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Chemoprofile and bioactivities of *Taverniera cuneifolia* (Roth) Arn.: A wild relative and possible substitute of *Glycyrrhiza glabra* L.

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Abstract

Chemoprofile of *Taverniera cuneifolia* (Roth) Arn. a wild relative of commercial licorice (*Glycyrrhiza glabra* L) is presented. Both *T. cuneifolia* and *G. glabra* L were found to be very similar phytochemically. At least eighteen chromatophores were found similar in both the plants including the sweetening principle, glycyrrhizin. The extracts of *T. cuneifolia* root, exhibited promising anti-inflammatory, anti-tumor, anti germ tube formation (in *Candida albicans*), protection from mutagen toxicity and cytotoxic activities comparable to that of *G. glabra*. In general, the results suggest that *T. cuneifolia* could be used as substitute of *G. glabra*.

Keywords: *Taverniera cuneifolia*; *Glycyrrhiza glabra*; Chemoprofile; Anti-HIV; Anti-inflammatory; Anti-tumor; Protective

Introduction

The genus *Taverniera* belonging to the family of Fabaceae, includes twelve species and is endemic to the Northeast African and Southwestern Asian countries (Naik, 1998; Stadler et al., 1994). Literature available on this plant is scarce, except for *Taverniera abbysinica*, which is used as a 'drug for sudden illness' in the African subcontinent (Stadler et al., 1994). *Taverniera cuneifolia* (Roth) Arn., is often referred as Indian licorice as its roots are sweet and taste very similar to that of *Glycyrrhiza glabra* L., popularly known as commercial licorice (Zore, 2005). The roots of *G. glabra* are very widely used in traditional systems of medicines all over the world (Grieve, 1992). *G. glabra* is rich in bioactivities like antiviral, anticancer, anti-ulcer, anti-diabetic, anti-inflammatory, anti-oxidant, anti-thrombic, anti-malarial, anti-fungal, anti-bacterial, estrogenic, immunostimulant, anti-allergenic and expectorant activities (Olukoga and Donaldson, 2000; Baltina, 2003; Sasaki et al., 2003, Cinatl et al., 2003, Rastogi and Mehrotra, 1989). The commercial licorice has a huge demand in the Indian system of medicine, Ayurveda and majority of the requirement of the Ayurvedic drug industry in India is met through import from Afghanistan and Pakistan (Rastogi and Mehrotra, 1989). A number of plants often

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referred as Indian licorices, could be potential alternatives to *G. glabra*. Not many studies are available on the scientific validity of indigenous alternatives or wild relatives of *G. glabra*. In this communication, we are presenting the chemoprofile and bioactivities of the root extracts of *T. cuneifolia* and it is compared with that of *G. glabra*.

Materials and methods

Roots of *T. cuneifolia* were collected from Osmanabad district of the Maharashtra state, India. Dried roots and runner pieces of *G. glabra* L. were obtained from local stores. Twenty grams of shade dried and powdered plant materials were extracted in 200 ml of 70% ethanol using a soxhlet extractor. The extracts were then filtered and evaporated to yield brown residues. The residues suspended in distilled dimethyl sulphoxide (DMSO) was used as crude extracts. Plant materials were also extracted sequentially using solvents with increasing polarity for 6–8 h. Flavonoids and Coumarins were extracted by refluxing 1-g root powder in 5 ml of methanol at 60 °C in a water bath for 30 min. Extracts were filtered, concentrated and re-suspended in 1 ml of methanol. Saponin extract residues were re-suspended in chloroform/methanol (1:1).

HPTLC analysis

Precoated TLC plates (Silica Gel 60 F254) of 0.2 mm thickness and 20×20 cm size were purchased from Merck KGaA, Germany. Standard Glycyrrhizin was purchased from Sigma (USA) and 1 mg/ml stock solution was prepared in ethanol. Ten microliter of the crude extracts and standard glycyrrhizin (5 µl) were loaded as 10-mm streak on HPTLC plates at 10-mm distance between two streaks, using a Linomat IV an automatic spotter (Camag, Pvt. Ltd., Switzerland). Plates were allowed to dry for few minutes and developed using *n*-butanol: acetic acid: water (7:1:2) as a solvent system. Plates were dried, observed under UV 254 and 366 nm and scanned using a Camag Scanner III (Switzerland). UV Spectra, Rf value, % AUC and λ max of each chromatophores were documented. Plates were derivatized using specific detection reagents (e.g. for glycyrrhizin, anisaldehyde: sulfuric acid reagent) and observed visually as well as under UV at 254 and 366 nm. All the extractions, chromatographic separations and analysis were done as per Wagner and Bladt (1996).

Anti-inflammatory assay

Wistar rats having an average body weight of 150–200 g were orally fed with ethanol/chloroform/petroleum ether extracts of *T. cuneifolia*, for 3 days prior to the injection of Carrageenan with doses of 250 mg and 500 mg/kg body weight. The animals were divided into eight groups of six each. Group I served as control, received distilled water, Group II and III received ethanol extract, Group IV and V received chloroform, Group VI and VII received petroleum extract. Group VIII served as positive control and received Na-Diclofenac, 9 mg/Kg (Cipla Pvt. Ltd., India). Drug was administrated orally, daily as a single dose. After pre-treating the animals for 3 days, on the day 4, 0.1-ml Carrageenan (10%) in distilled water was injected subcutaneously into the *plantar plexus* of the hind paw (Winter et al., 1962). Before injection, initial paw volume was recorded, plethysmographically. After injecting carrageenan, paw volume was recorded at 2, 4, 6 h and thereafter everyday for the next 5 days. Na-Diclofenac treatment was continued for the next 5 days. Statistical analysis was done by Students *t*-test.

Cytotoxicity testing

Cytotoxicity of crude extracts were determined in MT-2 cell line (T lymphoid cell line from NIH AIDS Research and Reference Programme, USA). Extract concentrations ranging from 0.24 to 500 µg/ml were prepared in 96 well flat-bottom tissue culture plates, using RPMI 1640 medium (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) containing 10% FCS (Moregate, Australia). To each well, washed, 2×10^4 MT-2 cells were added. The plates were incubated for 5 days at 37 °C. On the fifth day, plates were examined microscopically for cytotoxic effect and the cell viability was determined by trypan blue dye (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) exclusion method (Sasaki et al., 2003; Hu and Hsiung, 1989; Nokta and Pollard, 1992).

Virus titration

Seven serial four-fold dilutions of virus stock (ranging from 1:16 to 1: 65,536) were prepared. MT-2 cells were added to each well and the plates were incubated at 37 °C. After overnight adsorption, cells were washed and incubated at 37 °C for 7 days. At the end of incubation period, culture supernatant was tested for the presence of p24 antigen using a commercial ELISA kit (Coulter Inc., USA). The $TCID_{50}$/ml was calculated by Spearman-Karber method (Sasaki et al., 2003; Hu and Hsiung, 1989; Nokta and Pollard, 1992).

Anti-HIV activity

Using MT-2 cell line and HIV-1 III B strain, sub toxic concentrations of the extracts were tested for its ability to inactivate cell free HIV and to inhibit HIV replication