin test: To 1 ml of sample boiled with 0.1 % acetone solution of ninhydrin, appearance of violet colour shows the presence of amino acids

Test for Phenols

Ferric Chloride Test:
Extracts were treated with few drops of 5 % acidified ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Check the starch
Excerpts were treated with a solution of lugol (1 g iodine + 2 g potassium iodide) and water, the appearance of blue indicates the presence of starch.

Check Protein
Xanthoprotein Tests:
The extracts are treated with a few drops of conc. nitric acid. The yellow color indicates the presence of protein.

Check Amino Acids

Ninhydrin testing:
In 1 ml of boiled sample with a 0.1% acetone solution of ninhydrin, the appearance of pink indicates the presence of amino acids.
Bioactive compounds:

1) Flavonoid content:
   Flavonoids content of isolated crude (*Linum usitatissimum* and *Guizotia abyssinica* seeds) were determined this method (Jia *et al.*, 1999). Take a clean test tube and add 0.5 ml of the sample (Extract) containing 1.25 ml of distilled water. Then added 0.075 ml of 5 % sodium nitrite solution and allowed to stand for 5 min. Added 0.15 ml of 10 % aluminium chloride, after 6 min 0.5 ml of 1.0 M sodium hydroxide were added and the mixture were diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. The flavonoid content was expressed as mg catechins equivalents /g sample and same procedure were done with Extract of *Guizotia abyssinica* seeds.
2) Total phenolic content:
   Capacity of total phenolic contents was determined using method with slight modification. Total phenolic content of isolated crud (Fruit powder and Leaves) was determined by the method described by (Singleton *et al.*,

1965). 1.0 ml of sample was mixed with 1.0 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1.0 ml of saturated Na_2CO_3 (~35 %) was added to 2 3 the mixture and made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, observed under UV-Vis spectrophotometer at 760 nm absorbance. Gallic acid was used as a standard with varied concentration from 200 ppm to 1000 ppm. A calibration curve was constructed with different concentrations of catechol (0.01- 0.1 mM) as standard. The results were expressed as mg of catechol equivalents/g of extract and same procedure was done with Extract of *Guizotia abyssinica* seeds.

Results and Discussion

This study reports the in vitro plant regeneration protocol G. abyssinica through direct organogenesis and callus mediated through apical and axillary buds, leaf explants and internode and its phytochemical analysis.

Phytochemical Screening

Table No. 1: Phytochemical screening of extract of G. abyssinica

S. No.	Constituents Ethanolic extract	Ethanolic extract
1.	Alkaloids Mayer's Test Wagner's Test Dragendroff's test Hager's test	+ve +ve +ve +ve
2.	Glycosides Modified Borntrager's Test Legal's test	 +ve +ve
3.	Flavonoids Lead acetate Alkaline test	 +ve +ve
4.	Phenolics Ferric Chloride Test	+ve
5.	Proteins and Amino acids Xanthoproteic test Ninhydrin Test	+ve +ve
6.	Carbohydrates Molisch's Test Benedict's Test Fehling's test	 -ve -ve -ve
7.	Saponins Froth Test Foam test	 +Ve +ve
8.	Terpenoids Lieberman Burchardt test Salkowski test	 +ve +ve
9.	Test for Starch	-ve

10.	Test for Fatty Acids **a)** Spot test:	 +ve
11.	Test for Reducing Sugars Fehling's test:	 -ve
12.	Test for Phenols Ferric Chloride Test:	 +ve
13.	Test for Starch	-ve
14.	Test for Proteins **a)** Xanthoprotein test:	 +ve
15.	Test for Amino Acids Ninhydrin test:	 +ve

Total phenolic content (TPC)

The total phenolic content was determined on the basis of a colorimetric reduction based method using the Folin-Ciocalteu reagent. The TPC of the extracts was resolved by extrapolation from the calibration curve (Y = 0.007x+0.0211; R2 = 0.9932) prepared from the concentration of tannic acid (Figure.1) and expressed in mg per gram of tannic acid equivalence (TAE). The results are shown below as table no.2.

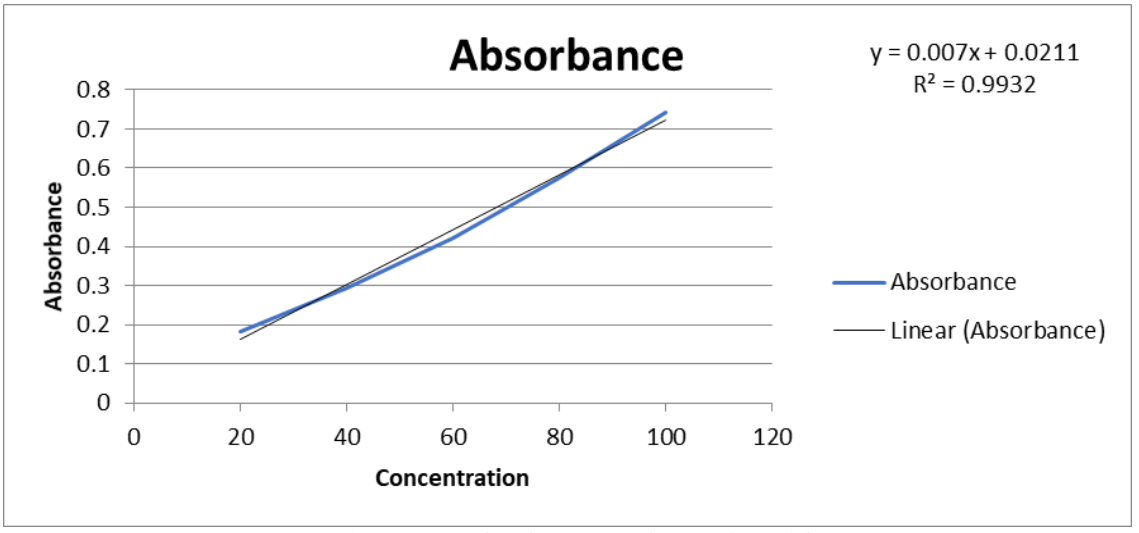

Fig.1: Standard Curve of Tannic acid

Table No. 2: Total Phenolic content of lemon peels Hydro alcoholic extract

QUANTITATIVE ANALYSIS	*Guizotia abyssinica*
TOTAL PHENOLS (µg of TAE/serving)	129.263±0.996

Total flavonoids content (TFC)

Total flavonoid extract content, estimated according to the method of aluminum chloride. For the determination of the total flavonoid content of the extracts, the aluminium chloride method (Chang et al., 2002) is used. The extract TFC was resolved by extrapolation from the calibration curve (Y = 0.0122x+0.6975; R2 = 0.9905) prepared from the concentration of quercetin (Figure 2) and expressed in mg of the equivalence of quercetin (QE) per gram. Below, the results are shown as and table no. 3.

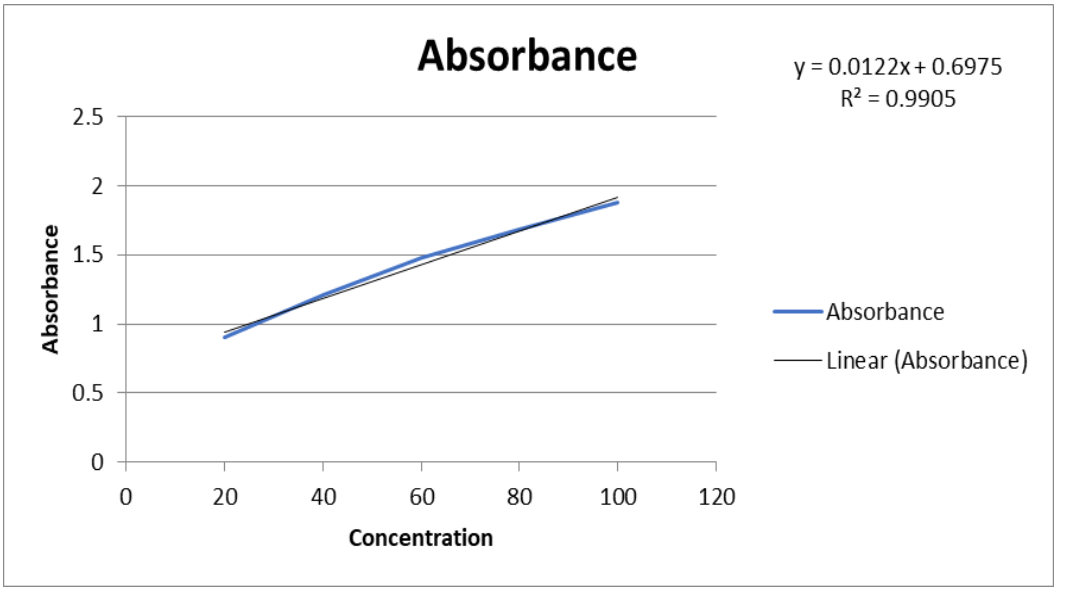

Fig. 2: Standard Curve of Quercetin

Table No. 3: Total Flavonoid content of *Guizotia abyssinica* seed extract

QUANTITATIVE ANALYSIS	*Guizotia abyssinica*
TOTAL FLAVONOIDS (mg of QE /serving)	48.293±1.384

Phytochemical experiments of G. abyssinica were examined. Phytochemical experiments are expected to assist in the accurate identification of high-quality materials (species, origin, individual plants or plant components) where plant chemistry differs between different species. All solvents namely. hexane, petroleum ether, methanol, chloroform, chloroform: methanol (2: 1) and seed water, natural leaf, in vitro leaf and callus produce highly variable effects on the presence of nutrients bioactive substances such as alkaloids. In addition, alkaloids and synthetic derivatives have been shown to have analgesic, antispasmodic and bactericidal effects. Plant extracts were also good for flavonoids. which are hydroxylated phenolic compounds known to be synthesized by plants in response to microbial infection and found to be antimicrobial

substances against a wide range of microorganisms in vitro. Their function is probably due to their ability to synthesize non-cellulite and soluble proteins as well as to the complexity of the bacterial cell wall. [65] They are also an effective antioxidant, antiinflammatory, anti-allergic, anti-analgesic cytostatic and show strong anti-cancer activity. In addition it is also known to remove LDL-c from the blood by increasing the concentration of LDL receptors in the liver and binding to polipoprotein B. However, alkaloids and flavonoids inhibit certain mammalian enzyme activities such as phosphodiesterase, which increase the activity of -cyclic-AMP. . Alkaloids also affect glucagons and thyroid hormones.

Plant extracts are known to be effective on steroids. which are very important compounds because they are related to compounds such as sex hormones and it has been reported that steroids have cardiotonic activities and antibacterial properties. Tannins are commonly described as polyphenolic compounds with a high molecular weight (over 1000) and can form a protein complex and disrupt protein formation.

mutagenicity of carcinogens and tumor promotion, host mediated antiviral activity without being disturbed by any obvious toxins, antibacterial and antiparasitic effect, and has been used to treat intestinal disorders such as diarrhea and diarrhea. The emergence of phenolic compounds in only in vitro-stimulated callus proved that callus cultures were able to produce secondary metabolites. Many studies focus on biological agents such as antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection and improve endothelial function and inhibition of angiogenesis and proliferative function. of cells. Phenolic compounds are widely used in disinfection and remain relatively similar to other bacteriocides.

Conclusion

It can be concluded from the present study that G. abyssinica contains a major bioactive compound of commercial value and can lead to great interest in phyto pharmaceuticals. Further research is needed to identify and identify the cellular structure that can improve production as the plant contains many important phytochemical chemicals.

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