Summary of the Minor Research Project Funded by UGC

Project Title: Phytochemical Study of *Cassia fistula* an important laxative medicinal plant

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Plants provide a variety of resources that contribute to the fundamental needs of food, clothing and shelter. Among plants of economic importance medicinal and aromatic plants have played a vital role in human sufferings. The history of herbal medicines is as old as human civilization. *Cassia fistula* L. belonging to family Caesalpiniaceae is locally known as Amaltas or Garmalo. *Cassia fistula* is mainly mild laxative. Both wild and cultivated plants are used for drug formulation.

The unscrupulous collection of medicinal plants from wild habitats by traders has threatened the very existence of valuable medicinal plant resources. Due to bio piracy and over exploitation, some of the medicinal plants are becoming rare.

Plant tissue Culture Technique is an important tool for a bypass or to overcome this difficulty. The production of useful metabolites from plant tissue culture has created a new methodology for their commercialization.

Phytochemicals are the naturally occurring biochemical in the plant that gives plant their color, flavor, smell and texture. They may help to prevent diseases like cancer and heart diseases besides their role to inhibit the microorganisms causing many diseases in human beings. One of the largest groups of chemical compounds produced by plants is alkaloid.

Alkaloids are more or less toxic substances. They have a basic character, containing heterocyclic nitrogen, and are synthesized in plants from amino acids or their immediate derivatives. In most cases they are of limited distribution in the plant kingdom. Flavonoids are another group of plant secondary metabolites which are present almost universally in higher plants and contribute to the flower and fruit colour. They impart mostly red, yellow, blue and violet colour to plant organs. Chemically they are phenolic compounds. Triterpenoids can be divided into at least four groups of compounds: True triterpenes, Steroids, Saponins and Cardiac glycosides.

The expanding universe of the chemistry of natural products is indicative of the organic chemist's interest in the plant kingdom for finding new phyto-constituents of therapeutic value, precursors for the synthesis of complex chemical substances, or new sources of compounds of economic value.

Cassia fistula is known for its laxative property. It is also effective in Skin fungal infection and constipation. Pulp of the pod contains anthraquinone glycosides, sennosides A & B, rhein and its glucoside, barbaloin, aloin, formic acid, butyric acid and their ethyl esters and oxalic acid. In the Present study presence of many important phytoactive substances was confirmed through spot test analysis.

Presence of Alkaloids, flavonoids, steroids, saponins, tanins, Cardiyac glycosides, anthraquinons and Iridoids are confirmed in the all four important part of plant viz. leaf, stem, bark and pod. Secondary metabolites are analyzed from the leaf, bark, stem and pod through HPTLC. 25 spots of alkaloids compound were detected from leaf of *C. fistula* through HPTLC under 254 nm wavelength. Bark showed 29 spots, Stem showed 27 and pod showed 26 spots of Alkaloids. Comparative study of alkaloids among the all four plant part indicated that there were 10 substances common. Results indicate that there is a great effect of storage condition on alkaloid production from pod. Under 366 nm and visible light 540 nm detection of alkaloid substance also indicated the maximum number of spots from 4 month old pod and gradually decrees the number of spot from old pod respectively. Alkaloids of leaf, bark and stem under storage showed maximum number of alkaloid spots from six month old leaf but less number of spots from fresh and old leaf. Bark and stem under 254 nm showed very less variation under storage with limited number of spots.

Flavonoid detected at 254 nm showed 25 spots from leaf, 29 from bark, 27 from stem and 25 from pod. Compounds detected at 366 nm revealed that 7 compounds were common among leaf, bark, stem and pod. Effect of the storage on the production of flavonoid from pod showed that fresh pod and 5 month old pod gave maximum number of flavonoid detected under 254 nm. However 11 month old pod also showed higher number of flavonoid but overall production of flavonoid decreased under the storage after 6 month. It is observed that flavonoids content is decreasing after six month. On the other hand leaf is showing less number of spots of flavonoids from fresh material but higher number of spots from the 9 month and 12 month old material.

Steroids of Cassia fistula leaf, bark, stem and pod detected under 254 nm wavelength showed maximum number of spots of steroidal compound from bark material followed by stem and pod with 27 spots from bark and 26 spots from stem and pod. Among the steroids produced from leaf, bark, stem and pod 8 spots were visualized common under 254 nm Among the steroids produced from leaf, bark, stem and pod 8 spots were visualized common under 254 nm. Production of steroid from pod under storage indicates that maximum steroid was produced from 4 month old pod. Under the 366 nm maximum steroid compound was detected from 4

month and 6 month old pod. 6 month old leaf produced maximum spots of steroids. Bark showed less variation in production of steroid. However stem produced steroid from old material stored up to 9 month.

MS media supplemented with combination of auxin (2,4-D) and kinetin were studied. Among of the five combinations (1 mg/l 2,4-D + 1mg/l kinetin, 2 mg/l 2,4-D + 2 mg/l kinetin, 3 mg/l 2,4-D + 3 mg/l kinetin, 4 mg/l 2,4-D + 4 mg/l kinetin, 5 mg/l 2,4-D + 5 mg/l kinetin) of PGRs, 2mg/l 2,4-D + 2mg/l kinetin was found most suitable media for the maximum production of callus through leaf culture in *C. fistula*.

Comparison of *in vivo* and *in vitro* produced alkaloids of *C. fistula* in UV – 200 nm revealed total 12 fluorescent zones (in blue to light blue background) between Rf 0.03 to 0.69. Nine spots were found common in *in vivo* and *in vitro.* Comparative study of in vivo and in vitro produced flavonoids of *Cassia fistula* detected in UV – 200 nm showed 15 fluorescent spots in blue background. 11 spots were common. Comparison of *in vivo* and *in vitro* produced Steroids of *C. fistula* in UV–254nm revealed total 8 fluorescent zones (brown to dark brown) between Rf 0.03 to 0.86. Six spots at Rf 0.03, 0.18, 0.26, 0.42, 0.54 and 0.62 were found common in both *in vivo* and *in vitro* sample. From the above result the following conclusions are drawn for *Cassia fistula*

- Leaf and bark is equally potent of produce the alkaloid substance as pod.
- Bark is more potent for the production of flavonoids from *C. fistula*
- Steroids are produce in higher amount from pod and stem.
- Storage condition affects the production of secondary metabolites. Maximum secondary metabolites of *C. fistula* were produced up to the six month of stored material. It requires the standardization of proper storage method for stabilizing the content of the *C. fistula*.
- Plant tissue culture technique can be used for the production of important secondary metabolites.
- Callus from the leaf explant of *C. fistula* can be produced on 2 mg/l 2,4-D and 2 mg/l kinetin.
- Comparative study of secondary metabolites produced from *in vivo* and *in vitro* plant material showed that important secondary metabolites of *C. fistula* can be produced through tissue culture. That will help to maintain the quality of the pharmaceutical important drug and sustainable supply of the medicinally important compound.