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PHYTOCHEMICAL AND MICROPROPAGATION STUDIES IN *HEMIDESMUS INDICUS* (L.) R. BR.

B. RAMA DEVI, CH. MOHAN, P. MANJULA, B. KIRAN KUMAR, B. NARESH AND B. PRATHIBHA DEVI*

Biotechnology and Molecular Genetics laboratory, Department of Botany, Osmania University, Hyderabad University,

Hemidesmus indicus (L.) R.Br. is a valuable medicinal plant belonging to Asclepiadaceae family. *Hemidesmus indicus* has anti-inflammatory, antioxidant, antipyretic, antimicrobial and antileprotic properties. It is used as blood purifier and has diuretic, anti rheumatic and antidiarrhoeal and anti viper venom activity. A protocol has been developed for its micropropagation from nodal explants. The explants were cultured on Murashige and Skoog (MS) medium containing different concentrations and combinations of growth regulators like Indole acetic acid (IAA), 6-benzylaminopurine (BAP), Kinetin (KN) and Adenine sulphate (AS). Multiple shoots were regenerated successfully from the nodal explants. Plant extracts were analyzed for phytochemical constituents. The qualitative analysis showed the presence of various phytochemicals like alkaloids, flavonoids, saponins, phenols and tannins. Quantitative analysis for total phenols and flavonoids produced interesting results.

Key words: *Hemidesmus indicus*, micropropagation, nodal explants.

Hemidesmus indicus is commonly known as Indian Sarsaparilla. It is a perennial climber, found throughout India, more common in eastern and southern parts of India and belongs to Asclepiadaceae family. It is a well known component of Ayurvedic system of medicine as blood purifier, diuretic, antirheumatic, antidiarrhoeal and anti Viper venom activity (Chopra *et al.* 1980 and Alam *et al.* 1996). It is also used in curing fever, leprosy, rheumatism and liver disorders (Prabhakaran *et al.* 2000). Root extract of *Hemidesmus indicus* has also proved to be efficient as snake venom antidote (Alam *et al.* 1998). It also has antimicrobial (Ahmad *et al.* 1999), antipyretic (Alam and Gomes 1998) and antioxidant effect (Alam and Gomes 1998 and Ravishankaran *et al.* 2002).

Literature survey revealed that *Hemidesmus indicus* contains lupeol octocosonoate, β-sitosterol, α and β amines, tetracyclic triterpene alcohols, small amount of resin acids, alkaloids, flavonoids tannins, saponins, glycosides, phenols and ketones in its roots (Subramanian and Nair 1968).Hemidesmin and Emidin (Mandal *et al.* 1991) are present in the stem. Tannins are present in leaves (Alam *et al.* 1998).

Due to its use in treating different diseases, *Hemidesmus indicus* has gained a lot of importance in medicinal industry. To meet the demand in medicinal industry for supply of different plant parts of *Hemidesmus indicus,* its conservation is needed. Therefore micropropagation method can be used for its conservation (Chatterjee *et al.* 2000).

The present study has been taken up to investigate the phytochemical characteristics of *Hemidesmus indicus* in view of its importance in phytomedicine and also to develop micropropagation protocol to aid in its conservation.

MATERIALS AND METHODS

Hemidesmus indicus plants were collected from Andhra Pradesh Medicinal Plant Board, N. G. Ranga Agricultural University and Osmania University Campus and planted in the Botanical Garden at Osmania University, Hyderabad. These plants were subjected to phytochemical analysis (both qualitative and quantitative) to estimate the medicinally important compounds. Further, a good protocol for micropropagation was developed to aid in its multiplication and conservation.

Phytochemical analysis

Qualitative analysis: Standard screening tests of plant extracts were carried out for different parts of *Hemidesmus indicus.* The crude extracts were screened for presence of secondary metabolites such as flavonoids, saponins, tannins, alkaloids and phenols.

Test for identification of Flavonoids: Ethyl acetate (5 ml) was added to the plant extract (0.5 gm of roots ground in 100 ml of water). The mixture was shaken

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and allowed to settle. Production of yellow colour is taken as positive for flavonoids.

Test for identification of Saponins: Roots (0.5 gm) were ground with 100 ml of distilled water and transferred to a test tube. The test tube was shaken vigorously for about 30 sec and allowed to stand in vertical position and observed for 30 min. If a honey comb froth above the surface of the liquid persists after 30 min, it indicates the presence of saponins.

Test for identification of Tannins: The leaf extract was prepared (by grinding 0.5 gm of leaves in 100 ml of water) and clarified by filtration. 10% ferric chloride solution was added to the clear filtrate, and it was observed for a change in colour to blue.

Test for identification of Alkaloids: The plant extract was prepared (0.5 gm of roots ground in 100 ml of water). It was dissolved in dilute HCl solution and clarified by filtration. The filtrate was tested with Drangendorff's and Mayer's reagent. The treated solution was observed for precipitation.

Test for identification of Phenols: The plant extract was taken in a test tube (0.5 gm of roots ground in 100 ml of water) and warmed. To this 2 ml of ferric chloride was added and observed for formation of green or blue colour.

Quantitative analysis: Quantitative analysis was carried out to estimate total phenols and total flavonoids.

Determination of total phenols: Total phenolic content of the extracts was determined by Folin Ciocalteu reagent method (Mc Donald *et al*. 2001) with some modifications. Plant extract (1.0 ml) (0.5 gm of root extract in 100 ml of water) was mixed with Ciocalteu reagent and allowed to stand for 15 min and 5 ml of saturated Na_2CO_3 was added. The mixture was allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of gallic acid equivalent (mg/ g of extracted compound).

Determination of total flavonoids: Aluminium chloride colorimetric method (Chang *et al.* 2002) with some modifications was used to determine flavonoid content. 1.0 ml Plant extract (0.5 gm of root extract in 100 ml of water) was mixed with 1.0 ml methanol, 0.5 ml aluminium chloride (1.2 %) and 0.5 ml potassium acetate (0.1176%). The mixture was allowed to stand for 30 min at room temperature. Later the absorbance was measured at 415 nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

Micropropagation studies

Nodal explants were collected from healthy, one year old plants of *Hemidesmus indicus* growing in glasshouse of Department of Botany, Osmania University, Hyderabad. They were thoroughly washed under running tap water for about 20 min, followed by shaking in tween-20 for 5 min. They were then washed with distilled water about 4-5 times and later placed in 0.1 % mercuric chloride for 5-8 min, followed by rinsing with sterile distilled water.

The surface sterilized nodal explants were cultured on MS medium (Murashige and Skoog 1962) containing 3% sucrose and 8% agar with various concentrations of growth regulators (Indole acetic acid (IAA), 6-benzylaminopurine (BAP), Kinetin (KN), Adenine sulphate (AS) etc.) with combinations such as BAP + IAA, BAP + KN and AS alone. The concentrations used in the study were the outcome of preliminary studies. The pH was adjusted to 5.8 and the medium autoclaved at 121°C for 15 lbs/cm ^{2} for 15 min. About 20 ml of culture medium was dispensed into each culture tube. The explants were inoculated (on a Laminar air flow bench) into the culture medium in culture tubes which were later incubated in a sterile growth room at controlled conditions of 25 ± 2°C under cool white fluorescent light (3000 lux with 14-16 hrs/8-10 hrs L/D period) with 50 - 60 % humidity. The response of the explants in terms of shoot regeneration was observed periodically. The efficiency of shoot regeneration was calculated as the percentage of nodal explants that have responded by the production of shoots out of the total number of inoculated explants.

Table -1. Qualitative analysis of phytochemical constituents of *Hemidesmus indicus*

S. No	Plant part	Phytochemical	Presence(+) / Absence(-)
1	Roots	Alkaloids	+
2	Roots	Flavonoids	+
3	Roots	Phenols	++
4	Roots	Saponins	-
5	Leaves	Tannins	+++

+++= present very strongly, ++=strongly present, += present, - = absent

Table-2. Quantitative estimation of phytochemicals of *Hemidesmus indicus*

Name of the compound estimated in roots	Estimation in mg/100gm*
Phenols	22.92 ± 0.2
Flavonoid	4.23 ± 0.6

* Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm.

Table - 3. Effect of culture media on shoot regeneration and production of multiple shoots from nodal explants and root induction in *Hemidesmus indicus.*

Culture medium	Percentage of shoot induction*	Number of shoots per explant
MS + BAP (0.5 mg/l) + IAA (0.5 mg/l)	30	1 – 3
MS + BAP (1.0 mg/l) + IAA (0.5 mg/l)	50	2 – 3
MS + BAP (2.0 mg/l) + IAA (0.5 mg/l)	70	3 – 6
MS + BAP (0.5 mg/l) + KN (1.0 mg/l)	55	2 – 5
MS + BAP (1.0 mg/l) + KN (2.0 mg/l)	80	8 – 10
MS + BAP (2.0 mg/l) + KN (4.0 mg/l)	60	1 - 2
MS + AS (1.0 mg/l)	40	2 – 3
MS+ AS (2.0 mg/l)	50	2 – 5
MS + AS (3.0 mg/l)	75	6 – 8
Rooting medium	**Percentage of rooting****	**Surviving plants in pots**
MS + IBA (1.0 mg/l)	60	3
MS + IBA (2.0 mg/l)	80	6

*The value was calculated as the percentage of nodal explants that have produced shoots out of the total number of inoculated explants.
** The value was calculated as the percentage of shoots with root induction out of the total number of inoculated shoots.

Regenerated shoots having 2-3 nodes (of about 2 cm long) were excised from the regenerated cultures and sub cultured on the same medium. Sub culture was repeated every 5 weeks for shoot elongation. The results are expressed in terms of percentage shoot induction and days to shoot induction.

Regenerated and elongated shoots about 3-4 cm long were cultured on rooting medium (MS medium supplemented with 1.0 mg/l or 2.0 mg/l of IBA) for induction of roots. The results are expressed in terms of percentage of root induction and days to root induction.

RESULTS AND DISCUSSION

Hemidesmus indicus is a climbing slender plant with twining woody stem and opposite petiolate leaves, entire, smooth shiny, varying in shape and size according to their age (Fig.1.a). Flowers are small, in axillary sessile racemes. The root is long, rigid and cylindrical.

The study comprises phytochemical analysis and micropropagation studies to develop a good protocol.

Phytochemical analysis:

Qualitative analysis of plant extract, indicated the presence of alkaloids, flavonoids, phenols and saponins in the roots of *Hemidesmus indicus* and tannins in leaves (Table-1). These results are similar to Rajan *et al*. (2011), Gopiesh and Kannabiran (2007) and Rekha and Parvathi (2012).

Aqueous extracts in the present study were positive

Figure 1(a – f): a. *Hemidesmus indicus* plant, b. Shoot regeneration from nodal explant in 12 days after inoculation, c. Multiple shoots in 25 days after inoculation, d. Rooting from regenerated shoot in 15 days after inoculation of shoot, e. Acclimatization of regenerated plantlet f. Regenerated plant

for alkaloids in contrast to Rajan *et al.* (2011). Review on *Hemidesmus indicus* also confirmed the presence of these compounds (Gayathri and Kannabiran 2009).

The plant extracts were quantitatively analyzed for secondary metabolites like phenols and flavonoids (Table–2). In quantitative analysis of plant extract, the percentage of phenols are higher in aqueous extract (22.92 mg/100 gm) than flavonoids (4.23 mg/100 gm) and these results are similar to Sameera *et al*. (2010).

Micropropagation studies: Different explants (node, internode and leaf segments) were cultured on full strength MS medium. Among these explants, only nodal explants responded with shoot bud initiation (Sarasan *et al*. 1994) within 5 days compared with shoot bud initiation in 10 days (Sree kumar *et al.* 2000). An efficient micropropagation protocol was developed with a high percentage of shoot regeneration (Fig.1.b) and multiple shoots (Fig.1.c). The highest response of production of multiple shoots was recorded with MS + BAP (1.0mg/l) + KN (2.0 mg/l) followed by MS + AS (3.0 mg/l) and MS + BAP (2.0 mg/l) + IAA (0.5 mg/l). (Table - 3). The explants proliferated by 5-8 days and shoot regeneration was observed by 10-15 days (Fig1.d). Shoots of about 2 cm with 2-3 nodes were produced by 30 days. These were cultured on root induction media containing IBA (1.0 mg/l or 2.0 mg/l) to induce roots (Fig.1.e). The higher concentration of IBA (2.0 mg/l) produced better rooting (Table - 3). The regenerated plants were transferred to the glasshouse for acclimatization (Fig.1.f). Out of a total of 30 explants (pooled from triplicates) inoculated, 24 explants could regenerate shoots and 22 shoots were inoculated on rooting media for root induction out of which 18 shoots could develop roots to enable 14 plants to be transplanted out of which 9 plants survived in pots. In the present study, three combinations of growth regulators were used (BAP + IAA, KN + BAP and AS alone) whereas, the combinations of BAP + KN, AS alone and BAP + IAA were reported to induce regeneration (Malathy and Pai 1998). Presently, the BAP + KN combination produced the highest shoot regeneration frequency (80%) in contrast to the BA + NAA combination as indicated by Misra *et al.* (2005). In the present study it was observed that MS + IBA combination produced efficient rooting compared to the reports using Indole acetic acid (IAA) (Sreekumar *et al.* 2000 and Patnaik and Debata 1996).

CONCLUSION

The qualitative and quantitative analysis of plant parts of *Hemidesmus indicus* for screening and estimation of various secondary metabolites provides valuable information to the pharmaceutical industry for preparation of drugs and stress the need for more intensive research since they play a great role in healthcare. The present study also describes the successful development of rapid micropropagation protocol of *Hemidesmus indicus*. This protocol provides a successful technique for conservation of the valuable medicinal plant which is used in treating various disorders.

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