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IN VIVO AND I*N VITRO* COMPARATIVE STUDY OF PRIMARY METABOLITES OF *COMMIPHORA WIGHTII* (ARNOTT.) BHANDARI

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ABSTRACT:*Commiphora wightii* (Arnott.) Bhandari is an endangered, slow growing medicinal tree. In present study callus was raised from the leaf. Maximum callus was obtained on MS medium supplemented with IBA 1.5 mg/liter. The callus and different plant parts were used for primary metabolite quantification. Maximum soluble sugars found in callus, maximum amount of starch, protein and phenolic contents were found in stem and maximum lipid found in leaf.
Keywords: *Commiphora wightii*, Callus culture, Primary metabolites.

INTRODUCTION

Commiphora wightii (Arnott.) Bhandari is an important medicinal plant of herbal heritage of India. *Commiphora wightii* belongs to family Burseraceae. Unfortunately the plant *Commiphora wightii* has become endangered because of its slow growing nature, poor seed setting, (Soni, 2010) lack of cultivation, poor seed germination rate (Kumar and Shankar, 1982; Kumar et al., 2003) and excessive and unscientific tapping for its gum resin by the pharmaceutical industries and religious prophets. This plant is incorporated in Data Deficient category of IUCN (2010) Red Data list. *C. wightii* occurs in Rajasthan, Gujarat, Maharashtra, Madhya Pradesh and Karnataka states of India. Many of the species produce resins of commercial importance. About five species occur in India of which *C. wightii* (Arnott) Bhandari and *C. roxburghii* yield guggul, an oleoresin gum (Anonymous, 1950). It yields guggul, an important oleo-gumresin used as incense, fixative in perfumery and in Ayurvedic medicine. Its antiarthritic, hypocholesterolaemic and hypolipidaemic (Dev, 1999; Wang et al., 2004) properties have been established (Satyavati, 1990). It is mainly used to treat diseases like atherosclerosis, leprosy, pneumonia, rheumatism etc. The gum resin also has aphrodisiac, diuretic and immunostimulant properties.

Material and Methods

Plant material

Healthy plants of *Commiphora wightii* were collected from herbal garden of Singhania University, Pacheri Bari, Jhunjhunju, Rajasthan. Plants were authenticated by the Herbarium, Department of Botany University of Rajasthan, Jaipur.

Fresh leaves were taken from pot grown two month old plants for callus culture. Callus and all the plant parts were shade dried and powdered with the help of pestle and mortar

Chemicals

All the chemicals and growth regulators were used are analytical grade and purchased from Hi Media Pvt. Ltd. Mumbai, India.

Callus induction

Leaves were surface sterilized by 1 % Teepol for 2-3 min followed by immersion in 70 % ethanol for 1 min and in 0.1 % mercuric chloride for ½ min and then rinsed thoroughly with sterile distilled water.

The leaves were then cut into pieces with sterile scalpel. Leaf discs were inoculated on to the MS medium (Murashige and Skoog, 1962) fortified with different concentrations of 2, 4-D and IBA. The pH of the media was adjusted to 5.8 before autoclaving. All media were autoclaved at 1.06 kg cm^{-2} and 121ºC for 15 min. The cultures were incubated in growth room at temperature of 25 ± 2 °C and 16-h photoperiod. 20 replicate cultures were established and each experiment was repeated twice and the cultures were observed at regular intervals.

Primary metabolite estimation

Callus, root, stem and leaf parts of *Commiphora wightii* were evaluated quantitatively to estimate the total levels of soluble sugars, starch, proteins, lipids and phenols following the established methods for the sugars, starch (Dubois et al., 1950), lipid (Jayaraman,1981), protein (Lowry, et al., 1951) and phenol (Bray and Thorpe, 1954). All experiments were repeated three times for precision and values were expressed in mean ± standard deviation in terms of shade dried material. (Table 2)

RESULTS

Callus induction

MS medium supplemented with different concentrations of 2, 4- D and IBA for callus induction. Leaf showed maximum callus formation on MS medium with IBA at the concentration of 1.5 mg/liter. Good amount of callus in creamish green healthy colour compact callus was obtained. But on 2, 4-D at 1.5 mg/liter concentration it was fragile and yellowish. Callus obtained after 8 weeks of culture from MS medium supplemented with IBA (1.5 mg/liter) was further evaluated for primary metabolites (Figure 1).

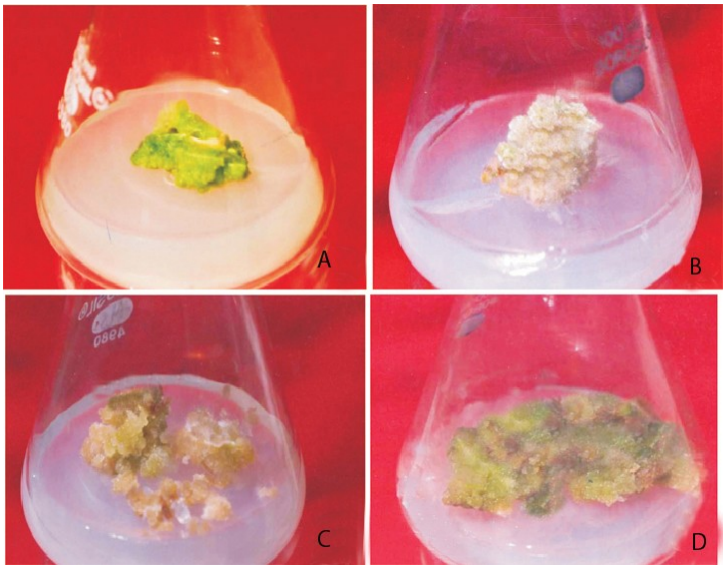


Figure: 1 Induction and proliferation of callus from leaf of *Commiphora wightii* A. leaf explant on MS medium after 7 days of inoculation B. Callus after 6 weeks (on MS medium supplemented with 2,4-D 1.5 mg/l) C. Callus after 4 weeks (on MS medium supplemented with IBA 1.5 mg/l) and D. Callus after 8 weeks (on MS medium supplemented with IBA 1.5 mg/l).

Primary metabolites

Maximum levels of soluble sugars (78±0.42 mg/gdw) was found in callus and minimum level of soluble sugar (21±0.64 mg/gdw) and protein (28.6±1.62 mg/gdw) was found in roots. Highest concentration of starch (35.2±1.22 mg/gdw) and proteins (42.8±1.83 mg/gdw) were in stem and lowest concentration of starch (24±0.29 mg/gdw) in leaf. Maximum level of lipid (30±0.45 mg/gdw) in leaf and phenolic contents (54.6±1.62 mg/gdw) in stem while minimum lipids (18±1.48 mg/gdw) in root and phenolic contents (17.25±1.16 mg/gdw) in callus were found. (Shown in table 2 and graph 1).

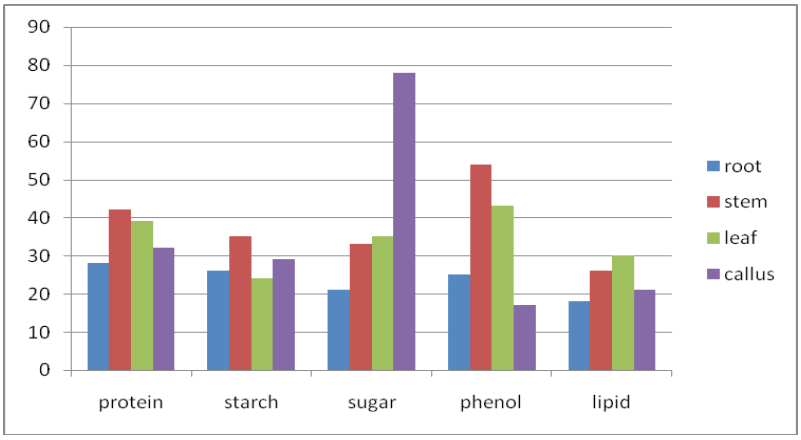
Table-1 Percentage of the callus induction from *Commiphora wightii* leaf under different levels of 2, 4-D and IAA after 8 weeks of culture.

S. No	Growth regulators	Concentration (µM/liter)	Percentage of the callus induction	Nature of callus
1	2,4-D	0.5 1.0 **1.5** 2.0 2.5 3.0 3.5	25±1.2 60±0.8 **70±1.4** 69±1.6 52±1.0 40±1.3 28±1.1	Prolific in growth, yellowish in color.
2	IBA	0.5 1.0 **1.5** 2.0 2.5 3.0 3.5	20±0.5 79±1.2 **85±1.7** 54±1.2 44±1.5 35±1.7 24±1.0	Good amount of callus in creamish green healthy colour compact callus was obtained.

Table: 2 Primary metabolites in *Commiphora wightii.*

Material	Root	Stem	Leaf	Callus
Protein	28.6±1.62	42.8±1.83	39.4±1.25	32.8±0.41
Starch	26.4±1.28	35.2±1.22	24.0±0.29	29.4±1.20
Sugar	21.0±0.64	33.6±1.29	35.0±1.65	78.0±0.42
Phenol	25.2±0.24	54.6±1.62	43.1±0.45	17.25±1.16
lipid	18.0±1.48	26.3±0.42	30.0±0.45	21.2±1.28

Graph: 1 Primary metabolites of *Commiphora wightii* in mg/ gram dry weight



DISCUSSION

The plant is an important medicinal plant and is used in large number of ayurvedic preparations to treat variety of diseases. Identification and preclinical/clinical development of novel antiangiogenic agents continues to be a topic of intense research (Dhanabal et al., 2005; De Smet et al., 2006). *In vitro* raised callus are being widely used. The callus was raised from explants of cotyledonary leaf and root segments of *Carthamus tinctorius* (Rani et al., 1996) and *Allium sativum* (Myers and Simon, 1998) were efficient for differentiation. In our study 2, 4- D 1.0 mg/liter and IBA 1.5 mg/liter both auxin concentrations showed callus formation but 2, 4-D at 1.0 mg/liter concentration showed maximum yield. *Alangium salviifolium* have been investigated for their primary metabolites (Tanwer and Vijayvergia, 2010). Comparative *in vitro* and *in vivo* biochemical performance has been evaluated in *Adhatoda vasica* **(**Chauhan and Vimala, 2009**)**. In present study callus was showed highest soluble sugars and proteins but less phenolic contents starch and lipids than *in vivo* plant parts. *In vitro* cells accumulate more sugar due to its easy availability in culture medium and these cells are in highly proliferating stage so they accumulate more primary metabolites than storage metabolites (starch, lipid) and secondary metabolites (phenolic contents). In our study also different plant parts as well as callus had showed superoxide radical scavenging activity according to their phenolic contents since presence of phenolic contents supports antioxidant status of the callus as also reported in Indian herbal tea.

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