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Direct Organogenesis of *Glinus lotoides* L. - Anti-helmintic Herb Using Nodal Segments

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Abstract

Glinus lotoides L. is a valuable medicinal herb for the treatment of human and livestock ailments. However, it was listed under endangered plant species due to over utilization and lower seed viability. The purpose of this study has been to develop a micropropagation method from nodal segments of *G. lotoides.* MS containing various concentrations of Kn was used for culture induction. Best culture establishment (95%) and highest mean shoot number (2.45 ± 0.37) were achieved on a medium containing 1.5 mg/l Kn. Even though different concentrations of 6-benzyl aminopurine (BAP), Kn and α-naphthalene acetic acid (NAA) were utilized for shoot proliferation, the highest number of shoots per nodal segment (3.66 ± 0.61) was attained in the medium containing 1.0 mg/l BAP in combination with 0.2 mg/l NAA. Shoots were rooted on half strength MS containing IBA, IAA and NAA. In a medium containing 1.5 mg/l IBA, 5.80 ± 1.10 roots per shoot, 1.05 ± 0.10 cm root length and 100% root generation frequency were obtained. Plantlets were successfully established under greenhouse conditions with 94% survival rate and no aberrant plants were detected.

Introduction

Glinus lotoides is semi-erect to prostrate herb, stem up to 45 cm long, most parts with stellate hairs and belongs to Molluginaceae family (Gilbert 2000). It is widespread in the tropics and subtropics worldwide. In Ethiopia, it grows at an altitude of 530-1650 m.a.s.l. (Gilbert 2000). Tablet formulation (Abebe 2005; Abebe et al. 2008), 10% of crude saponin (Abebe et al. 1998), 14% of fat (Biftu et al. 1979; Abebe et al. 2004), antitumour, anti-helmintic and taenicidal activity (Chopra et al. 1956, Mulatu 1978, Berhanu and Berhane 1980, Abebe et al. 1997,

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Kavimani et al. 1999), anticancer and nutrition value (Abebe and Youan 2010) of *G. lotoides* were reported. Endophytic fungus (*Chaetomium globosum*) isolated from its leave was used in laccase production (El-Zayat 2008). Toxicological study (Jemal et al. 2007) and its toxicity effect on nymphs of the desert locust (Ould Ahmedou et al. 2001) have been described. Different hopane-type saponins and flavonoids were isolated from its seed (Abebe 2005) and root (Hamed et al. 2005). It is used to treat *Oestrus ovis* (Gidey et al. 2011), *Moniezia* and *Thysaniezia* spp. (Mesfin and Obsa 1994), purgative, boils, bilious attack, wounds, pains, and urinary disorders (Kumar et al. 2011; Shanmugam et al. 2012), wounds, inflammation, syphilis and intestinal worms (Qureshi and Bhatti 2008; Qureshi et a*l*. 2010).

There is a great demand of *G. lotoides* in Ethiopia (Kloos et al. 1978; Endashaw 2007) and this market demand has been increasing (Lehoux and Chakib 2012). So far, there has been no report on the conventional cultivation of this plant and it is continuously collected from the wild. Poor seed germination (Teshome and Feyissa 2015b), over exploitation and harvesting the whole plant by uprooting or collecting before seed setting, threatened this species and may led it to be extinct in the near future. In order to meet the increasing demand of the plant materials and germplasm conservation of this endangered species, using biotechnological tools such as *in vitro* mass propagation technique is very important. A few works have been reported on the *in vitro* propagation of *G. lotoides*. *In vitro* propagation from shoot tips (Teshome and Feyissa 2015b) and indirect shoot organogenesis from leaf (Teshome and Feyissa 2015a) of this plant were reported. However, mass propagation from nodal explant of *G. lotoides* was not reported to date. Therefore, the purpose of this study was to develop micropropagation method for *G. lotoides* from nodal segments.

Materials and Methods

Four-week-old nodal explants (1-2 cm) of *G. lotoides* were obtained from stock plant maintained under aseptic culture condition at Plant tissue culture laboratory of Institute of Biotechnology, Addis Ababa University, Ethiopia.

Cultures were induced on MS medium containing 30 g/l sucrose, 8.0 g/l agar and Kn (0.0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.25, 2.5 mg/l). Five nodes per culture vessels in four replications were used.

Multiplication media contained BAP (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 mg/l), Kn (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 mg/l), BAP (0.0, 0.5, 1.0, 1.5, 2.0 mg/l) in combination with NAA (0.2 mg/l), Kn (0.0, 0.5, 1.0, 1.5, 2.0 mg/l) in combination with NAA (0.2 mg/l), 30 g/l sucrose and 8.0 g/l (w/v) agar. Ten

nodes from *in vitro* initiated shoots per culture vessels in three replications were planted.

Root induction was initiated in half strength MS medium supplemented with NAA (0.0, 0.3, 0.5, 1.0, 1.5, 2.0 mg/l), IBA (0.0, 0.3, 0.5, 1.0, 1.5, 2.0 mg/l), IAA (0.0, 0.3, 0.5, 1.0, 1.5, 2.0 mg/l), 15 g/l sucrose and 8.0 g/l agar. Ten shoots per culture vessels and 30 shoots per treatment in three replications were used. Magenta culture vessels (7 cm diameter) containing 50 ml medium were used in all experiments.

For this experiment, pH of the medium was adjusted to 5.8 before the addition of agar and autoclaved at 121°C under a pressure of 105 kpa for 15 min. Cultures were maintained at 25 ± 2ºC under light intensity of 22 μmol $m^{-2}s^{-1}$ and 16h photoperiod provided by cool-white fluorescent lamp. Data were collected on the number of shoots per explant, shoot length, number of roots, root length and leaf number every four weeks for each subculture.

Following acclimation, the roots of the plantlets were washed under running tap water and planted in plastic pots filled with sand, red soil and compost in 1:3:1 ratio respectively. The potted plantlets were enclosed with polyethylene bags and transferred to the greenhouse maintained at 25 ± 2°C and 50% - 60% relative humidity. One week later, polyethylene bags were removed and data on the plants that survived were recorded after a month.

Data were subjected to analysis of variance (ANOVA) to differentiate the significant differences among means using statistical data analysis software SPSS version 22.0 at 5% probability level.

Results and Discussion

Within 14-16 days, in all concentrations of KIN, shoot regeneration from the nodal segments were observed. In the presence of 1.5 mg/l Kn, 95% shoot induction frequency, 2.45 ± 0.37 mean shoot number, mean number of leaf (7.00) were obtained (Table 1, Fig. 1A). Mean of longest shoot (1.25 ± 0.08) was recorded in a medium containing 0.25 mg/l Kn. In plant growth regulator free medium, lowest (0.60±0.11) bud induction was observed. Thus, it was mandatory to add Kn in media in order to obtain multiple shoot. This may be attributed to high level of endogenous Kns which was resulted in the induction of new shoots. Shoot stimulating effect of Kn has been reported in *Tinospora cordifolia* (Sivakumar et al. 2014). Mean number of shoot, leaf and shoot length were declined with increased concentrations of Kn. The data suggest that an elevated level of Kn exhibits the negative effect on the frequency and number of regenerated buds.

Table 1. Shoot initiation from nodal segments of *G. lotoides*.

Treatment (mg/l) Kn	Regeneration (%)	Shoot number	Shoot length (cm)	Leaf number
0.0	60	0.60 ± 0.11^{c}	0.75 ± 0.16^{b}	4.80 ± 1.11^{ac}
0.25	100	1.30 ± 0.14^{c}	1.25 ± 0.08^{a}	3.85 ± 0.63^{bc}
0.5	45	0.55 ± 0.16^{c}	0.42 ± 0.11^{bc}	2.70 ± 0.83^{c}
0.75	85	2.00 ± 0.30^{ab}	1.12 ± 0.10^{a}	6.20 ± 0.84^{a}
1.0	30	0.30 ± 0.10^{c}	0.35 ± 0.15^{c}	0.60 ± 0.25^{c}
1.5	95	2.45 ± 0.37^{a}	0.75 ± 0.13^{b}	7.00 ± 1.39^{a}
2.0	40	1.00 ± 0.34^{bc}	0.23 ± 0.08^{c}	4.15 ± 1.34^{bc}
2.25	70	1.60 ± 0.31^{b}	0.48 ± 0.08^{bc}	5.60 ± 1.21^{ab}
2.5	65	1.30 ± 0.28^{bc}	0.57 ± 0.12^{bc}	3.35 ± 0.72^{bc}

Means followed by different letters within a column are significantly different at $p < 0.05$. The values represented as mean ± SE.

Shoot multiplication

At different concentration of BAP and Kn, the number of shoots per explant was significantly affected. The highest shoot number per explant (2.23 ± 0.31, 2.40 ± 0.27, 2.13 ± 0.32, 2.53 ± 0.46) was observed in the presence of BAP (0.25, 1.25 mg/l) and Kn (1.0, 1.25 mg/l), respectively (Table 2, Fig. 1B). Statistically, there is no significant difference among these means at p=5%. The results show the main effects of cytokinins in which they play a very important role in releasing lateral bud dormancy as compared to the effect of growth regulators-free medium. The reports of Thiyagarajan and Venkatachalam (2012), Teshome and Feyissa (2015a, 2015b), Teshome and Soromessa (2015) agree with the present observations. However, with an increase in the concentration of BAP and Kn, the mean number of shoots per explant declined. The best shoot length (1.0 ± 0.0) was noticed on PGRs free medium. This might be due to the effect of cytokinins that inhibit shoot length and promote bud formation. Teshome and Feyissa (2015a, 2015b) reported the same results.

Effect of Kn and NAA on shoot multiplication

Even though the highest mean number of shoots (1.83±0.13) per nodal segment was achieved in a medium containing 1.5 mg/L Kn in combination with 0.2 mg/L NAA. In such poorly developed cultures a lower mean number of shoots was observed (Table 3). The results show that, the combined action of Kn and NAA has a negative impact on shoot proliferation rate. Almost all cultures produced callus at the base of shoots and spontaneous roots. This could be due to the

influence of NAA. Comparable results were reported in *Physalis peruviana* (Otroshy et al. 2013) and *S. abyssinica* (Teshome and Soromessa 2015).

Table 2. Effect of BAP and Kn **on shoot multiplication.**

Treatment (mg/l) **BAP**	Mean of shoot	Mean of shoot length (cm)	Mean of leaf
0.0	1.43 ± 0.11^{b}	1.00 ± 0.00^{a}	8.73 ± 0.84^{b}
0.25	2.23 ± 0.31^{a}	0.70 ± 0.06^{b}	11.33 ± 1.50^{a}
0.5	2.06 ± 0.20^{a}	0.36 ± 0.05^{c}	11.90 ± 1.52^{a}
0.75	1.40 ± 0.25^{b}	0.43 ± 0.09^{c}	9.16 ± 1.80^{ab}
1.0	1.26 ± 0.22^{b}	0.40 ± 0.07^{c}	8.73 ± 1.35^{b}
1.25	2.40 ± 0.27^{a}	0.56 ± 0.04^{b}	12.16 ± 1.01^{a}
1.5	1.76 ± 0.21^{b}	0.53 ± 0.02^{c}	11.40 ± 1.20^{a}
1.75	1.66 ± 0.16^{b}	0.55 ± 0.02^{b}	9.70 ± 0.81^{a}
2.0	1.13 ± 0.19^{b}	0.43 ± 0.05^{c}	6.36 ± 0.96^{bc}
Kn			
0.25	2.06±0.27^{a}	0.80±0.08^{b}	6.30 ± 0.76^{bc}
0.5	1.70±0.19^{b}	0.71±0.04^{b}	6.30 ± 0.97^{bc}
0.75	1.96±0.19^{a}	0.88±0.14^{ab}	6.73 ± 0.99^{bc}
1.0	2.13±0.32^{a}	0.63±0.05^{b}	6.70 ± 0.91^{bc}
1.25	2.53±0.46^{a}	0.86±0.04^{ab}	4.63 ± 0.73^{c}
1.5	1.66±0.16^{b}	0.61±0.03^{b}	5.66 ± 0.68^{bc}
1.75	1.73±0.19^{b}	0.61±0.03^{b}	8.66 ± 0.97^{b}
2.0	1.20±0.07^{b}	0.71±0.04^{b}	7.40 ± 0.92^{bc}

Means followed by different letters within a column are significantly different at $p < 0.05$. The values represented as mean ± SE.

Table 3. Effect of Kn and NAA on shoot multiplication of nodal segments of *G. lotoides*.

Treatment (mg/l)		Shoot number	Shoot length (cm)	Leaf number
Kn	NAA			
0.0	0.0	1.43 ± 0.11^{b}	1.00 ± 0.00^{a}	8.73 ± 0.84^{b}
0.5	0.2	0.83 ± 0.09^{c}	0.40 ± 0.040^{c}	6.70 ± 0.74^{b}
1.0	0.2	1.66 ± 0.16^{ab}	0.65 ± 0.040^{b}	9.80 ± 0.98^{ab}
1.5	0.2	1.83 ± 0.13^{a}	0.61 ± 0.40^{b}	11.43 ± 1.17^{a}
2.0	0.2	1.33 ± 0.11^{b}	0.71 ± 0.40^{b}	10.30 ± 0.88^{ab}

Means followed by different letters within a column are significantly different at $p < 0.05$. The values represented as mean ± SE.

Effect of BAP and NAA on shoot multiplication

The highest mean number of shoots per nodal segment (3.66 ± 0.61) and the leaf number (11.16 ± 2.03) were achieved on MS medium fortified with 1.0 mg/l BAP in combination with 0.2 mg/l NAA (Table 4, Fig. 1C). The positive interaction effect of BAP and NAA resulted in the release of lateral bud dormancy factor as compared to Kn and NAA, BAP and Kn. These results indicated that, addition of auxin (NAA) in medium promotes shoot multiplication rate. BAP was found to be more effective than Kn when combined with NAA. Shoot production efficiency of BAP×NAA was reported in *Orthosiphon spiralis* (Mohanakrishnan et al. 2014), *Vernonia amygdalina* (Khalafalla et al. 2007), *Lagerstroemia indica* (Niranjan et al. 2010) and *Eclipta alba* (Sharma et al. 2013). Highest concentration resulted in the reduction of shoot numbers per nodal segment. This indicates the inhibitory effect of BAP at 0.2 mg/l concentration of NAA and the supra-optimal concentration is not recommended. In plant growth regulators free-medium, the best shoot length (1.0 ± 0.0) was recorded.

Table 4. Effect of BAP and NAA on shoot multiplication from nodal segments of *G. lotoides*

Treatment (mg/l)		Shoot number	Shoot length (cm)	Leaf number
BAP	NAA			
0.0	0.0	1.43 ± 0.11^{c}	1.0 ± 0.00^{a}	8.73 ± 0.84^{a}
0.5	0.2	1.06 ± 0.17^{c}	0.40 ± 0.05^{c}	7.16 ± 1.29^{a}
1.0	0.2	3.66 ± 0.61^{a}	0.46 ± 0.02^{c}	11.16 ± 2.03^{a}
1.5	0.2	2.70 ± 0.29^{b}	0.58 ± 0.04^{b}	11.26 ± 1.28^{a}
2.0	0.2	2.16 ± 0.29^{bc}	0.51 ± 0.04^{bc}	10.76 ± 1.87^{a}

Means followed by different letters within columns are significantly different at $p < 0.05$. The values represented as mean ± SE.

Root induction

Production of plant with abundant root is very vital for hardening in greenhouse as well as in field. After two-weeks, roots were observed in culture media. There was a significant root induction competency among IBA, NAA and IAA (Table 5). The highest mean numbers of root (5.80 ± 1.10), root length (1.05 ± 0.10), highest regeneration frequency (100%), vigorous and fast growing roots were obtained on a medium supplemented with 1.5 mg/l IBA. IBA (0.5 mg/l) and PGRs free medium produced maximum mean number of shoot (1.35 ± 0.13) and shoot length (1.10 ± 0.08) respectively (Fig. 1D). Although NAA and IAA were capable of inducing root formation, IBA was found to be superior over them in all measured parameters. Better performance of IBA was also reported in

Orthosiphon spiralis (Mohanakrishnan et al. 2014), *Hybanthus enneaspermus* (Sudharson et al. 2015), *Eclipta alba* (Sharma et al. 2013), *G. lotoides* (Teshome and Feyissa 2015a, 2015b), *S. abyssinica* (Teshome and Soromessa 2015). However, the root stimulating effect of NAA and IAA were reported in *Stevia rebaudiana* (Thiyagarajan and Venkatachalam 2012) and in *Mentha viridis* (Rahman et al. 2013) respectively.

Plantlets were successfully established under greenhouse conditions with 94% survival rate. Aberrant plants were not observed (Fig. 1E).

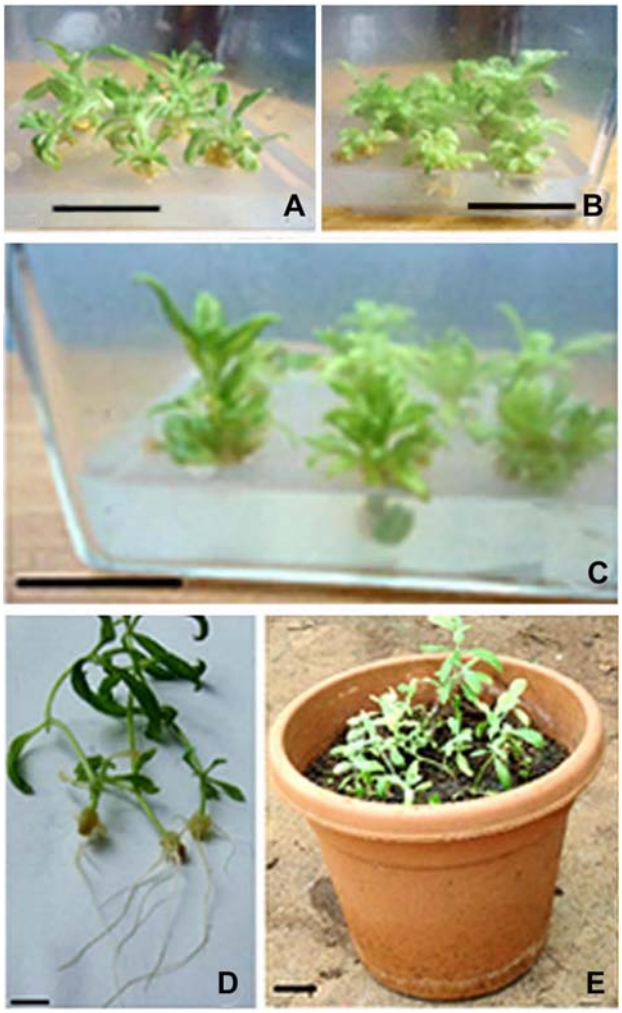

Fig. 1. Regeneration of *G. lotoides* from nodal segments. Initiation of shoots (A. 1.5 mg/l Kn), multiplication (B. 1.25 mg/l BAP, C. 1.0 mg/l BAP + 0.2 mg/l NAA), root formation (D. 1.5 mg/l IBA) and acclimatization (E). Bars represent 2 cm.

Table 5. Effect of NAA, IBA and IAA on root induction of *G. lotoides*

Treatment (mg/l) **NAA**	Root formation (%)	Mean of root	Mean of root length (cm)	Mean of shoot	Mean of shoot length (cm)
0.0	25	0.40 ± 0.18^{c}	0.37 ± 0.18^{bc}	1.00 ± 0.00^{b}	1.10 ± 0.08^{a}
0.3	30	1.75 ± 1.01^{bc}	0.32 ± 0.12^{bc}	0.90 ± 0.10^{bc}	0.85 ± 0.08^{b}
0.5	20	0.25 ± 0.12^{c}	0.17 ± 0.08^{c}	0.20 ± 0.09^{d}	0.12 ± 0.06^{d}
1.0	15	0.30 ± 0.17^{c}	0.07 ± 0.04^{c}	0.65 ± 0.10^{c}	0.50 ± 0.09^{c}
1.5	45	2.40 ± 0.79^{b}	0.32 ± 0.09^{bc}	0.85 ± 0.08^{bc}	0.55 ± 0.07^{c}
2.0	30	0.65 ± 0.26^{c}	0.17 ± 0.06^{c}	0.30 ± 0.10^{d}	0.22 ± 0.08^{d}
IBA					
0.3	25	0.50 ± 0.24^{c}	0.15 ± 0.06^{c}	1.25 ± 0.12^{ab}	0.57 ± 0.04^{c}
0.5	30	0.90 ± 0.35^{bc}	0.37 ± 0.14^{bc}	1.35 ± 0.13^{a}	0.70 ± 0.05^{bc}
1.0	45	1.00 ± 0.29^{bc}	0.60 ± 0.16^{b}	1.15 ± 0.10^{ab}	0.76 ± 0.06^{bc}
1.5	100	5.80 ± 1.10^{a}	1.05 ± 0.10^{a}	1.05 ± 0.05^{ab}	0.85 ± 0.06^{b}
2.0	30	1.15 ± 0.55^{bc}	0.30 ± 0.12^{bc}	1.15 ± 0.10^{ab}	0.72 ± 0.08^{bc}
IAA					
0.3	30	0.35 ± 0.13^{c}	0.17 ± 0.06^{bc}	0.65 ± 0.10^{c}	0.47 ± 0.09^{c}
0.5	10	0.35 ± 0.24^{c}	0.17 ± 0.13^{c}	0.80 ± 0.18^{bc}	0.45 ± 0.10^{c}
1.0	25	1.20 ± 0.60^{bc}	0.27 ± 0.12^{bc}	1.00 ± 0.17^{b}	0.55 ± 0.08^{c}
1.5	20	0.40 ± 0.19^{c}	0.20 ± 0.08^{c}	0.80 ± 0.13^{bc}	0.50 ± 0.08^{c}
2.0	20	2.35 ± 1.22^{b}	0.32 ± 0.20^{bc}	1.10 ± 0.20^{ab}	0.57 ± 0.08^{c}

Means followed by different letters within columns are significantly different at $p < 0.05$. The values represented as mean ± SE.

G. lotoides is an important medicinal plant which has different traditional uses in different countries. It is used to treat both human and livestock ailments. Addition of 1.0 mg/L BAP × 0.2 mg/L NAA and 1.5 mg/ L IBA in MS medium were found to be the best for shoot multiplication and root induction, respectively. The method reported here will be highly helpful for the production of clonal plant material and conservation.

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References

Abebe E (2005) Isolation, Structural Elucidation, Quantification and Formulation of the Saponins and Flavonoids of the Seeds of *Glinuslotoides*, PhD Dissertation, Eberhard Karls University, Tubingen, Germany.

Abebe E and **Youan B C** (2010) Anticancer activity and nutritional value of extracts of the seed of *Glinuslotoides*. J. Nutr. Sci. Vitaminol. **56:** 311-318.

Abebe E, Getachew M and **Tsige G/M** (1997) *In vitro* fungicidal activity of the seeds of *Glinus lotoides* on *Hymenolepis nana* worms. Eth. Pharm. J. **15:** 46-51.

Abebe E, **Moges K** and **Tsige G/M** (1998) *In vivo* anthelmintic activity of the extract of the seeds of *Glinus lotoides* in Albino Mice infested with *Hymenolepis nana* Worms. Eth. Pharm. J. **16:** 34-41.

Abebe E, Schmidt PC and **Tsige G/M** (2004) Standardisation and physicochemical characterisation of the extracts of seeds of *Glinus lotoides*. Pharmazie. **59:** 34-38.

Abebe E, Tsige G/M and **Schmidt PC** (2008) Granulation by Roller Compaction and Enteric Coated Tablet Formulation of the Extract of the Seeds of *Glinus lotoides* Loaded on Aeroperl® 300 Pharma. AAPS. **9:** 31-38.

Berhanu A and **Berhane T** (1980) A new triterpenoid glycoside from the seeds of *Glinus lotoides*. Phytochemistry. **19:** 1553-1554.

Biftu T, Berhanu A and **Teffera S** (1979) Fatty acid composition of "mettere" (*Glinus lotoides* Linne) seeds by gas liquid chromatography (GLC). SINET: Eth. J. Sci. **2:** 19-22.

Chopra RN, Nayar SI and **Chopra IC** (1956) Glossary of Indian medicinal plants. CSIR, New Delhi, India.

El-Zayat SA (2008) Preliminary studies on laccase production by *Chaetomium globosum,* an endophytic fungus in *Glinus lotoides*. AEJSA. **3:** 86-90.

Endashaw B (2007) Study on actual situation of medicinal plants in Ethiopia. Prepared for JAICAF (Japan Association for International Collaboration of Agriculture and Forestry).

Gidey Y, Mekonen T and **Mezgebe K** (2011) Survey of medicinal plants used to treat human ailments in Hawzen district, Northern Ethiopia. Int. J. Biodivers. Conserv. **3:** 709-714.

Gilbert MG (2000) Magnoliaceae to Flacourtiaceae, in *Flora of Ethiopia and Eritrea* (eds) S Edwards, T Mesfin, D Sebsebe and I Hedberg (The National Herbarium, Addis Ababa University, Ethiopia). p. 234.

Hamed AI, Piacente S, Autore G, Marzocco S, Pizza C and **Oleszek W** (2005) Antiproliferative hopane and oleanane glycosides from roots of *Glinus lotoides*. Planta Med. **71:** 554-560.

Jemal D, Tsige G/M, Kaleab A, Wondwossen E and **Ephrem E** (2007) Toxicological study on *Glinus lotoides*: A traditionally used taenicidal herb in Ethiopia. J. Ethnopharmacol. **111:** 451–457.

Kavimani S, Manisenthilkumar KT, Ilango R and **Krishnamoorthy G** (1999) Effect of the methanolic extract of *Glinus lotoides* on Dalton's ascitic lymphoma. Biol. Pharm. Bull. **22:** 1251-1252.

Khalafalla MM, Elgaali EI and **Ahmed MM** (2007) *In vitro* Multiple Shoot Regeneration from Nodal Explants of *Vernonia amygdalina*-An important medicinal plant. Afr. Crop Sci. Conf. **8:** 747-752.

Kloos H, Tekle A, Yohannes L, Yosef A and **Lemma A** (1978) Preliminary studies of traditional medicinal plants in nineteen markets in Ethiopia. Eth. Med. J. **16:** 33-43.

Kumar G, Banu G S, Rajarajan T and **Sathishkumar G** (2011) Medicinal Flora of Palayapalayam, Namakkal District, Tamil nadu. Indian J. Applied & Pure Bio. **26:** 135-158.

Lehoux H and **Chakib A** (2012) Non wood forest products. Ethiopia.

Mesfin T and **Obsa T** (1994) Ethiopian traditional veterinary practices and their possible contribution to animal production and management. Rev. - Off. Int. Epizoot. **13:** 417-424.

Mohanakrishnan L, Ramasamy D and **Balasundaram J** (2014) Rapid and efficient plant regeneration from nodal explants of *Orthosiphon spiralis* Murr. Int. J. Curr. Biotechnol. **2:** 40-44.

Mulatu D (1978) Taenicidal activity of *Glinus lotoides* **(**Aizoaceae). J. Eth. Pharm. Assoc. **3:** 9-11.

Niranjan MH, Sudarshana MS and **Girisha ST** (2010) *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. **2:** 212-217.

Otroshy M, Mokhtari A, Mehdi Khodaee MS and **Bazrafshan AH** (2013) Direct regeneration from leaves and nodes explants of *Physalis peruviana* L. Intl J Farm & Alli Sci. **2:** 214-218.

Ould Ahmedou ML, Bouaichi A and **Idrissi Hassani LM** (2001) Evidence of deterrent and toxic effects of *Glinus lotoides* (Aizoacées) on nymphs of the desert locust *Schistocerca gregaria* Forskål *(Orthoptera, Acrididae).* Zool. Baetica. **12:** 109-117.

Qureshi R and **Bhatti GR** (2008) Ethno botany of plants used by the Thari people of Nara desert, Pakistan. Fitoterapia. **79:** 468-473.

Qureshi R, Bhatti GR and **Memon RA** (2010) Ethno medicinal uses of herbs from northern part of Nara Desert, *Pakistan.* Pak. J. Bot. **42:** 839-851.

Rahman MM, Ankhi UR and **Biswas A** (2013) Micropropagation of *Mentha viridis* L.: An aromatic medicinal plant. Int. J. of Pharm. & Life Sci. **4:** 2926-2930.

Shanmugam S, Rajendran K and **Suresh K** (2012) Traditional uses of medicinal plants among the rural people in Sivagangai district of Tamil Nadu, Southern India. Asian Pac. J. Trop. Biomed. 429-434.

Sharma A, Bhansali S and **Kumar A** (2013) Micropropagation of *Eclipta alba* (L.) Hassk. an important medicinal plant of traditional medicine. IJLPR. **3:** 47-51.

Teshome S and **Soromessa T** (2015) *In Vitro* Propagation of *Satureja abyssinica* (Benth.) Briq. – A Valuable Medicinal Plant. Adv. Life Sci. Technol. **34:** 100-109.

Teshome S and **Feyissa T** (2015a) *In Vitro* Callus Induction and Shoot Regeneration from Leaf Explants of *Glinus lotoides* (L.) - An Important Medicinal Plant. Amer. J. Plant Sci. **6:** 1329-1340.

Teshome S and **Feyissa T** (2015b) Micropropagation of *Glinus lotoides* L.: An Endangered Medicinal Plant. Adv. Life Sci. Technol. **34:** 32-41.

Sivakumar V, Dhana Rajan MS, Mohamed Sadiq A and **Jayanthi M** (2014) *In vitro* micropropagation of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms - an important medicinal plant. J. Pharmacogn Phytochem. **3:** 5-10.

Sudharsan S, Anbazhagan M, Balachandran B and **Arumugam K** (2015) *In vitro* propagation of *Hybanthus enneaspermus* (L.) Muell: an important medicinal plant. Int. J. Curr. Sci. **14:** 1-6.

Thiyagarajan M and **Venkatachalam P** (2012) Large scale *in vitro* propagation of *Stevia rebaudiana* (bert) for commercial application: Pharmaceutically important and antidiabetic medicinal herb. Ind Crops Prod. **37:** 111-117.