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Micropropagation studies and phytochemical analysis of the endangered tree *Commiphora wightii*

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ABSTRACT

Commiphora wightii is an endangered tree valued for its gum guggul-resin that has cholesterol-reducing activity is well documented in alternative systems of medicine. Phytochemical investigations were taken up in six accessions of *C. wightii* and micropropagation protocol developed for its conservation. The leaves and gum of *C. wightii* were qualitatively and quantitatively tested for secondary metabolites and an elite accession with very high contents of total sterols and phenols was identified. An efficient HPLC method was utilized in the present study to estimate the valuable and pharmaceutically important steroid compounds present in its gum, viz. E-Guggulsterone and Z-Guggulsterone, which are hypolipidemic agents. The estimation resulted in the values of 2.45 mg/L and 2.17 mg/L for E- and *Z*-guggulsterone respectively. A highly efficient micropropagation protocol with good rooting and a high plantlet survival was developed from the nodal explants to aid its conservation and 360 plants survived out of 396 plants (transferred to the field) with a high percentage of survival (92.8%). The micropropagation efficiency reported presently in *C. wightii* far exceeds all the earlier reports and was mainly achieved due to strong rooting and healthier state of plantlets.

1. Introduction

Commiphora wightii (Arnold) Bhandari (known as guggul, guggal, gum guggulu, and gugulipid) is considered endangered in India and is listed in the IUCN Red Data list (IUCN, 2010). The United Nations Development Programme has listed this species as "Critically endangered" in 2008. The Government of India has banned its export and also other over-exploited species (http://www.iucnredlist.org/details/31231/0) in the country (Billore, 1989). *C. wightii* belongs to family Burseraceae. It is a slow growing high branching shrub that grows to a height of 2–3 m with silvery and paper like gray-brown bark peeling off in small pieces (Barve and Mehta, 1993). The stem is thorny with small leaves. The leaves are alternate or fascicled small, sessile, rhomboid-(ob)ovate, compound, 1–3 (or more)-foliolate, imparipinnate; leaflets sessile or subsessile, serrate, crenate, or entire, highly aromatic, leathery, shinning green on top and greyish below. The plant has poor seed set and germination and has been subjected to indiscriminate and harsh methods of gum-harvesting leading to tree death (Soni, 2010). The oleo gum (resin) is collected by tapping guggul plants in summer and the yield is about 200–800 g per plant. The composition of Guggul is 61% resin, 29.3% gum, 0.6% volatile oils, 6.1% moisture, and 3.2% foreign matter (The Ayurvedic Pharmacopoeia of India, 2001). The guggulosterones present in the guggul gum of *C. wightii* plants have antioxidant activity (Bhati, 1950).

The diuretic activity of isolated fractions from the leaf petroleum ether extract of Commiphora berryi on healthy albino rats was studied with frusemide as reference drug (Selvamani et al., 2005). The leaf and stem extracts of different species of *Commiphora* were tested positive for the anti-oxidant (ABTS and DPPH assays), antimicrobial (MIC and death kinetic assays), anti-inflammatory (5-LOX assay), anticancer (SRB assay) properties, as well as the cytotoxic effects (tetrazolium-based assay) (Paraskeva et al., 2008) and hence, the leaves must be analyzed for medicinal compounds. The Guggulipids from the resin have been reported to be effective as anti-inflammatory, anti-bacterial, antimicrobial, anti-oxidant,

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anti-arthritic, anti-malarial, antimycobacterial, anti-schistomal, hepatoprotective, muscle relaxant, larvicidal, and molluscicidal (Atal et al., 1975; Satyavati, 1988; Satyavati, 1990; Sahni et al., 2005; Saxena et al., 2007; Pradhan and Dash, 2011). Studies have reported the cardiac and neuronal protective activity of the steroid guggulsterone (Kaul and Kapoor, 1989).It was found to be beneficial in diabetes (Lather et al., 2011) and also for prevention of cancer (Ramawat et al., 2008; Urizar et al., 2002). Extracts from *C. wightii* (guggal) are popular in Asia as cholesterol lowering agents and are gaining popularity in the United States (Jachak and Saklani, 2007).

Since the plant is very difficult to grow and is also slow growing, there is a need to develop alternative conservation methods like micropropagation for effective production of planting material. Very few reports are available on plant regeneration from cotyledonary node segments (Kant et al., 2010), shoot tip and nodal explants (Barve and Mehta, 1993) and stem cuttings (Soni, 2010). Somatic embryos were reported by Kumar et al. (2004, 2006) from immature zygotic embryo and leaf explants. *In vitro* regeneration of *C. wightii* and the influence of growth regulators was reported by Tejovathi et al. (2011). However, more efforts are still needed to establish efficient *in vitro* regeneration of this endangered medicinal plant. The present study was carried out to investigate the phytochemical characteristics of six accessions of *C. wightii* with a view to identify the elites in terms of the quality of secondary metabolites, estimate the total steroids and to utilize an efficient HPLC method for the quantitative determination of the E- and *Z*-guggulsterones in the gum exudate of the identified elite accession (s). Further, the study included the development of an efficient micropropagation protocol of *C. wightii* to aid in its conservation.

2. Materials and methods

Six accessions of *Commiphora wightii*, growing in the Botanical garden, at Osmania University were taken up for the study. These 8 year old accessions were originally sourced from different places of south India, Cw-P (Herbal garden, Rajendranagar, Hyderabad, TS); Cw-R (Karthikavanam Dhulapally, Hyderabad, TS); Cw-S (Hyderabad Urban Forestry, Erragadda, Hyderabad, TS); Cw-U (Andhra Pradesh Medicinal Plants Board, Chilkur, Hyderabad, TS); Cw-Q (Pragathi Green Nursery, Proddutur, AP) and Cw-T (Herbal garden, Rajahmundry, AP) and authenticated by the Head, Department of Botany, Osmania University, Hyderabad.

The leaves and gum exudates of the six accessions of *C. wightii* were screened using qualitative, quantitative and HPLC methods for various phytochemicals of immense medicinal value, especially the E- and *Z*-guggulsterones with a view to identify an elite which was subsequently multiplied *in vitro* by the development of a micropropagation protocol for conservation of this valuable, endangered medicinal plant.

2.1. Phytochemical analysis

2.1.1. Preparation of extracts

The extracts were prepared from healthy leaves and gum of the all the accessions of *C. wightii.* Leaf samples were collected, washed under tap water and dried in shade. They were powdered and stored in bottles for future use. The gum was collected from all the accessions by nicking the stem carefully without injuring the phloem. The resin canals are outer to the phloem and therefore deep cuts are not needed. The gum was allowed to dry for a week and then washed thoroughly under running tap water for removal of soil particles and shade dried for about 10 days. After complete drying of the gum, it was ground into a fine powder by passing through a sieve after each grinding (Fig. 1). The samples was kept in air tight containers and protected from light until used. The leaf/gum extract was prepared (by grinding 0.5 gm of leaf/gum powder in 100 mL of water) and filtered through a muslin cloth before subjecting them to the phytochemical analysis.

2.1.2. Qualitative phytochemical analysis

Qualitative tests were carried out for the presence of Alkaloids, Flavonoids, Steroids, Terpenoids, Tannins, Saponins, Glycosides, Phenols and Carbohydrates in the leaves and gum of *C. wightii* using standard procedures to identify the constituents present in these extracts.

2.1.2.1. Test for Alkaloids. The leaf/gum extract (5 mL) was dissolved in 5 mL dilute HCl solution and filtered. The filtrate was tested with Dragendroff's and Mayer's reagent. The treated solution was observed for precipitation.

2.1.2.2. Test for Flavonoids. 5 mL ethyl acetate was added to 10 mL of leaf/gum extract, the mixture was shaken and allowed to settle. Production of greenish yellow color is taken as positive for Flavonoids.

2.1.2.3. Test for Terpenoids. Presence of Steroids and terpenoids in the leaf/gum extract was tested by a mixture of acetic anhydride and chloroform (5 mL each) in presence of concentrated sulphuric acid (2 mL). Appearance of a blue-green ring at the interface between the two liquids indicated the terpenoids.

2.1.2.4. Test for Sterols. 10 gm of leaf/gum powder was added to 10 mL of Liberman-Burchard reagent (0.5 mL of sulphuric acid dissolved in 10 mL of acetic anhydride and stored covered in an ice bucket). The development of a characteristic green color confirmed the presence of sterols.

2.1.2.5. Test for Tannins. To 100 mL of the leaf/gum extract, 10% ferric chloride solution was added and was observed for a change in color to blue.

2.1.2.6. Test for Saponins. Leaf/gum powder (0.5 g) was ground with 100 mL of distilled water and transferred to a test tube. The test tube was shaken vigorously for about 30 s 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.

2.1.2.7. Test for Glycosides. Presence of Glycosides was tested by adding 5 mL dilute sulphuric acid to 10 mL leaf/gum extract. It was boiled for 5 min, filtered and cooled. Equal volume of chloroform was added and shaken well to separate into two layers. The lower chloroform layer was collected and half volume of ammonia solution added to it which turns pink due to the presence of glycosides.

2.1.2.8. Test for Phenols. The leaf/gum extract (2 mL) was taken in a test tube and warmed. To this, 2 mL of 1% ferric chloride was added and observed for formation of green or blue color.

2.1.2.9. Tests for carbohydrates. Three tests were carried out.

2.1.2.10. Molisch's test. Presence of carbohydrate was indicated when 5 mL of the extract was slowly mixed with 5 mL Molisch reagent, and later, a small amount of concentrated sulphuric acid was added slowly, leading to the formation of purple ring at the interface.

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