Indian J. Pl. Genet. Resources 8(1): 49-56, 1995; Special Issue (2)

VARIABILITY IN TOTAL BITTERS IN *TINOSPORA CORDIFOLIA* (WILLD.) MIERS POPULATIONS

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Giloe wood is used in number of formulations in Indian system of medicine and has a large growing demand in the market. Its pharmacological activity is evaluated in terms of its total- bitters (TB) content. Accessions collected from wide areas of UP, Delhi and other regions were evaluated for TB contents. It is observed that there is a wide variability in it. Promising accessions were identified in terms of its TB content which can be grown for commercial cultivation.

Key words : *Tinospora cordifolia*, total bitters, variability

Giloe (*Tinospora cordifolia*) is a glabrous woody, climbing shrub, bearing large alternate and cordate leaves. It is a fairly common plant of the dry deciduous forests growing over hedges and small trees. This species is distributed throughout tropical and sub-tropical India from Punjab to North eastern states and extending to Deccan plateau including Konkan, Karnataka and mesophytic forests in Kerala in South India. This plant grows in varying soil types with great ease and its woody stems attain a thickness of 6cm diameter. At present, it is nowhere cultivated commercially but is raised, sometimes, as ornamental climber through stem cuttings. As a drug plant, it has large bulk demand in the market, ranging from 300-500 tonnes of dry woody stem annually.

Giloe is used in the Ayurvedic system of medicine since remote past. It is reported to be useful in treating 'Kushta, Meha, Jwara, Svasa, kasa and Aruchi' (Bhavmisra, 1969; Raghunathan and Mitra, 1982). It is known as bitter-tonic, astringent, diuretic aphrodiasic, promote longevity with curative properties against infection, jaundice and provides relief in diabetes, piles, diaorrhea, dysentry etc. (Chopra *et al.*, 1982; Gupta *et al.*, 1967; Singh *et al.*, 1975; Sharma, 1982). Although a large number of chemical compounds are reported in this plant (Bisset and Nwaiwu, 1983; Chatterjee and Ghosh, 1960; Dixit and Khosla, 1971; Hanuman *et al.*, 1986; Kidwai *et al.*, 1949; Qudrat-i-Khuda *et al.*, 1964, 1966), yet no authentic information on the identity of pharmacologi-

cally active chemical constituent(s) in *Tinospora cordifolia* is known. It is considered that the bitter extract obtained from the plant contains pharmacological activity (Wlth. India, 1976), hence a study of chemical nature of total-bitters in plants, its chemical assay and the factors affecting its synthesis and accumulation was undertaken. Utilising this newly developed methodology, woody stems of plants of *Tinospora cordifolia* collected from Uttar Pradesh, Delhi and those obtained from different parts of the country, were screened for total-bitters content. This will help in the identification of superior genotypes for domestication and commercial cultivation.

MATERIALS AND METHODS

1. Plant material

Seventy-seven wild growing populations of Giloe were sampled from foothills and eastern part of UP and Delhi state. A large number of samples were obtained from other parts of the country (Table 2, 3) also. These vines were found associated with several tree species as support and along roadsides. For this study, twigs of nearly same age group were collected in the month of February. Woody stems of 1 cm. diameter thickness were cut into small pieces, dried in shade and powdered for chemical analysis.

2. Extraction of TB

The dried and powdered stems of two materials (50 gm each) were subjected to successive extraction for 8 hours with solvents. Continuous hot extractions with successive solvents were carried in soxhlet apparatus (Table 1).

Table 1. Successive solvent extarction of *Tinospora cordifolia*

Solvent	Extractive (%)		Taste	Compound	TLC
	Sample-1	Sample-2			
Petroleum Ether (60-80)	1.07	1.52	Tasteless	-	-
Benzene	090	0.70	Tasteless	-	-
Chloroform	0.60	0.52	Faintly bitter	Dragen-droff+	Four spots
Ethyl acetate	0.65	0.61	Bitter		Four spots
Ethyl alcohol	4.45	3.58	Bitter	Dragen-droff+ Saponins	Five spots
Water	6.50	7.22	Tasteless	-	-

Table 2. Total-bitters content in *Tinospora cordifolia*

Collection from U.P.	Total-bitters(%)
Nawabganj (Gonda)	0.2313
Madhavpur (Gonda)	0.7320
Manakpur (Gonda)	0.7572
Dalpatpur (Gonda)	0.8120
Murta range Forest (Bahraich)	0.3365
Katarnia Forest (Bahraich)	0.4542
Amba (Baharaich)	1.0115
Chikwa (Balrampur)	0.2127
Purshotampur (Gonda)	0.5857
Katra (Gonda)	0.5150
Dhabrua (Basti)	0.8395
Bisraolia (Basti)	0.4538
Kaptanganj (Basti)	0.5980
Haraiya (Basti)	0.5302
Chawani (Basti)	0.9025
Rauja (Barabanki)	1.0292
Ram Snehi Ghat (Barabanki)	0.2770
Purwa (barabanki)	1.1488
Ashram (Barabanki)	0.6792
Bhathat (Gorakhpur)	0.6640
Bhathul (Gorakhpur)	0.3000
Fareda (Gorakhpur)	0.7736
Sahjanwa (Gorakhpur)	0.7300
Saraya (Gorakhpur)	0.9448
Halalgarh (Gorakhpur)	0.6460
Dalalganj (Gorakhpur)	0.6780
Shyamdevra (Maharajganj)	0.4140
Parasa (Maharajganj)	0.1312
Pipra (Maharajganj)	0.3524
Mahadeva (Maharajganj)	0.4328
Bhitauli (Maharajganj)	0.5620
Khalilabad (Basti)	0.6070
Pauri (Ratora)	0.4425
Tehri (Pao)	0.2352
Tehri (Chaih)	0.3455
Almora (Bheta)	0.2735

Both the materials were also repeatedly refluxed with water. Solvents of extractives were distilled off and residues weighed. These residues were tasted for bitterness. These were also tested with different reagents prepared in the laboratory for determining the nature of compound present in each extractives. These extractives were also run over thin-layer chromatography (TLC).

3. Column chromatography

In order to isolate compounds constituting total bitters of *T. cordifolia*, the alcoholic extract was run over column of silica gel. 50 g dried and powdered material of *T. cordifolia* was subjected to repeated refluxing with ethanol (95%) to extract out TB. These extracts were combined, concentrated to 5 ml, filtered and run over silica-gel filled (175 g) glass column with chloroform with increasing concentration of methanol ($CHCl_3$, $CHCl_3/CH_3OH$ 3/1, $CHCl_3/CH_3OH$ 1/1, $CHCl_3/CH_3OH$ 1/3, CH_3OH). 22 fractions of volume 25 ml each were collected, concentrated and run over TLC for qualitative analysis.

4. Thin layer chromatography (TLC)

TLC was performed on silica gel coated (0.25 mm thick) glass plates (20 × 5cm). The plates were developed with solvents to a distance of 15 cm from the point of application. The following solvents were used:

1. $CHCl_3$: CH_3OH (90/10)
2. Ethyl acetate : CH_3OH : H_2O (77/15/8)
3. Toluene : Ethyl acetate : Diethylamine (70/20/10)

The extracts were spotted (2-5 μ/l) on the plate and run. Attempts were made to identify the compounds by spraying the plates with following reagents prepared in the laboratory, 1. Dragendorff reagent 2. Vanillin Sulphuric acid reagent and 3. Concentrated Sulphuric acid followed by heating at 100° C for 2 min.

RESULTS AND DISCUSSIONS

1. Chemical studies

To evaluate the qualitative and quantitative nature of total bitters (TB) in *Tinospora cordifolia*, the dried powdered material was extracted with solvents successively over Soxhlet apparatus (Table-1). By testing, it was found that the extractives of ethyl acetate and alcohol were distinctly bitter. It was found that ethyl alcohol extracted all the bitters from the material by repeated refluxing (This was confirmed by testing successive extracts until no more bitterness found in the extract).

To isolate chemical compounds constituting TB, the extracts were combined, concentrated and run over column chromatography. It was found, by TLC, that bitter components were present in eluants 11-16, 20-22 and in final methanol. It was also noted that these extracts responded positively to the test for saponins and to Dragendorff reagent. One of the Dragendorff positive compound was highly basic, moved very little on TLC and responded to the test of berberine alkaloid. Furthermore, it was also observed that all these spots showed myriad of colour under UV-light. It was found that total bitters (TB) consisted of five spots shown over TLC, indicating five chemical components.

2. Chemical evaluation

It was found that, though ethyl alcohol extracted all the bitters from the material, it also contained many other impurities. A method was worked out to remove impurities from the extracts without affecting TB, Washing the extract repeatedly, first with $CHCl_3$ and then with C_6H_6 impurities removed from the extract. This method (Fig-1) was found to be simple, rapid and highly reproducible. Utilizing this method, the accessions were chemically evaluated.

3. Factors Affecting TB

(a) *Season of collection*

It was earlier, shown that material collected during winter when the plant was defoliated, showing maximum TB (Srivastava *et al.*, 1990) Fig. 2.

(b) *Thickness of stem*

Since thickness of the stem is directly related with its age, TB was found to be increasing with thickness of stem (Fig. 3)

(c) *Host plant*

It is a traditional belief amongst the Ayurvedic practitioner that this plant grown over 'Neem' and mango trees, would have increased medicinal value due to higher blitterness (Watt, 1972). However, it was found that host plants did not produce any appreciable effect over TB content of this woody climbers.

Extent of total bitters in collections

There is great variation in TB content (Table 2) in the collections of UP. The range of variation is from 0.1312 to 1.1488 per cent. Amongst the collections analysed, seven accessions were showing more than 0.8 per cent TB whereas four were as low as 0.2 per cent. By and large accessions collected from UP hills were showing average TB content ranging from 0.2352 to 0.4425 per cent. It was concluded that genotypes from Dalpatpur (Gonda), Amba (Bahraich),

Table 3. Total-bitter content in *Tinospora cordifolia* collections from Delhi and other parts of the country

Place of collection	Total bitters		Total bitters
Delhi		**Outside Delhi**	
Talkatora Garden	0.6157	Regional Research Centre (Ayurveda), Jaipur	0.2458
ITO Cemetry	0.3519	RRC (Ayur.), Thinuvananthapuram	0.5938
Devnagar	0.2758	RRC. (Ayur), Vijaywada	0.8085
Gulabibagh	0.2563	RRC (Ayur), Gwalior	0.2130
Zakhira	0.1538	RRC (Ayur), Itanagar	0.4280
Lohamandi	0.2721	RRC (Ayur), Gauhati	1.4227
FCI Gate	0.1206	NBPGR, Shillong	0.2028
Shankar Road Ridge	0.8715	Anand	0.1472
Rajendra Nagar (R-Block)	0.4702	Kerala Agril. Univ., Vellanikkara	0.4701
Bikaner House, India Gate	0.3369	RRC (Ayur), Coimbatore	0.2115
Mausam Bhawan, Lodhi Rd.	0.1118		
Jhandewalan	0.3885		
Panchkuin Road	0.2745		
Rajendra Nagar, Ridge Road	0.2062		
Mycology Div., IARI	1.0287		
Punjabi Bagh	0.2493		
Behind Red Fort	0.3820		
Azadpur	0.3995		
Tees Hazari	0.2375		
Gulmohar Park	0.2133		
Shankar Road	0.5362		
Mandir Marg	0.2340		
Lodhi Road	0.2242		
Subramaniam Bharti Marg	0.5939		
Hauz Khas Tomb	0.2460		
Khirkee Village	0.2898		
Kalkaji Mandir	0.2356		
Inderpuri (Military Road)	0.7360		
Inderpuri A-Block	0.5660		
Mayapuri LIG Flat	0.5027		
Sarita Vihar	0.7156		

Dhabrua (Basti), Chawani (Basti), Raoja (Barabanki), Purwa (Barabanki), Saraya (Gorakhpur) of UP were very promising. Accessions collected from Delhi were showing wide variation in TB (Table 3). It ranges from 0.1118 to 1.0287 per cent. Only two accessions collected from Shankar Road ridge (0.8715%) and

from IARI (1.0287%) were observed to be promising. Similarly materials received from other parts of the country exhibited great variation i.e., from 0.1472 to 1.4227 per cent TB. Only two accessions obtained from RRC (Ayurveda), Vijayawada (0.8085%) and RRC (Ayur), Guwahati (1.4427%) were found to be promising.

The variability in TB can be utilised in making selection of elite type for raising of new plants under domestication leading to crop improvement programme. At present raw material for pharmaceutical industry are usually collected from wild growing plants. Pharmaceutical establishments, on the other hand require a regular supply of enormous quantities of raw material which only commercial cultivation can produce. Hence information generated through this study will help in making selection of promising genotypes for commercial cultivation leading to improved yield of better raw material.

ACKNOWLEDGEMENTS

The authors express sincere thanks to Dr. O.P. Singh, Plant Breeder, and Dr. J. Prasad, Assoc. Professor (Hort.), N.D.U.A.T., Kumarganj, Faizabad for providing all assistance in collection tour of eastern Uttar Pradesh. The authors are also grateful to Director, NBPGR, New Delhi for providing the facilities.

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