WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 2.786
Volume 4, Issue 1, 1193-1217. Research Article ISSN 2278 – 4357

THIDIAZURON PROMOTES *IN VITRO* PLANT REGENERATION AND PHYTOCHEMICAL SCREENING OF *GUIZOTIA ABYSSINICA* CASS. - A MULTIPURPOSE OIL CROP

Sapna Baghel and Yogendra Kumar Bansal*

Plant Tissue Culture Laboratory, Dept. of Biological Science, R. D. University, Jabalpur (M.P.) India.

Article Received on
01 Nov 2014,

Revised on 25 Nov 2014,
Accepted on 20 Dec 2014

***Correspondence for Author**
Yogendra Kumar Bansal
Plant Tissue Culture Laboratory, Dept. of Biological Science, R. D. University, Jabalpur (M.P.) India.

ABSTRACT

Objective: *Guizotia abyssinica* Cass. belonging to family Asteraceae is an herbaceous crop with a lot of industrial as well as medicinal importance. The present study describes a simple, efficient and reproducible regeneration protocol for *in vitro* propagation of *G. abyssinica* as well as comparative phytochemical analysis of natural seed, leaf (nature grown as well as *in vitro* regenerated) and callus of *G. abyssinica*. **Methods:** Different explants viz. apical and axilllary buds, leaf and internode were selected for *in vitro* regeneration study to observe the effect of different concentrations of TDZ. Different parts of the plant like seed, natural leaf, *in vitro* leaf and callus were dried and extracted in different solvents and were subjected to various phytochemical analysis. **Results:** Among all the four explants used, apical bud proved best in terms of shoot regeneration and multiplication. The best multiple shoot regeneration (4.44±0.1) was observed on TDZ (0.45 µM) supplemented medium. *In vitro* regenerated callus showed the presence of phenol. It may be concluded that further standardization of hormonal combinations could be helpful for large scale propagation and extraction of drugs for pharmaceutical application on a commercial scale. **Conclusion:** The results obtained provide a support for the potential utility of tissue culture strategies for production of bioactive compounds but further studies are required as well.

KEYWORDS- TDZ, Phytochemicals, *Guizotia abyssinica*, Callus, *in vitro* regeneration.

INTRODUCTION

Niger (*Guizotia abyssinica* (L.f.) Cass.) is an oilseed crop cultivated for more than 5000 years. It is widely grown in South India and Ethiopia, which are the two major countries producing Niger.[1] In India, it is grown on the hill slopes and in coastal plains of Odisha, Chhattisgarh, some parts of Madhya Pradesh, Jharkhand, Bihar, Maharashtra, Karnataka, Andhra Pradesh and West Bengal.[2] It is a dicotyledonous herb, moderately to well branched, growing up to 2 m in height.[3] The crop grows best on poorly drained, heavy clay soils.[4] The important feature of this crop is that it gives reasonable seed yield even under poor marginal growing conditions. Niger is mainly cultivated for extraction (about 30-50%) of oil which is used for soap making, lighting, lubrication and also used as a biodiesel.[5, 6] The niger oil is good absorbent of fragrance of flowers used as a base oil by perfume industry. The plant is used by the various tribal communities of India in the treatment of rheumatism, arthritis, microbial infections applied to treat burns, used for birth control and treatment of syphilis.[7, 8] The plant *G. abyssinica* is treated as neglected crop despite being nutritionally rich, medicinally and economically important.[9] Besides, the plant suffers from low yield due to self incompatibility, lodging, shattering, indeterminate growth habit, instability at higher temperature and susceptibility to diseases.[10, 11]

Plants have been an important source of medicine for thousands of years. Even today majority of world's population still relies mainly on traditional remedies such as herbs. Plants are also the source of many modern medicines. To a large extent, medicinal plant species are gathered and collected from the wild and relatively few genera are cultivated on a commercial scale.[12] Commercial exploration by the method of micropropagation for biopharmaceuticals and bio-energy production are some of the prospective future potential of this plant. It is used as an important tool in both basic and applied studies. *In vitro* culture of plant cells or tissues has many applications in crop improvement, preservation and breeding industries. Plants have been a major focus of investigations for novel biologically active compounds from natural resources and in recent years many pharmaceutical companies have been looking to develop these natural products to produce more affordable and cost effective remedies. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from the plants.[13] Use of volatile and penetrating plant extracts in therapeutic applications for psychological and physical well being has been in practice from ancient times. Indian Ayurveda medicines have used herbs such as turmeric

since as early as 1900 BC. Ayurvedic medicines continue to be the system of health care in India along with Western Medicine.[14]

Growth regulators play a key role for developing a specific mode of growth in the cultured cells or tissues, which may be due to accumulation of specific biochemical contents in them. The single or combination of different hormones in the medium causes maintenance of specific, balanced inorganic and organic contents in the growing tissue. This leads the cells or tissues to develop either into shoots/roots or even death.[15]

Phytochemicals are responsible for medicinal activity of plants.[16] These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. Primary metabolites comprise of common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on.[17, 18] As previously reported, niger seed contain phytochemical compounds such as fixed oil, fats, protein, amino acids, and flavonoids.[19, 20]

TDZ is a substituted phenyl urea (N-phenyl-1, 2, 3-thidiazol-5-yl urea) compound, shown to possess potent activity as a cytokinin in the regulation of shoot organogenesis in several plant species.[21] Although the mode of action of TDZ is not known, the present data provides further evidence that TDZ may modulate endogenous auxin and cytokinin metabolism during regeneration. Our study seeks to identify the regeneration potential and the role as well as interaction of explant source and growth regulators in direct and callus-mediated organogenesis and preliminary analysis from different parts viz. Seed, natural as well as *in vitro* regenerated leaves and *in vitro* regenerated callus of *G. abyssinica* Cass.

MATERIALS AND METHODS

Collection and Sterilization of Explants

Seeds of niger var. JNC 6 were obtained from Jawaharlal Nehru Krishi Vishwavidyalaya (JNKVV) Jabalpur. Seeds were soaked in water for 24 hrs and washed thoroughly under running tap water for 30 mins and kept in 1 % (w/v) Bavistin (Carbendenzim Powder, BASF, India Ltd) for 10 mins and then treated first with wetting agent labolene (1 %) followed by rinsing in running water (1 hr). They were then surface sterilized using (w/v) 0.1 % $HgCl_2$ for 6–8 mins followed by three rinses with sterile distilled water. Seeds were then inoculated on sterilized moist cotton with liquid MS[22] medium.

The seeds germination started within 7-8 days. Four types of explants viz. apical, axillary buds (1.0 cm each), leaf (1 cm) and internode (0.8-1 cm) were isolated from 4 weeks old *in vitro* germinated seedling.

Shoot Multiplication

The explants were cultured on Murashige and Skoog (MS) medium fortified with different concentrations and combinations of plant growth regulators. Sucrose (3 %) was used as the carbon source and gelled with 0.8 % agar. The pH of the medium was adjusted to 5.6-5.8 before autoclaving at 121 ^{0}C for 20 mins. All explants viz. apical bud, axillary bud, leaf and internode were inoculated on MS medium containing different concentrations of TDZ (0.45-22.7 µM). For shoot elongation and rooting the regenerated shoots were transferred in PGR free medium. All cultures were maintained at 16 hrs photoperiod with 3000 Lux light intensity at 25±2 ^{0}C. Seeds, callus, natural and *in vitro* leaves were shade dried. Each sample of the material was ground separately into fine powder and stored in air tight containers at ambient temperature.

In vitro regenerated plantlets (3–4 months) possessing well developed shoot and root systems were washed with tap water to remove adhering agar without damaging the delicate root system and treated with 1 % (w/v) fungicidal (Bavistin) solution for 5 mins. The plants were then transferred to plastic pots containing sterilized sand, soil and farm yard manure (1:1:1). To maintain humidity, hardened plantlets were completely covered with plastic bags and irrigated regularly with distilled water. After 2 weeks, the plastic covers were perforated with small holes. The bags were removed intermittently (5–10 mins) to aid adaptation of *in vitro* plantlets to normal environment conditions. Subsequently exposure time was increased in the following weeks and after 7–8 weeks plastic covers were removed completely. After 8 weeks of hardening the plantlets were transferred to the field.

Experimental Design, Data Collection and Analysis

The experiments were set according to completely randomized design and all experiments were performed in triplicates with 12 treatments. The data recorded within 25-30 days of inoculation. The data was analyzed using analysis of variance (ANOVA) and means were separated at p=0.05 level of significance using Duncan's multiple range test (DMRT) employing the statistical software IBM SPSS 20.

Phyto-Chemical Screening

Preparation of Plant Extracts

Approximately 2 g of sample was weighed and dissolved with solvents viz. Hexane, petroleum ether, methanol, chloroform, chloroform: methanol (2:1) and water separately and was allowed to stay for 24 hrs. After 24 hrs incubation the sample was filtered by Whattman filter paper, the filtrate was used for phytochemical screening.

Phytochemical Tests

The phytochemical analysis was carried out to determine the presence of following bioactive compounds using the standard qualitative procedures.[23]

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. $K_2Cr_2O_7$ and 6 ml of 6 M H_2SO_4. A blue green colour indicates the presence of flavonoids.

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. H_2SO_4 slowly through the sides of the tube, a purple to violet ring appears at the junction.

Test for Saponins

a) **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

a) **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

a) **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.

b) **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 Reducing Sugars

a) **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 Anthocyanins and Anthocyanidins

Aqueous extract (2 ml) was added to 2 ml of 2 N HCl and ammonia. The appearance of pink red turns blue violet indicates the presence of anthocyanins and anthocyanidins.

Test for Anthracene Glycosides

Ammonium hydroxide (25 %) was added to 5 ml of extract formation of red colour indicates the presence of anthracene glycosides.

Test for Coumarins

About 3 ml NaOH (10 %) was added to 2 ml of extract formation of yellow colour indicates the presence of coumarins.

Test for Emodins

NH_4OH (2 ml) and benzene (3 ml) were added to the extract. Appearance of red colour indicates the presence of emodins.

Test for Carotenoids

Development of blue green colour after adding conc. HCl: Phenol (1:1) shows the presence of carotenoids.

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.

Test for Cardiac Glycosides

Kellar Kiliani test: Conc. H_2SO_4 (1 ml) was taken in a test tube then 5 ml of extract and 2 ml of glacial acetic acid with one drop of ferric chloride were added which results in formation of a blue colour.

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 transparence on filter paper indicates the presence of fatty acids.[24]

RESULTS AND DISCUSSION

This study reports an efficient *in vitro* plant regeneration protocol for *G. abyssinica* through direct as well as callus mediated organogenesis through apical and axillary buds, leaf and internode explants and its phytochemical analysis.

Effect of Type of Explants on Shoot Regeneration

The shoot induction response could be dependent on the developmental state and gene expression pattern of the explants.[25] Of the four explants (Apical bud, axillary bud, leaf and internode) used, maximum regeneration frequency and multiplication was obtained from apical bud explants (Fig. 1(a)). The variations in the regeneration potential of explants are attributable to the differences in the physiological and genetic makeup of cells [26]. In the present study, the highest bud proliferation was observed in apical bud explants within 30 day of culture (4.44 shoots, Fig. 1 (c)). The reason for the better efficacy of apical bud is probably the presence of meristematic shoot bud at the already grown shoot tip. But in case of nodal explants new shoot buds from the nodes are needed to be induced through purely hormonal control, or in other words new shoot buds develop after inoculation in the absence of apical dominance. In many cases, the hormone concentrations can not be the sole mechanism controlling *in vitro* developmental processes. It may be related to differences in tissue and cell differentiation and organization in all explants culture. Similar results were also reported by us previously in this plant[27, 28] and other plants such as in *Heliotropium indicum*[29], *Stevia rebaudiana*[30], *Lagerstroemia indica*[31] and *Solanum nigrum.*[32]

Effect of Thidiazuron on Shoot Multiplication and Elongation

In tissue culture, plant growth regulators are important media components in determining the development and developmental pathway of the plant cells. Growth regulators are used in different proportions to break dormancy and enhance shoot formation since it is well demonstrated that the apical dormancy is under control of these growth regulators.[33] Thidiazuron is a phenyl urea that has gained importance as being more or just as potent as combined auxin and cytokinin in evoking morphogenic responses *in vitro*.[21] It is well established that the concentration of TDZ is highly sensitive for the induction of multiple

shoots and their proper growth or development as compared to other cytokinins. TDZ has been successfully used in plant regeneration systems for many herbs like *Curcuma longa,*[34] *Cineraria maritime,*[35] *Hydrastis Canadensis,*[36] *Clitoria ternatea,*[37] *Spilanthes acmella*[38] and *Withania somnifera*.[39]

The frequency and response of *in vitro* shoot regeneration differed depending on the type of explants and both the type and concentration of growth regulators added to the regeneration medium.[40, 41] Thus, in the second experiment, wide ranges of different concentrations of TDZ (0.45-22.7 µM) were attempted for the selection of optimal concentration to induce multiple shoots in *G. abyssinica*. Initial response was swelling and enlargement of the explant tissue within 1 week of inoculation (Fig. 1). Differentiation of multiple shoot buds started after 2 weeks of culture from apical bud explant on TDZ (0.45 µM) (Fig. 1 (g)). Whereas shoot buds were induced directly from the axil of axillary bud explant and later on grew in size to develop into healthy shoots (Fig. 1 (c, f)). The leaf and internode explants developed callus (Within 2-3 weeks) which led to the formation of green shoots. These three explants (viz. axillary bud, leaf and internode) produced maximum multiple shoots on TDZ (2.27 µM). Induction of multiple shoots occurred on lower concentrations of TDZ and the number of multiple shoots increased with increase in its concentration from 0.45 to 2.27 µM (Table 1). The role of TDZ in inducing regeneration is attributed to the ability of TDZ in enhancing the synthesis of adenine type cytokinins. These results corroborate the fact that TDZ is an effective plant growth regulation for induction of shoot bud regeneration. The possible reason for the higher activity of individual TDZ treatment might be its high stability due to its resistance to cytokinin oxidase.[21] The stimulatory effect of TDZ on multiple shoot formation has been reported earlier in this plant,[9] in *Arnebia euchroma,*[42] *Salvia brachyodon,*[43] *Artemisia vulgaris,*[44] *Artemisia annua,*[45] *Stevia rebaudiana*[46] and in *Curculigo latifolia*.[47] TDZ-induced regeneration is linked to accumulation and transport of certain endogenous signals such as auxins or the related compounds like melatonin and serotonin.[48]

However, beyond the 2.27 µM of TDZ a sudden decrease in explant response was noticed which ultimately affected the number of shoots per explant as well as shoot length and produced callus at the base of the explants (Fig. 2). Moderate concentration of PGRs have been shown to inhibit morphogenic responses, possibly through negative feed-back mechanism. Thidiazuron could have inhibited growth through elevation of endogenous ethylene which is a hormone that has been reported to promote degradative processes in

addition to causing stem shortening and thickening.[49, 50] Similar effect of high concentration of TDZ was reported in *Ocimum americanum,*[51] *Cassia angustifolia,*[52] *Abelmoschus moschatus,*[53] *Clitoria ternatea,*[37] *Saussurea involucrate,*[54] *Solanum aculeatissimum*[55] and in *Aerva lanata*.[56]

In the present study, it was observed that the multiple shoots induced by TDZ exhibited stunted growth and also the prolonged exposure to TDZ supplemented medium resulted in distortion and fasciation in the regenerated shoots which has also failed to elongate further (Fig.1(p)). The inhibition of shoot elongation may be due to the high cytokinin activity of TDZ and the presence of a phenyl group in TDZ may be the possible cause of shoot bud fasciation.[21] The inhibitory effect of TDZ on shoot elongation has also been reported for *Salvia fruticosa,*[57] *Salvia brachyodon*[43] and *Artemisia vulgaris*.[44] To overcome this problem, the clumps of multiple shoot buds were separated from the parent tissue and transferred to growth regulator free MS basal medium for elongation. A similar phenomenon has been observed previously in *Curcuma longa,*[34] *Hedychium coronarium,*[58] *Thymus bleicherianus*.[59] Elongation and rooting was achieved in 4-6 weeks on PGR free MS medium (Fig. 3 (b)) and flower bud initiation started on the apex and axils of some branches of *in vitro* regenerated shoots. In the present study i*n vitro* flowering was also obtained from the explants only on those shoots (5-8 %) which were raised on TDZ (0.45 µM) that were subcultured to basal medium. Maximum number of flower buds (2–5) were initiated in plantlets which developed on TDZ supplemented medium (Fig. 3 (a)). However, *in vitro* flowering was low as compared to an earlier report[28] which showed much higher (70 %) one. It is known that the plant growth regulator requirement of plants for *in vitro* flowering varies. *In vitro* flowering has been induced in *Rosa rubiginosa*[60] and *Withania somnifera*[39] were cultured on TDZ supplemented medium. Well developed plantlets were transferred to poly cups containing sterile sand, soil and farm yard manure (1:1:1) under high humidity. After 4–5 weeks, approximately 90 % plantlets survived upon hardening (Fig. 3 (c)). The hardened plantlets were transferred to field after 2 months (Fig. 4 (d, e)) as already discussed by us.[27, 28]

Phytochemical Screening

Phytochemical screening of *G. abyssinica* was evaluated and results shown in Table 2. Phytochemical screening is expected to facilitate the accurate identification of elite materials (species, provenances, single plants or plant parts) where plant chemistry varies between

different specimens.[61] All solvents viz. hexane, petroleum ether, methanol, chloroform, chloroform: methanol (2:1) and water of seeds, natural leaf, *in vitro* leaf and callus yielded quite variable results in the presence of bioactive constituents such as alkaloids (Fig. 4 (a, b)) that contain highly reactive substances with biological activity in low doses. Many alkaloids have remarkable effects on the central nervous system, gastrointestinal tract [62] and also used for the treatment of cancer.[63] Moreover alkaloids and their synthetic derivatives are documented to possess analgesic, antispasmodic and bactericidal effects.[64] The plant extracts were also positive for flavonoids (Fig. 4 (c)) which are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.[65] They also are effective antioxidant, anti-inflammatory, anti allergic, anti analgesic cytostatic and shows strong anticancer activities.[66, 67] Moreover it is also known to remove LDL-c from blood by increasing the LDL receptor densities in liver and binding to a polipoprotein B.[68] However, alkaloids and flavonoids inhibit certain mammalian enzymatic activities such as those of phosphodiesterase, prolonging the action of cyclic-AMP. Alkaloids also affect glucagons and thyroid stimulating hormones.[69]

The plant extract is known to be positive for steroids (Fig. 4 (d)) which are very important compounds because it has a relationship with compounds such as sex hormone[70] and it has also been reported that steroids have cardiotonic activities and antibacterial properties.[71] Tannins are generally defined as polyphenolic compounds having high molecular weight (over 1000) and can form a complex with the protein and interfere with protein synthesis.[72] Tannins found in the plant (Fig. 4 (e)) have been recognized for their beneficial effects e.g., inhibition of lipid peroxidation, mutagenicity of carcinogens and tumour promotion, host-mediated antitumor activity, antiviral activity without being troubled by any obvious toxicity, antibacterial and antiparasitic effect, and also been used for treating intestinal disorders such as diarrhoea and dysentery.[73, 74] Foods which are rich in tannins can be used in the treatment of HFE hereditary hemochromatosis which is a hereditary disease characterized by excessive absorption of dietary iron, resulting in a pathological increase in total body iron stores. It can also be effective in protecting the kidneys and certain tannins were able to inhibit HIV.[64, 75] Furthermore the occurrence of phenolic compounds in only *in vitro* regenerated callus (Fig. 4 (f)) proved that callus cultures are able to produce secondary metabolites. It is one of the

largest and most ubiquitous groups of plant metabolites.[76] Primarily phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics,[77] bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including certain types of cancer, cardiovascular problems and decrease cholesterol level. A number of studies have focussed on the biological properties such as antiapoptosis, anti-ageing, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection and improvement of the endothelial function as well as inhibition of angiogenesis and cell proliferation activity.[78] Phenolic compounds have been extensively used in disinfections and remain the standards with which other bacteriocides are compared.[79]

A broad range of the biological properties of terpenoids were observed in extract (Fig. 4 (g)) which described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, antiinflammatory and antiparasitic activities.[73] Moreover terpenoids have effect on common cold, fever, inflammation, cardiovascular effects and effects on central nervous system, brain, respiratory system and antifertility effect.[80] The higher amount of plant lipid can be used as essential oils, spice oleoresins and natural food colours. With a strong foundation in research and development, plant lipids have developed products that work with diverse requirements which could be culinary, medicinal or cosmetic.[81]

Amino acids are critical to life and have many functions in metabolism. Amino acids are very important in nutrition. These are commonly used in food technology and industry. Very often in plants during diseases conditions, the free amino acid composition exhibits a change and hence the measurement of the total free amino acids gives the physiological and health status of the plants.[82] In the present study comarins was also present (Fig. 4 (h)). It known to act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity.[67] Various studies have been demonstrated that coumarin is a potential antioxidant and its antioxidant activity is due to its ability to scavenge free radicals and to chelate metal ions.[16]

Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact they exploit their rich supply of potential energy to maintain life.[83] The presence of carbohydrates (Fig. 4 (i)) reducing sugars (Fig. 4 (j)) and proteins (Fig. 4 (k)) in nature grown

and *in vitro* plant in different fractions is also shown in this study. Other phytochemicals such as saponins, anthracene glycoside, emodin, carotenoid, cardiac glycosides and gum and mucilage were not found to be present in the all extracts. These results suggest that the phytochemical properties for curing various ailments, leads to the isolation of new and novel compounds. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against various diseases. These observations also support the usefulness of *G. abyssinica* in folklore for the treatment of various infections.

Table 1 - Effect of TDZ on shoot proliferation and elongation from apical bud, axillary bud, leaf and internode explants of Guizotia abyssinica Cass.

TDZ Conc. (µM)	Frequency of shoot initiation (%)				Shoot number (Mean±SE)				Shoot length (Mean±SE)			
	ApB	AxB	Leaf	Internode	ApB	AxB	Leaf	Internode	ApB	AxB	Leaf	Internode
0.45	72.22±2.27^{c}	63.88±2.26^{b}	-	30.55±2.26^{b}	4.44±0.1^{d}	2.55±0.1^{c}	-	1.13±0.01^{b}	1.18±0.1^{d}	1.38±0.1^{d}	-	0.19±0.01^{b}
2.27	52.66±2.17^{b}	61.10±2.26^{b}	30.55±2.26^{b}	69.44±2.27^{c}	2.77±0.2^{c}	2.72±0.2^{c}	1.05±0.01^{b}	2.49±0.3^{c}	0.71±0.1^{c}	0.52±0.1^{c}	0.20±0.01^{c}	0.27±0.01^{c}
4.54	61.10±4.53^{bc}	55.55±4.53^{b}	27.77±2.26^{b}	25.00±0.01^{b}	2.38±0.1^{b}	2.33±0.1^{b}	0.97±0.1^{b}	1.16±0.1^{b}	0.61±0.01^{b}	0.25±0.01^{b}	0.14±0.01^{b}	0.12±0.01^{b}
22.7	11.10±2.26^{a}	-	-	-	1.0±0.01^{a}	-	-	-	0.1±0.01^{a}	-	-	-

Experiment was repeated thrice with 12 replicates. Values represent mean ± standard error. Mean values followed by different superscript letters is significantly different at$P \leq 0.05$, as determined by Duncan's Multiple range Test. The bold data showed the best results

Table 2. Phytochemical screening of *G. abyssinica.*

S. No.	Tests	Seeds						Natural leaf						*In vitro* leaf						Callus					
		PE	ME	CL	CL: ME	HE	WE	PE	ME	CL	CL: ME	HE	WE	PE	ME	CL	CL: ME	HE	WE	PE	ME	CL	CL: ME	HE	WE
1.	**Alkaloids**	-	+	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-
	Mayers	-	+	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-
	Wagners																								
2.	**Flavonoid**	-	+	-	-	-	+	+	-	+	-		+	+	-	+	-	-	+	+	+	+	+	-	+
3.	**Carbohydrates**	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
	Molish's Test	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Benedict's Test																								
4.	**Saponins**	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	**Tannins**	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+

6.	**Steroids**	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	-
	Salkowski test Libbermans Burchad test	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+	-	-
7.	**Terpenoids Libbermans Burchad test**	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-
8.	**Reducing Sugars**	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-	+
9.	**Proteins**	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-
10.	**Amino acids**	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-
11.	**Anthocyanine & Anthocyanidine**	-	+	+	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
12.	**Anthracene glycoside**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13.	**Coumarins**	-	+	+	+	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	+	+	+	-	-
14.	**Emodin**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15.	**Carotenoid**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16.	**Phenolics**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+
17.	**Cardiac Glycosides**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18.	**Starch**	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19.	**Fatty acids**	+	+	+	+	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
20.	**Gums & mucilage**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Petroleum Ether Extract = PE, Methanolic Extract = ME, Chloroform = CL, Hexane= HE, Water extract = WE. (+) Positive test and (-) Negative test.

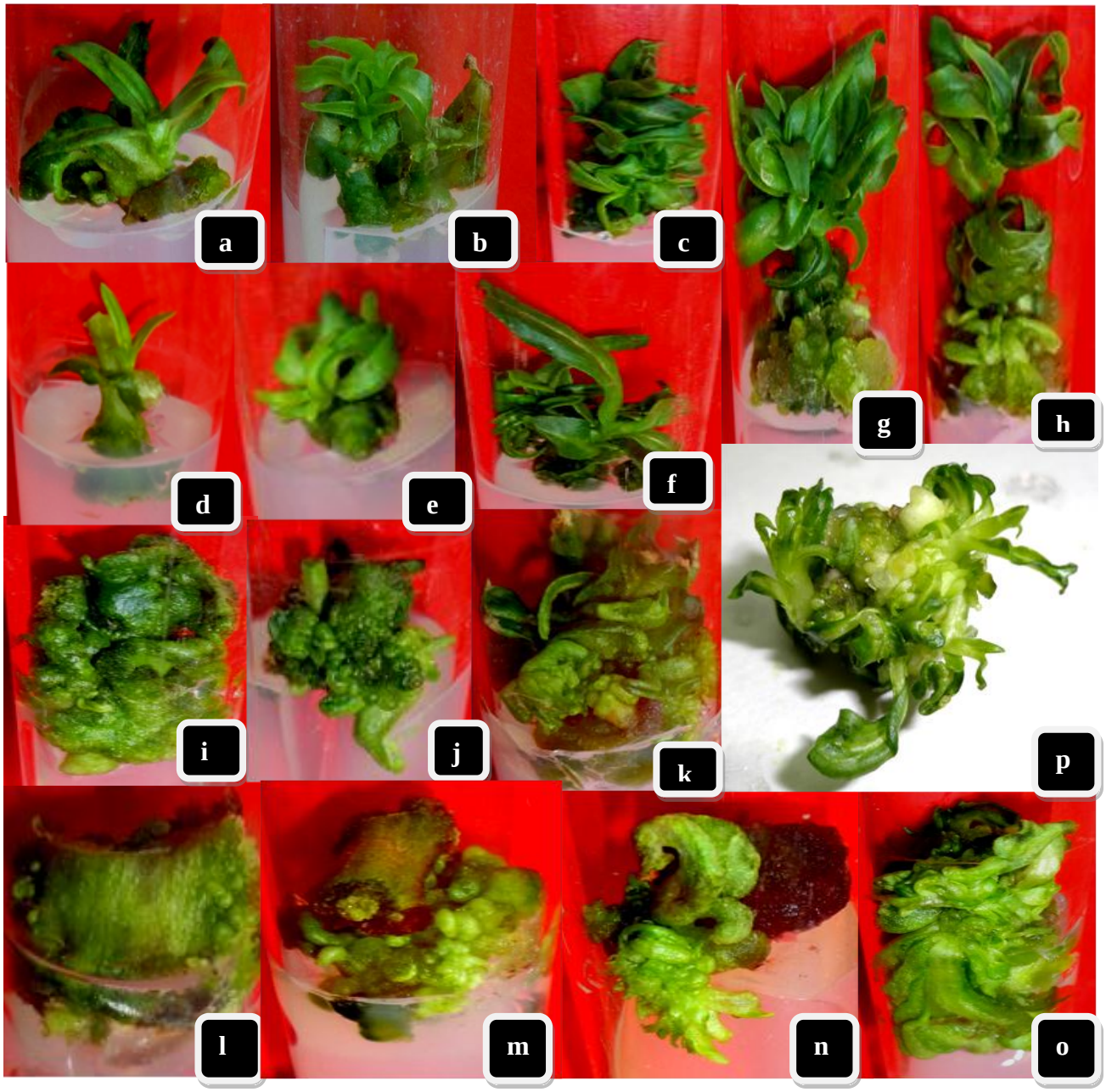

Fig. 1 *In vitro* regeneration in *Guizotia abyssinica* Cass. a-b Shoot bud initiation from apical bud on TDZ (0.45 µM) 2 week. c Multiple shoot formation from apical bud with TDZ (0.45 µM) 3-4 week. d-e Bud break from axillary bud on TDZ (2.27 µM) 2 week. f Shoot multiplication from axillary bud on TDZ (2.27 µM) 3-4 week. g-h Shoot elongation on TDZ (0.45µM) from apical and axillary bud explants 4 week. i Swelling in leaf on TDZ (2.27 µM) 1 week. j-k Shoot initiation and Multiple shoot formation with TDZ (2.27 µM) 3-4 week. l-m Initiation of bulb like structure on TDZ (2.27 µM) from internode. n-o Shoot initiation and complete multiple shoot formation on TDZ (2.27 µM) 4 week. p Rosette multiple shoot formation on TDZ (2.27 µM) in 1st Subculture.

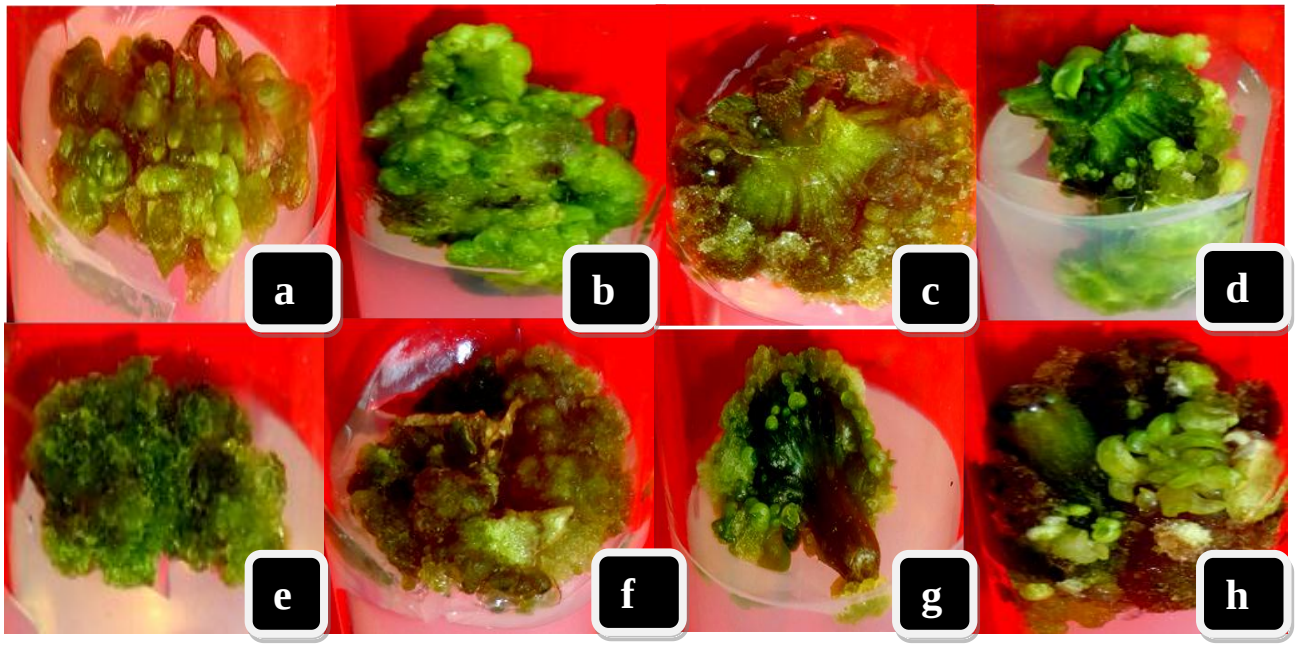

Fig 2 Callogenic study in *G. abyssinica* a-b Callus initiation and proliferation of callus (compact green colour) from apical bud on TDZ (22.7 µM) 3-4 week. c-d Callus initiation and proliferation from axillary bud (compact green colour) on TDZ (22.7 µM) 3-4 week. e-f Callus initiation (green colour) and complete callus formation (compact brown-green in colour) from leaf on TDZ (22.7 µM) 3-4 week. g-h Initiation of callus (light green in colour) and complete callus formation (light green-brown) from internode on TDZ (22.7 µM) 3-4 week.

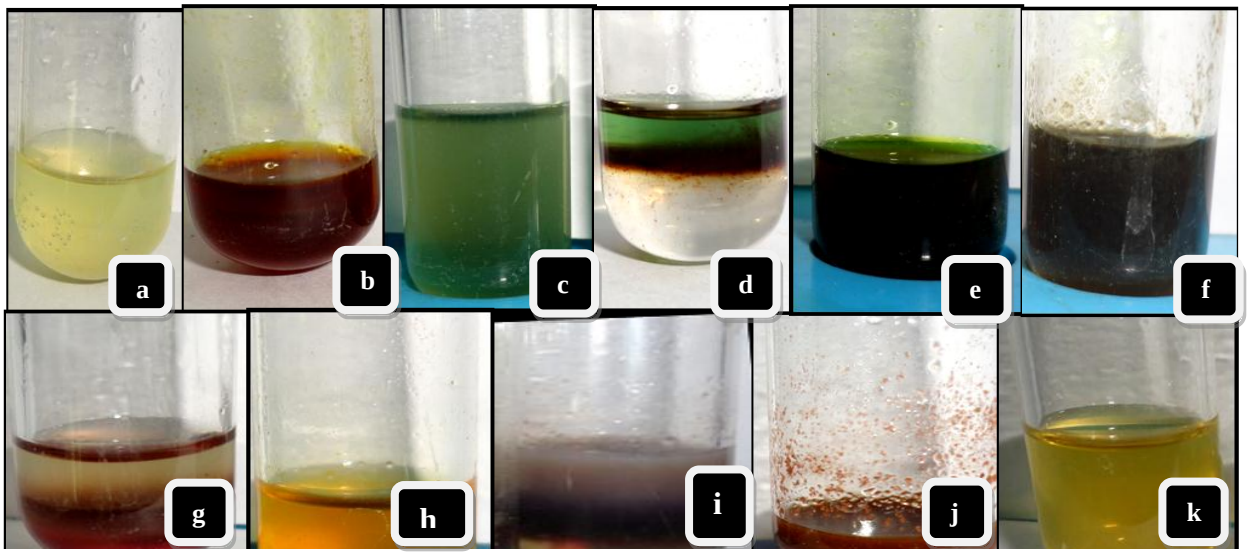

Fig 4 a Mayer's test of alkaloids (Cream white precipitate). b Wagner's test of alkaloids (Reddish brown precipitate). c Jone's Test of flavonoids (Blue green colour). d Libermann-Buchard test of steroids and terpenoids. e Ferric chloride test of tannin (Army green colour). f Phytochemical test of phenols (Bluish black colour). g Libermann-Buchard test of triterpenoids (Purple colour). h Phytochemical test of coumarins (Pale yellow colour). i Molisch's test of carbohydrates (Purple violet ring). j Fehling's test of reducing sugars (Brick red colour). k Xanthoprotein test of proteins (Yellow colour).

Fig 3 a Flowering of *in vitro* regenerated plantlets. b Plantlets with well developed roots in full strength MS medium c. Hardened *in vitro* regenerated plants in poly cup covered with perforated polythene. d Hardened *in vitro* regenerated plants after 15 days in natural environment. e Full developed *in vitro* regenerated plantlet after 25 days.

CONCLUSION

It can be concluded from the present study that *G. abyssinica* contains primary bioactive compound of commercial importance and can result in great interest in phyto pharmaceuticals. Further investigation is required to identify and characterize their molecular structure which can enhance the production as the plant contains many important phytochemicals.

ACKNOWLEDGMENTS

One of us (S. Baghel) acknowledges funding received under Rajiv Gandhi National Fellowship (RGNF) scheme of University Grants Commission (UGC), New Delhi India for carrying out this research.

REFERENCES

1. Ramadan MF. Functional properties, nutritional value, and industrial applications of niger oilseeds (*Guizotia abyssinica* Cass.). Crit Rev Food Sci Nutr, 2012; 52:1-8.
2. Mishra JS, Moorthy BTS, Bhan M, Yaduraju NT. Relative tolerance of rainy season crops to field dodder (*Cuscuta campestris*) and its management in niger (*Guizotia abyssinica*). Crop Prot, 2007; 26:625–29.
3. Getinet A, Teklewold A. An agronomic and seed-quality evaluation of niger (*Guizotia abyssinica* Cass.) germplasm grown in Ethiopia. Plant Breed, 1995; 114: 375–76.
4. Francis CM, Campbell MC. New high quality oil seed crops for temperate and tropical Australia. A report for the Rural Industries Research and Development Corporation, RIRDC 2003 Publication No 03/045.
5. Mohan Kumar BN, Basavegowda, Vyakaranahal BS, Deshpande VK, Kenchanagoudar PV. Influence of sowing dates on production of seed yield in niger (*Guizotia abyssinica* Cass.). Karnataka J Agric Sci, 2011; 24(3):289 – 93.
6. Sarin R, Sharma M, Khan A. Studies on *Guizotia abyssinica* L. oil: biodiesel synthesis and process optimization. Bioresour Technol, 2009; 100: 4187-92.
7. Getinet A, Sharma SM. Niger *Guizotia abyssinica* (L. f.) Cass. Promoting the conservation and use of underutilized and neglected crop. 5; Institute of Plant Genetics and Crop Plant Research, Gatersleben, International Plant Genetic Resources Institute, Rome, 1996; 1-59.
8. Jain SK. Dictionary of Indian folk medicine and ethnobotany. Deep Publications: New Delhi, 1991; 97.
9. Bhandari HR, Sujatha M, Dangi KS, Sivasankar A. Efficient plantlet regeneration from seedling explants of niger (*Guizotia abyssinica* Cass). Int J Agr Environ Biotech, 2009; 2(4): 438 –44.
10. Sarvesh A, Reddy TP, Kavikishor PB. Androclonal variation in niger (*Guizotia abyssinica*). Euphytica, 1994; 79: 59-64.

11. Murthy HN, Jeong JH, Choi YE, Paek KY. Agrobacterium-mediated transformation of niger [*Guizotia abyssinica* (L. f.) Cass.] using seedling explants. Plant Cell Rep, 2003; 21: 1183–87.
12. Parveen S, Shahzad A. TDZ–induced high frequency shoot regeneration in *Cassia sophera* Linn. via cotyledonary node explants. Physiol Mol Biol Plants, 2010; 16(2): 201-06.
13. Raaman N, Divakar S, Jeyam P, Hariprasath L, Baskar M, Mathiyazhagan K. Micropropagation, antimicrobial activity and phytochemical analysis of *Clerodendron phlomidis* Linn. medicinal plants. Int J Phytomedicines and Related Industries, 2011; 3:,119-27.
14. Dwivedi G, Dwivedi S. Sushrutha-the Clinician- Teacher Par Excellence. Indian J Chest Dis Allied Sci, 2007; 49: 243-44.
15. Ikram-ul-Haq, Dahot MU. Morpho-physiological aspects of micro-propagating Banana under different hormonal conditions Asian J Plant Sci, 2007; 6(3):496-501.
16. Savithramma N, Linga Rao M, Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle East J Sci Res, 2011; 8(3):579-84.
17. Jigna P, Sumitra CV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol, 2007; 31: 53-8.
18. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N, Krishna MRV. Phytochemical investigation on a tropical plants. Pak J Nutri, 2009; 8: 83-5.
19. Kuo WL, Chen CC, Chang PH, Cheng LY, Shien BJ, Huang YL. Flavonoids from *Guizotia abyssinica*. J Chin Med, 2007; 18(3,4): 121-28.
20. Dwivedi S, Balakrishnan BR, Thenmozhi S. Quantitative estimation and analysis of fixed oil obtained from seeds of *Guizotia abyssinica* (L.F) Cass. Pharma Chem, 2010; 10: 24-6.
21. Huettman CA, Preece JE. Thidiazuron- a potent cytokinin for woody plant tissue culture. Plant Cell Tissue Organ Cult, 1993; 33: 105-19.
22. Murashige T, Skoog FA. Revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plantarum, 1962; 15: 473-97.
23. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis, third edition. London: Chapman and hall, 1998; 107-08.
24. Kiran Kumari SP, Sridevi V, Chandana Lakshmi MVV. Studies on phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. J Chem Biol Phys Sci, 2012; 2(3):1326-32.

25. Vijayakumar J, Shobana Rathi GS, Bhuvaneshwari M, Ranjitha Kumari BD, Castano E. Thidiazuron-induced shoot organogenesis of *Cleome viscosa* (L) through cotyledonary explants culture. Afr J Biotechnol, 2014; 13(9): 1027-36.
26. Thangavel P, Britto SJ, Senthilkumar SR. Adventitious shoot regeneration from leaf explants of the valuable medicinal herb *Plectranthus barbatus* Andrews. Afr J Biotechnol, 2011; 10(43): 8562-69.
27. Baghel S, Bansal YK. Micropropagation and *in vitro* flowering of a biodiesel plant niger *Guizotia abyssinica* (Cass.). Asian J Exp Biol Sci, 2013; 4(4): 532-39.
28. Baghel S, Bansal YK. Synergistic effect of BAP and GA_3 on *in vitro* flowering of *Guizotia abyssinica* Cass.-a multipurpose oil crop. Physiol Mol Biol Plants, 2014; 20(2): 241–47.
29. Kumar MS, Rao MV. *In vitro* micropropagation of *Heliotropium indicum* Linn. - an ayurvedic herb. Indian J Biotechnol, 2007; 6: 245-49.
30. Hossain MA, Shamim Kabir AHM, Jahan TA, Hasan MN. Micropopagation of Stevia. Int J Sustain Crop Prod, 2008; 3(3): 1-9.
31. Niranjan MH, Sudarshana MS, Girisha ST. *In vitro* multiple shoot induction from excised shoot tips and nodal segment explants of *Lagerstroemia indica* (L) A medicinal cum ornamental shrub. J Biomed Sci and Res, 2010; 2(3): 212-17.
32. Kavitha MS, Wesely EG, Mehalingam P. Direct multiple shoot regeneration from shoot tip and nodal explants of *Solanum nigrum* L. a medicinal herb. J Ornam Hortic Plants, 2012; 2(2): 65-72.
33. Madhulatha P, Anbalagan M, Jayachandran S, Sakthivel N. Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of Banana (Musaspp. AAA). Plant Cell Tissue Organ Cult, 2004; 76(2): 189-92.
34. Prathanturarug S, Soonthornchareonnon N, Chuakul W, Phaidee Y, Saralamp P. High-frequency shoot multiplication in *Curcuma longa* L. using thidiazuron. Plant Cell Rep, 2003; 21: 1054–59.
35. Banerjee S, Tripathi J, Verma PC, Dwivedi PD, Khanuja SPS, Bagchi GD. Thidiazuron induced high-frequency shoot proliferation in *Cineraria maritime* Linn. Curr Sci, 2004; 87(9): 1287-90.
36. He SS, Liu CZ, Saxena PK. Plant regeneration of an endangered medicinal plant *Hydrastis canadensis* L. Sci Hortic, 2007; 113: 82–6.

37. Mukhtar S, Ahmad N, Khan MI, Anis M, Aref IM. Influencing micropropagation in *Clitoria ternatea* L. through the manipulation of TDZ levels and use of different explant types. Physiol Mol Biol Plants 2012; DOI 10.1007/s12298-012-0136-4.
38. Sharma S, Shahzad A. Efficient micropropagation of *Spilanthes acmella* (L.) Murr.: A threatened medicinal herb. Br Biotechnol J, 2013; 3(3): 405-15.
39. Sharma N, Dhiman M, Koshy EP. TDZ induced multiple shoot regeneration and *in vitro* flowering from nodal explant in *Withania somnifera* (L) Int J Sci Res, 2014; 3: 50-2.
40. Gubis J, Lajchova Z, Farago J, Jurekova Z. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). Biol Brat, 2004; 59(3): 405–08.
41. Ishag S, Osman MG, Khalafalla MM. Effects of growth regulators, explant and genotype on shoot regeneration in tomato (*Lycopersicon esculentumc*. v. Omdurman). Int J Sustain Crop Prod, 2009; 4: 7–13.
42. Jiang BO, Yang YG, Guo YM, Guo ZC, Chen AZ. Thidiazuron induced *in vitro* shoot organogenesis of the medicinal plant *Arnebia euchroma* (Royle) Johnst. Vitro Cell Dev Biol Plant, 2005; 41: 677–681.
43. Misic D, Grubisic D, Konjevic R. Micropropagation of *Salvia brachyodon* through nodal explants. Biol Plantarum, 2006; 50(3): 473-76.
44. Sujatha G, Ranjitha Kumari BD. High-frequency shoot multiplication in *Artemisia vulgaris* L. using thidiazuron. Plant Biotechnol Rep, 2007; 1: 149–54.
45. Lualon W, De-Eknamkul W, Tanaka H, Shoyama Y, Putalun W. Artemisinin production by shoot regeneration of *Artemisia annua* L. using thidiazuron. Z Naturforsch C, 2008; 63 (1-2): 96-100.
46. Lata H, Chandra S, Wang YH, Raman V, Khan IA. TDZ induced high frequency plant regeneration through direct shoot organogenesis in *Stevia rebaudiana* Bertoni: an important medicinal plant and a natural sweetener. Am J Plant Sci, 2013; 4: 117-28.
47. Babaei N, Abdullah NAP, Saleh G, Abdullah TL. An efficient *in vitro* plantlet regeneration from shoot tip cultures of *Curculigo latifolia*, a medicinal plant. Sci World J 2014; http://dx.doi.org/10.1155/2014/275028.
48. Jones MPA, Yi ZJ, Murch SJ, Saxena PK. Thidiazuron induced regeneration of *Echinacea purpurea* L.: Micro propagation in solid and liquid culture systems. Plant Cell Rep, 2007; 26: 13-9.
49. Beyer EM Jr, Morgan PW, Yang SF. Ethylene. In: Wilkins MB, editor *Advanced Plant Physiology*. Pitman Publishing Ltd. London, 1984; 111-26.

50. Eisinger W. Regulation of pea internode expansion by ethylene. Annu Rev Plant Physiol, 1983; 34: 225-40.
51. Pattnaik S, Chand PK. *In vitro* propagation of the medicinal herbs *Ocimum americanum* L. syn. O. canum Sims. (hoary basil) and *Ocimum sanctum* L. (holy basil). Plant Cell Rep, 1996; 15(11): 846-50.
52. Siddique I, Anis M. *In vitro* shoot multiplication and plantlet regeneration from nodal explants of *Cassia angustifolia* (Vahl.): a medicinal plant. Acta Physiol Plant, 2007; 29 (3): 233-38.
53. Sharma R, Shahzad A. Thidiazuron (TDZ) induced regeneration from cotyledonary node explant of *Abelmoschus moschatus* Medik. L. (A Valuable Medicinal Plant). World J Agr Sci, 2008; 4(4): 449-52.
54. Guo B, Stiles AR, Liu CZ. Thidiazuron enhances shoot organogenesis from leaf explants of *Saussurea involucrata* Kar. et Kir. Vitro Cell Dev Biol Plant, 2012; 48(6): 609-12.
55. Ghimire BK, Yu CY, Chung IM. Direct shoot organogenesis and assessment of genetic stability in regenerants of *Solanum aculeatissimum* Jacq. Plant Cell Tissue Organ Cult, 2012; 108(3), 455-64.
56. Varutharaju K, Soundar Raju C, Thilip C, Aslam A, Shajahan A. High efficiency direct shoot organogenesis from leaf segments of *Aerva lanata* (L.) Juss. ex Schult by using thidiazuron. Sci World J 2014; http://dx.doi.org/10.1155/2014/652919.
57. Arikat NA, Jawad FM, Karam NS, Shibli RA. Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa* Mill.). Sci Hortic, 2004; 100(1): 193-02.
58. Verma M, Bansal YK. Effect of a potent cytokinin thidiazuron (TDZ) on *in vitro* regeneration of *Hedychium coronarium* J. Koenig–A Valuable Medicinal Plant. Int J Rec Biotech, 2014; 2(1): 38-4.
59. Aicha N, Abdelmalek EM. Rapid *in vitro* regeneration and clonal multiplication of *Thymus bleicherianus* Pomel, a rare and threatened medicinal and aromatic plant in Morocco. Med Aromat Plants, 2014; 3(1): 1-5.
60. Wang GY, Yuan MF, Hong Y. *In vitro* flower induction in roses. Vitro Cell Dev Biol Plant, 2002; 38(5): 513-18.
61. Sarasan V, Kite GC, Sileshi GW, Stevenson PC. Applications of phytochemical and *in vitro* techniques for reducing over-harvesting of medicinal and pesticidal plants and generating income for the rural poor. Plant Cell Rep, 2011; 30(7): 1163-72.

62. Obaineh OM, Shadrach A. Phytochemical constituents and medicinal properties of different extracts of *Anacardium occidentale* and *Psidium guajava*. Asian J Biomed Pharmaceut Sci, 2013; 3(16): 20-3.
63. Gawri S, Upadhyay A. A comparative study on the antimicrobial activity and the presence of phytochemicals in the petioles and callus of *J. curcas*. J Phytol, 2012; 4(3): 18-20.
64. Ravi Kiran C, Madhavi Y, Raghava Rao T. Evaluation of phytochemicals and antioxidant activities of *Ceiba pentandra* (Kapok) seed oil. J Bioanal Biomed, 2012; 4(4): 68-3.
65. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol, 2011; 3(12): 10-4.
66. Okwu DE. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. J Sustain Agric Environ, 2004; 6(1): 30-37.
67. Mohamed Sham Shihabudeen H, Hansi Priscilla D, Thirumurugan K. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Int J Pharma Sci Res, 2010; 1(10): 430-34.
68. Baum JA, Teng H, Erdmn JW, Weigel RM, Klein BP, Persky VW. Long term intake of soy protein improves blood lipid profile and increases mononuclear cell low-density lipoprotein receptor messenger RNA in hypercholesterolemic postmenopausal women. Am J Clin Nut, 1998; 58: 545.
69. Okaka JC, Enoch NJ, Okaka NC. Human nutrition: an integrated approach. ESUT publications, 1992.
70. Okwu DE. Evaluation of the chemical composition of medicinal plants belonging to Euphorbiaceae. Pak Vet J, 2001; 14: 160-62.
71. Epand RF, Savage PB, Epand RM. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochem Biophys Acta, 2007;1768 (10): 2500-09
72. Mailoa MN, Mahendradatta M, Laga A, Djide N. Tannin extract of Guava leaves (*Psidium guajava* L) variation with concentration organic solvents. Int J Sci Technol Res, 2013; 2: 106-10.
73. Satdive RK, Chimata R, Namdeo A, Fulzele DP. *In vitro* screening for phytochemical and antimicrobial activity of poisonous plant *Ficus tseila* Roxb. Int J Pharm Bio Sci, 2012; 3: 213-21.
74. Dharmananda, S. 2003.Gallnuts and the Uses of Tannins in Chinese Medicine http: //www. itmonline. org/ arts/ gallnuts. Htm.

75. Gowri SS, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J Pharm Tech Res, 2010; 2(2):1569-73.
76. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chem Toxicol, 2007; 45(7): 1216-23.
77. Gupta VK, Singh GD, Singh S, Kaul A, Medicinal Plants: Phytochemistry, Pharmacolgoy and Therapeutics, Daya Publishing House, Delhi, 2010; 1: 1-31.
78. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci, 2007; 8: 950-88.
79. Okwu DE. Evaluation of Chemical composition of indigenous species and flavouring agents. Global J Pure Appl Sci, 2001: 458-59.
80. Narasimhan R, Mohan A. Phytochemical screening of S*esamum indicum* seed extract. World J Pharm Pharmaceut Sci, 2003; 1: 1298-08.
81. Birajdar S, Kedarnath, Chimkod V, Patil CS. Phytochemical screening and characterization of *Pongamia pinnata* (L) seed oil. Int J Pharma Anal, 2011; 3: 17-20.
82. Sadasivam S. and Manickam A. Biochemical Methods, II ed., New Age International (P) Limited Publishers, New Delhi, 1996.
83. Bhumi G, Savithramma N. Screening of pivotal medicinal plants for qualitative and quantitative phytochemical constituents. Int J Pharm Pharm Sci, 2014; 6: 63-5.