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Effect of activated charcoal on shoot bud induction and callus induction in different explants of guggul [*Commiphora wightii* (Arnott)]: A medicinal plant

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Abstract
Commiphora wightii (Arnott) is a medicinally important plant which is now considered as critically endangered species of the family *Burseraceae* having the chromosome number 2n = 26. One of the major problem associated with plant tissue culture is browning of the culture medium and the explants, which invariably leads to death of plants. In order to control browning of medium and explants *in vitro*, many workers have tried to incorporating non specific absorbents like activated charcoal. Activated charcoal is often used in tissue culture to improve cell growth and development.

Keywords: Phenolic, browning, nodal segment, shoot apex, leaf, callus, *in vitro*, antioxidants

Introduction
Commiphora wightii (Arnott) is also an important plant of the herbal heritage of India. It is commonly known as Guggul because of the production of oleo-gum resin 'guggul', used for the treatment of inflammatory disorders, rheumatoid arthritis, lipid disorders, obesity, hyperchloesterolemia and other ailments Arora *et al.* (1973) [1], Kuppurajan *et al.* (1978) [5] and Satyavati (1988) [10]. Guggul, is an endangered species because of its over exploitation for gum resin, slow growth of the plant, poor seed set and excessive tapping Kumar and Shankar (1982)[4], Ramawat *et al.* (1991)[8] and Sharma *et al.* (1999) [12].
The majority of woody plants and some herbaceous species under *in vitro* culture shows browning of medium. If this browning was so extreme, the explants turn its colour brown to black and become growth of explants. It might be due to immediate absorption of nutrients from medium. Activated charcoal is a strong phenol adsorbent Zhou *et al.* (2010) [14] that reduces phenolic browning in explants by way of absorption of toxic substances and phenols Fernando *et al.* (2010) [2] in culture media.

Materials and Methods
The present research work was conducted on *Guggul [Commiphora wightii (Arnott)]*. Three explants *viz.*, nodal segments, shoot apex and leaves were used as explant in the present investigation. All the explants were washed with liquid detergent under running tap water for 20 minutes to remove dust particles. These were again washed with liquid detergent (Rankleen) for ten minutes with vigorous shaking. After washing with detergent, explants were again washed with running tap water to remove any trace of detergent for 5 minutes. After it were sterilized with bavistin for 5-10 minutes and then washed with double distilled water 4-5 times, then sterilized with 0.1 per cent $HgCl_2$ for 2-5 min depending upon the nature of explants. Thereafter, the explants were again washed 4-5 times with autoclaved distilled water. After sterilization the explants were inoculated on culture media aseptically.
Antioxidants activated charcoal was added to control the accumulation of phenolic compounds in the culture medium to enhance the rate of micropropagation were worked out at most responsive level of plant growth regulators differentiation (Nodal segment, 1.5 mg/l BAP and shoot apex, 2.0 mg/l Kn and callus induction in leaf explants, 2.0 mg/l 2, 4-D). The following levels of activated charcoal were tested.

I. Activated charcoal (50, 100, 150, 200, 250 and 300 mg/l).

Result
Maximum number of shoot bud (2.1) was observed at 200 mg/l activated charcoal when nodal segment was used as explant and maximum shoot bud indication (1.9) was observed at 150

mg/l activated charcoal when shoot apex explant was used. Maximum callus proliferation (1.15 g) was observed at 250 mg/l activated charcoal when leaf explant was used. (Table 1 & 2)

Effect of activated charcoal

When activated charcoal (50 - 300 mg/l) was added in the basal medium with micropropagation protocol for nodal segment (1.5 mg/l BAP), it induced shoots at all the level of activated charcoal ranging from 1.7 – 2.1 within 16 – 20 days of incubation. Maximum number of shoot bud (2.1) was observed at 200 mg/l activated charcoal with low browning intensity in the culture medium (Fig. 1). All other level showed medium browning except 200 mg/l BAP. Similarly, when shoot apex explant incubated on MS medium supplemented with 2.0 mg/l Kn along with different levels (50-300 mg/l) of activated charcoal. Maximum shoot bud indication (1.9) was observed at 150 mg/l activated charcoal followed by 200 mg/l activated charcoal with low intensity of browning in the medium (Fig. 2). All other levels of activated charcoal showed medium browning in the medium, however, (>150 mg/l) activated charcoal showed low to medium browning in the medium with comparatively less number of shoot buds due to inhibitory effect on growth through hampering the nutrient absorption.

When activated charcoal (50 - 300 mg/l) was added in the basal medium for callus induction in leaf explants (2.0 mg/l 2, 4-D), it induced callus at all the level of activated charcoal. Maximum callus proliferation (1.15 g) was observed at 250 mg/l activated charcoal followed by (0.98 g) at 200 mg/l activated charcoal with low intensity of browning in the medium (Table 2 and Fig. 3).

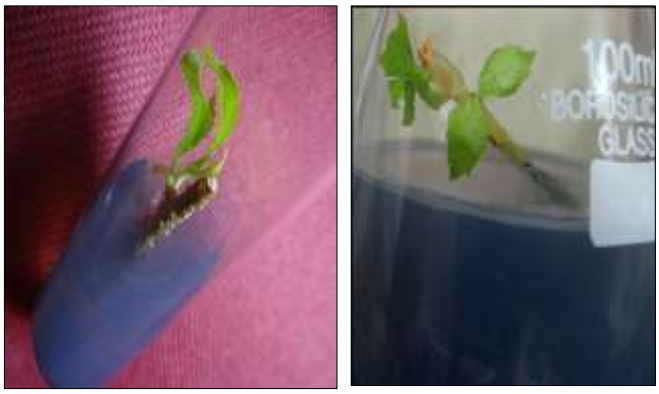
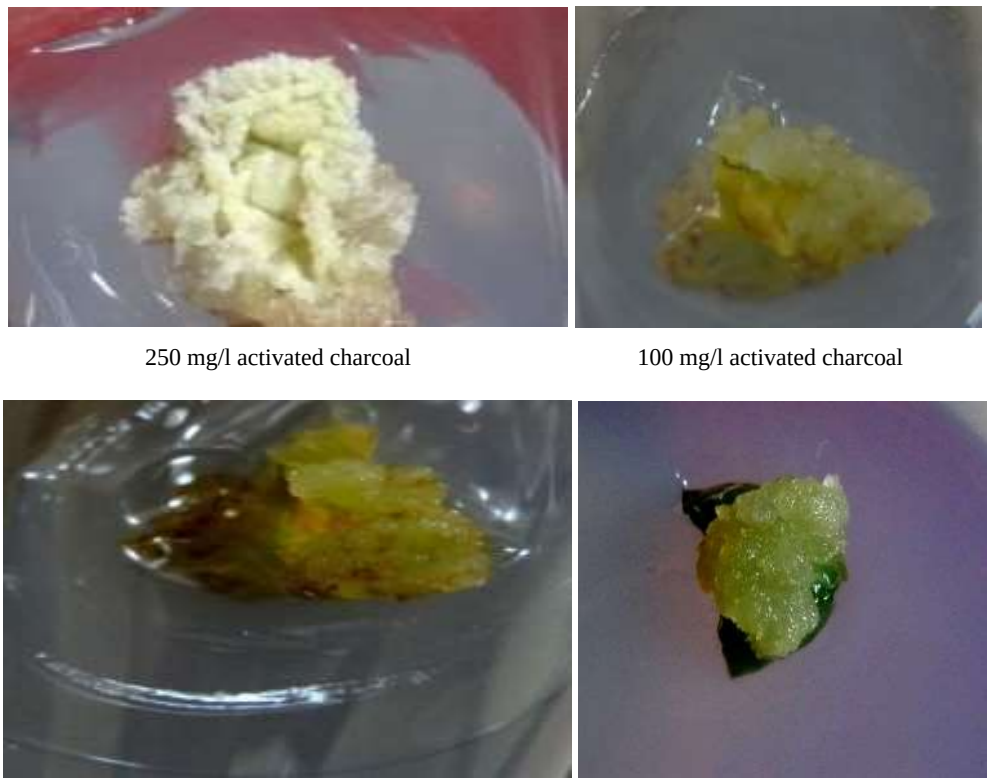


Fig 1: Effect of activated charcoal (200 mg/l) on shoot bud induction in nodal segment explants of guggul, supplemented with 1.5 mg/l BAP.

Fig 2: Effect of activated charcoal (150 mg/l) on shoot bud induction in shoot apex explant of guggul, supplemented with 2.0 mg/l Kn



250 mg/l activated charcoal 100 mg/l activated charcoal

200 mg/l activated charcoal 50 mg/l activated charcoal

Fig 3: Effect of activated charcoal on callus induction in leaf explant on MS medium supplemented with 2.0 mg/l 2, 4-D.

Table 1: Effect of antioxidants on *in vitro* shoot bud break in nodal segment and shoot apex explants on MS medium supplemented with responsive levels of plant growth regulators

Antioxidant	Concentration (mg)	Days taken in shoot induction		Number of shoot bud induction		Shoot length (cm)		Browning intensity	
		Nodal segment	Shoot apex	Nodal segment	Shoot apex	Nodal segment	Shoot apex	Nodal segment	Shoot apex
Activated Charcoal	50	18.6	18.8	1.7±0.26	1.6±0.22	1.75±0.03	1.68±0.04	++	++
	100	17.5	18.4	1.8±0.24	1.7±0.26	1.79±0.04	1.71±0.06	++	++
	150	17.3	16.8	2.0±0.14	1.9±0.23	1.85±0.04	1.97±0.02	++	+
	200	17.1	17.1	2.1±0.23	1.8±0.20	1.93±0.05	1.89±0.02	+	+
	250	18.1	18.7	1.9±0.18	1.7±0.15	1.83±0.03	1.79±0.04	++	++
	300	18.8	19.1	1.8±0.20	1.6±0.16	1.81±0.04	1.74±0.04	++	++

Table 2: Effect of antioxidants on callus proliferation from leaf explant in MS medium supplemented with 2.0 mg/l 2, 4-D in guggul

Antioxidant	Concentration (mg)	Days taken in callus induction	Fresh callus weight (g)	Browning intensity
Activated Charcoal	50	22.9	0.89	+++
	100	21.1	0.92	++
	150	20.5	0.91	++
	200	19.9	0.98	+
	250	19.1	1.15	+
	300	21.7	0.88	+++

Discussion

Activated charcoal is often used in plant tissue culture to improve cell growth and development by absorbing the beneficial plant growth promoting substances from the media. The majority of woody plants and some herbaceous species under *in vitro* culture shows browning of medium. If this browning was so extreme, the explants turn its colour brown to black and become necrotic and finally lead to die Ko *et al.* (2009) [3]. The browning of the medium is due to releasing phenol by the explants which get oxidized, and this oxidation product could be phytotoxic. Thus, it needs a scrutinized investigation before incubating explants in the culture medium. The degree of browning is different from species to species and depend on the age of the tissue (old tissues show more browning than the younger one), season of culture initiation (more in winters and autumn) and composition of the medium Saenz *et al.* (2010) [9]. Thomas (2008) [13] reported that activated charcoal can alleviate toxic metabolites, phenolic exudation, their accumulation and promotes regeneration by absorbing inhibitory compounds. Activated charcoal could promote growth by releasing substances that are naturally present in the charcoal. It also immediately adsorbed PGRs and vitamins from medium and gradually releases them again in the medium.

In the present investigation different antioxidants activated charcoal was incorporated singly in MS medium supplemented with responsive level of plant growth regulators, elicited different response for shoot bud induction and callus differentiation because it controls the accumulation of inhibitory substances (phenolic compounds) in the growth medium. Antioxidants activated charcoal was found better in reducing browning of medium and explant. The results of present investigation for use of activated charcoal were in close agreement with observation of antioxidant by Madhusudhanan and Rahiman (2000) [6] in piper species (*P. longum, P. attenuatum, P. betle, P. nigrum*). They observed effective role of activated charcoal in minimization and elimination of browning of culture medium.

Activated charcoal is a strong phenol adsorbent Zhou *et al.* (2010) [14] that reduces phenolic browning in explants by way of absorption of toxic substances and phenols Fernando *et al.* (2010) [2] in culture media. Parmar and Kant (2012) [7] and Sharma *et al.* (2012) [11] reported positive response of activated charcoal (0.3 per cent) for shoot bud break in nodal segment of *Commiphora wightii* and root induction *in vitro* derived shoot of *Acacia leucophloea*, respectively.

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