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Physicochemical evaluation of male and female plants of Guduchi (*Tinospora cordifolia* (Willd.) Miers)

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Abstract

Guduchi (*Tinospora cordifolia* (Willd.) Miers.) is a dioecious creeper, commonly known as 'Giloe', with significant medicinal importance in the traditional systems of medicine. The plant is dioecious, however, this factor is not given proper consideration while collecting the plant material for medicinal purposes. Minimal studies are available on male and female varieties of Guduchi and no published information on the comparative physicochemical study of these varieties is available so far. Considering this, the present study is planned to comparatively evaluate the physicochemical profiles of male and female varieties of *Guduchi*. Both male and female varieties were collected, authenticated and subjected to morphological/organoleptic screening, relevant physico-chemical parameters, qualitative testing for various functional groups, and quantitative estimation of total alkaloids, total starch and mucilage contents. The present study differentiated both male and female varieties in morphological and organoleptic characters. The values of water and alcohol soluble extractives was found relatively higher in male variety, while the total alkaloid, total starch and total mucilage contents were higher in female variety. All functional groups were found to be same in both varieties; however, more extensive works on quantification of chemical constituents and secondary metabolites are needed.

Keywords: *Guduchi*, Dioecious, physicochemical evaluation, *Tinospora cordifolia*.

Introduction

Guduchi is an incredibly versatile vine in Ayurvedic system of medicine since ancient times. In Ayurveda, it is designated as *Rasayana* drug recommended to enhance general body resistance and promote longevity and as antistress and adaptogen.^{1,2} Several reports on its chemical constituents, medicinal properties, and validation of therapeutic claims have already been published.³⁻⁵ The accepted botanical source of *Guduchi* throughout India is *Tinospora cordifolia* (Willd.) Miers., which belongs to family Menispermaceae. The genus *Tinospora* is derived from the Latin word *tinnio* and Greek word "*spora*" means subglobose or ellipsoidal seed. In species '*Cordifolia*', the word *folia* means leaves having cordial-heart shape. Family: Menispermaceae means drug having moon or crescent shaped seed.⁶ *Tinospora cordifolia* is a large glabrous, dioecious climber with succulent, corky, grooved stems. The plant is dioecious, where male and female flowers are borne on separate plants, however, this factor is not considered generally while collecting the plant for medicinal purposes. The phenomenon of dioecy in plants is rarer and is confined to about 7% of the known taxa, widely distributed in different orders and families.⁷ It is notable that nearly twenty five plant species with known dioecism are presently being used traditionally. Different genders are reported to exhibit few differences such as in growth rates, morphological and microscopical characters, and phytoconstituents levels.^{8,9} Recent reports suggest that female *Guduchi* is having higher concentration of secondary metabolites and superior antioxidant potential than male variety.¹⁰ Another report also concluded that there are many morphological, genetical, microscopical, and phytochemical differences present in male and female *Guduchi*, however, this difference has failed to influence the expression of pharmacological activity.¹¹ Nevertheless, future experimental and clinical studies are needed to validate the findings and to understand the difference in mechanism of biological expression of activities of both varieties.

In spite of numerous medicinal attributes, wide use in practice, and several reported studies on the physicochemical profile of various dosage forms of *Guduchi*,¹²⁻¹⁴ information on the comparative physicochemical profile of male and female varieties is not available. Considering this, the present task is undertaken to ascertain the physicochemical standards of both genders of *Guduchi*.

Materials and Methods

Collection of raw materials

Fresh plants of both male and female varieties of *Guduchi*, spreading over *Nimba* (*Azadirachta indica*)

tree were collected from the non-polluted, wild areas near the campus of Abhilashi Ayurvedic college and research institute, Abhilashi University, district Mandi, Himachal Pradesh, in between date 05/02/2015 to 06/02/2015. After collection, the plants were authenticated by the concerned authority in the Pharmacognosy

laboratory of the same institute and the voucher specimen was kept for reference. For identification and authentication help was taken from various available official Databases and floras.¹⁵⁻¹⁸ Few morphologically differentiating characters are observed in stem of male and female plants as mentioned in table 1.

Table 1: Comparative morphological /organoleptic characters of male and female stem

Characters	Male plant stem	Female plant stem
General Appearance	Succulent, ridged, studded with warty tubercles as a result of the development of vertical and longitudinal rows of lenticels, with characteristic three beaked nodal swellings	Similar characters as that of male one but more succulent and less flaky in nature.
Shape	Slender, dextrorotatory twinned	Similar
Size (2 years mature plant)	1.38-2.06 cm	1.74-2.32 cm
Surface	Jagged due to longitudinal fissures of cracks along the rows of lenticels	Similar
Mucilage content on cut surface	Less	More
Pith proportion of cut surface (Pith α 1/age)	More than half in young plants	Comparatively larger pith area
Touch	Rough	Similar
Colour (of outer exfoliating bark)	Light-grey to Creamish-white	Dark green to Creamish-white
Odour	Not specific but characteristic bitter smell after removal of exfoliating outer bark	Comparatively strong
Taste	Bitter (+++)	Bitter (++)

Matured stems were separated from other parts of the plant like roots, leaves, flowers, fruits and other physical impurities and washed thoroughly with potable water for three times. Some stem samples of both male and female plants were allowed to dry in hot air oven at 45-500C and stored for further analysis.

Analytical study

Both fresh and dry form of *Guduchi* stem of both genders was analyzed by employing various analytical parameters. Morphological/organoleptic characteristics (general appearance, shape, size, surface, color, odor, taste, touch etc) and physicochemical analysis like loss on drying at 110⁰C,¹⁹ ash value,²⁰ acid insoluble ash,²¹ sulphated ash, pH value,²² and various extractive values (water soluble,²³ methanol soluble,²⁴ Chloroform soluble, Benzene soluble, Diethyl ether soluble²⁵) were carried out. Samples were further subjected to qualitative tests for various functional groups,^{26,27} quantitative estimations of total alkaloids,²⁸ total starch contents²⁹ and mucilage contents.

Results

Organoleptic evaluation revealed that the taste of both plants was very bitter, where slight more bitterness was perceived in male variety. Observations of physicochemical data of fresh and dry samples of both varieties are detailed in tables 2 and 3. pH of both plants did not revealed much difference and showed to be weak acidic in nature (table 3). Loss on drying values was found 5.68 %w/w and 7.52 %w/w in male and female samples respectively. Ash values for male and female samples were 6.72 and 6.90 %w/w respectively. Water soluble extractive was 12.63 %w/w for male and 11.25 %w/w for female, and alcohol soluble extractive was 8.37 %w/w for male and 6.18 %w/w for female plant. Total alkaloid contents were 0.18% in male and 0.10% in female variety. Starch content was 0.216 % in male and 0.381 in female plant. Mucilage content was 10.28 cS-ml/g in male and 12.86 % in female plant. Qualitative test for various functional groups showed no difference between both samples.

Table 2: Comparative physicochemical parameters of fresh male and female stem

S. No.	Parameters	Male stem	Female stem
1	Loss on drying at 110 ⁰ C (%w/w)	71.2	75.8
2	Ash value (%w/w)	1.69	1.74
3	Acid insoluble ash (%w/w)	1.78	1.91
4	Sulphated ash (%w/w)	3.87	4.05
5	Water soluble extractive (%w/w)	7.31	7.06
6	Alcohol soluble extractive (%w/w)	14.30	14.05

Table 3: Comparative physicochemical parameters of powders of male and female stem

S. No.	Parameters	Male stem	Female stem
1	pH (5% aqueous sol)	5.56	5.58
2	Loss on drying at 110°C (%w/w)	5.68	7.52
3	Ash value (%w/w)	6.72	6.90
4	Acid insoluble ash (%w/w)	0.65	0.91
5	Water insoluble ash (%w/w)	3.22	3.37
6	Sulphated ash (%w/w)	5.46	5.30
7	Water soluble extractive (%w/w)	12.63	11.25
8	Alcohol soluble extractive (%w/w)	8.37	6.18
9	Chloroform soluble extractive (%w/w)	0.51	0.53
10	Benzene soluble extractive (%w/w)	0.06	0.06
11	Diethyl ether soluble extractive (%w/w)	0.04	0.05

Table 4: Quantitative estimation of total alkaloid, starch and mucilage contents

Parameters	Male stem	Female stem
Total Alkaloid content (%)	0.10	0.18
Starch content (mg / ml) (%)	0.216	0.381
Mucilage content (cS-ml/g)	10.28	12.86

Table 5: Results of qualitative test for various functional groups

Sr.no.	Functional gp	Test/ Reagent	Observation	Male stem	Female stem
1.	Cardiac glycosides	Legal's test	No color change	-ve	-ve
2.	Alkaloids	Dragendorff's reagent	Orange Brown ppt	+ve	+ve
		Wagner's reagent	Reddish brown ppt		
3.	Tannins and Phenols	5% fecl ₃ sol.	Deep blue black colour	+ve	+ve
4.	Proteins	Biuret reagent	No color change	-ve	-ve
5.	Carbohydrates	Molish's test	Violet ring is formed at the junction	+ve	+ve
6.	Steroids	Liebermann-buchard	First red, then blue and finally green color appears	-ve	-ve
7.	Flavanoids	Shinoda test	Yellow ppt	-ve	-ve
8.	Saponins	Shaking in test-tube	Frothing with honeycomb appearance	+ve	+ve
9.	Amino acids	Ninhydrin test	Purple or bluish colour observed	-ve	-ve
10.	Starch	Iodine test	Bluish colour appeared	+ve	+ve

+ve =present, -ve =absent

Discussion

In earlier studies, many scholars attempted to establish the pharmacognostical and the physicochemical profile of *Guduchi*,^{30,31} however, published data on gender based differences of the physicochemical profile of *Guduchi* is not available so far, considering which an effort has been made by this study to throw some more light toward development and comparative evaluation of physicochemical profile of the same.

For the present study, fresh stems of both male and female plants of *Guduchi* were collected as per classical guidelines.³² *Guduchi*

creeping over *Nimba* is said to be the best as the synergy between these plants enhance its efficacy, accordingly *Nimba Guduchi* was preferred.³³ Freshly collected raw drug was authenticated and analyzed before processing because good quality products mainly depend on genuine raw materials. Before analysis, separation of physical impurities and outer skin was done for quality maintenance. Ayurvedic classics advocate to use '*Angustha pramana*' (thumb size) of *Guduchi* stem,³⁴ accordingly thumb sized or medium size stem diameter (1.6-2.1 cm) was selected for study.³⁵

The organoleptic characters which correspond to the *panchagyanedriya pariksha* (perception by the five sense organs) of

Ayurveda, and few morphological factors differentiating both male and female varieties were recorded (table 1). Male variety was found more bitter and the bitterness of the stem of *Tinospora cordifolia* is largely due to the clerodane glycosides.³⁶⁻³⁸ Other key bitter principles of *Tinospora* are berberine, giloin, giloinin, columbin, chasmanthin, palmarin, tinosporon, tinosporic acid and tinosporol³⁹⁻⁴²

The pH conventionally represents the acidity and alkalinity. pH of both plants was weak acidic and showed insignificant difference from each other. Loss on drying indicates the moisture content. Material absorbs moisture during the storage. In conjunction with a suitable temperature, moisture will lead to the activation of enzymes and given suitable condition, to the proliferation of living organism. Hence, moisture contents may affect the quality of the drug. Although the weight loss in the samples is principally due to water, small amount of other volatile materials will also contribute to the weight loss. High loss on drying value in female variety indicates rich moisture contents and hygroscopic nature of it.

Ash value depends upon the inorganic substances present in the particular drug. The higher the inorganic substances present in drugs, more will be the ash value. Here, the relatively higher ash value was observed in female variety.

Various components have their solubility in particular media. In both samples, soluble principles were more in water and methanol extractives. In the solubility test, increase in water soluble extractive was found, which depicts its more bioavailability in a water medium. Other obtained extracts (chloroform, benzene and diethyl ether) were fewer quantitatively in both varieties, which signify that they might be playing a minor role in expression of biological activities of *Guduchi*.

A study suggested that female plant is best for its therapeutic phyto-constituents as it is having a higher concentration of metabolites than that of the male variety.¹⁰ Findings of the present study support the same wherein the total alkaloid contents were found more in female variety than male (table 4). Relatively higher starch and mucilage contents in female variety suggest that more yield of *Guduchi Satva* (aqueous extractable starchy substance of *Tinospora cordifolia*) can be expected from the female variety, which need to be investigated further.

Qualitative tests were carried out to detect the functional groups (table 5). The study reveals the presence of alkaloids, tannins, phenols, carbohydrates, saponins and starch, whereas an absence of cardiac glycosides, proteins, steroids, flavanoids and amino acids in both varieties. Though qualitative study didn't revealed any difference in both genders, further comparative studies should be undertaken for quantitative estimation of levels of present phytoconstituents in these plants.

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Conclusion

Organoleptic parameters, physicochemical and phyto-chemical analysis are essential parameters for quality assessment; thus were carried out as per the norms of WHO guidelines. Present study differentiated both male and female varieties in morphological and organoleptic characters. The values of water and alcohol soluble extractives was found relatively higher in male variety, while the total alkaloid, total starch and total mucilage contents were found higher in female variety. All functional groups were found to be same in both varieties. The phytochemical screening reported in the present study is only qualitative; more extensive works on quantification of chemical

constituents and secondary metabolites are needed. The data obtained by present study may prove a torch bearer for future studies.

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