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Comparative Study Between *Tinospora cordifolia* Male and Female Species with Physico-Chemical Analysis

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ABSTRACT

Guduchi, commonly known as *Tinospora cordifolia* or *Giloy*, is a highly esteemed medicinal plant in traditional Indian medicine, known for its many therapeutic properties. Although it is frequently exploited, detailed comparative studies of the male and female plants of this species are lacking. This study aims to reveal the differences between male and female *T. cordifolia* plants by a comprehensive physiochemical analysis. Also, a comparative study between the *Kwatha* prepared by both samples were analyzed. Physicochemical analysis revealed that while both genders contain similar types of bioactive compounds, there are notable quantitative differences between them. Both male and female *T. cordifolia* plants exhibit nearly identical results, showing minimal discernible differences like pH values, alcohol soluble extractive values and ash values in which female variety showing slightly better values than male plant of *Guduchi*. These findings emphasize the need to consider plant gender when using *T. cordifolia* medicinally, suggesting that targeting the use of male or female plants could improve therapeutic effects for specific health conditions.

Keyword: Guduchi, Giloy, Physico-chemical

Introduction:

Tinospora cordifolia (T.cordifolia), known as *Guduchi*, is a medicinal plant that has been used in traditional Ayurvedic medicine for centuries. *T. cordifolia* is known for its immunomodulatory, antioxidant, and anti-inflammatory properties. It is also believed to be effective in treating various conditions such as fever, diabetes, and skin diseases. In this comparative study, we will analyze the physico-chemical differences between *T. cordifolia* male and female plants. This study aims to provide a better understanding of any variations in the chemical composition of the two genders of

the *T. cordifolia* plant and their potential impact on the medicinal properties (Radwan et al., 2021). A study is conducted to compare the chemical profiles of *T. cordifolia* male and female plants. This study will contribute to the existing knowledge of *T. cordifolia* and may have implications for its traditional and modern medicinal uses (Upadhyay et al., 2010).

The physico-chemical analysis of *T. cordifolia* male and female plants involves examining various parameters such as moisture content, pH, total ash, acid insoluble ash, water-soluble extractive, and alcohol soluble extractive. By comparing these parameters between *T. cordifolia* male and female plants, significant differences in their chemical composition can be determined in present (Sampaio et al., 2016). Additionally, it is important to conduct microbial tests to ensure the safety of the *T. cordifolia* plants, especially if they are intended for medicinal use. Pathogen tests is also conducted to detect any harmful microorganisms that may be present (Choudhry et al., 2014). Moreover, heavy metal tests is also carried out to assess the levels of heavy metals in the plants, as their presence can be detrimental to human health if consumed. These tests will provide valuable information regarding the safety and quality of the *T. cordifolia* male and female plants, contributing to a comprehensive understanding of their suitability for medicinal purposes (Shen et al., 2017).

Moreover, heavy metal tests will be conducted to determine the levels of heavy metals such as lead, cadmium, and mercury in the *T. cordifolia* male and female plants. Elevated levels of heavy metals can pose serious health risks to consumers, and thus, evaluating the heavy metal content is essential in determining the safety of the plants for medicinal applications.

In addition to the before mentioned analyses, it is crucial to assess the microbial load in both *T. cordifolia* male and female plants. Microbial tests will examine the presence of any bacteria, yeast, mold, or other microorganisms that could compromise the safety and quality of the plants(Dhami & Mishra, 2015). Pathogen tests will also be performed to detect any harmful microorganisms that may be present, ensuring that the *T. cordifolia* plants meet safety standards for medicinal use.

In addition to the physico-chemical analysis of *T. cordifolia* male and female plants, another important aspect to consider is the preparation of *T. cordifolia Kwath* using the stems of both genders (Moniruzzaman et al., 2020). The preparation and comparative analysis of *T. cordifolia Kwath* can provide valuable insights into any variations in the medicinal properties of the male and female *T. cordifolia* stems.

The study will involve the evaluation of *T. cordifolia* Kwath prepared by using Male and Female *T. cordifolia* separately, using standardized methods for both male and female *T. cordifolia* stems (Kawlni et al., 2018). After the preparation, a comparative analysis was conducted to evaluate the phytochemical composition, especially focusing on Ph, Viscosity, Total Solid content, Refractive index and specific gravity. *T. cordifolia Kwath* will be assessed to determine if there are any gender-specific differences in their efficacy.

The comparative study of *T. cordifolia Kwath* prepared with male and female *T. cordifolia* stems will provide an in-depth understanding of any potential differences in their medicinal properties. This comparative analysis will offer valuable insights into the gender-specific efficacy and safety of *T. cordifolia* in traditional and modern medicinal practices, contributing to the existing knowledge of this valuable medicinal plant.

Material and Method

Collection of samples:

Sample of Male and Female *T. cordifolia* were collected from herbal garden Dayanand Ayurvedic College Jalandhar and nursery of horticulture department Punjab cantonment road near BSF campus Jalandhar.

Physico-chemical analysis: Physico-chemical analyses were carried out by following parameters like 'loss on drying, acid insoluble ash, water soluble extractive, alcohol soluble extractive, Sulphated ash' was performed.

Loss On Drying:

Accurately weighed 10gm of the sample was taken into a completely dried pre- weighed dish. Record the total mass of the dish with the sample. Dish was placed in oven for drying. Sample was dried at 105°C for 30 mins. After 30 mins, weight was checked and again the dish was kept in oven and the weight was checked after every 15 mins till the constant weight is observed. After drying, the final weight was taken and loss on drying was determined, using the formula:

$$\boldsymbol{LOD}(\%) = \frac{(\boldsymbol{W2} - \boldsymbol{W3})}{(\boldsymbol{W2} - \boldsymbol{W1})} \times \mathbf{100}$$

W1 = Mass of the empty dish.

W2 = Mass of the dish with the sample before drying.

W3 = Mass of the dish with the sample after drying.

Total Ash content:

2 gm of the sample was taken in a Clean and dry pre-weighed crucible. Then the crucible was placed in the pre heated muffle furnace at 550-600°C for 4 hours. The crucible was removed from the furnace and was placed in a desiccator to cool to room temperature. After complete cooling the weight was taken, and as value was calculated by using the formula:

$$\boldsymbol{Total\ Ash\ Content}\ (\%) = \frac{\boldsymbol{W2} - \boldsymbol{W1}}{\boldsymbol{W3} - \boldsymbol{W1}} \times \mathbf{100}$$

W1 = Mass of the empty crucible.

W2 = Mass of the crucible with the sample before ash.

W3 = Mass of the crucible with the ash after ash.

Alcohol Soluble Extractive Value:

5 grams of the powdered sample was weighed accurately using the analytical balance and taken into a conical flask. 100 mL of alcohol (ethanol) was added to the flask containing the sample. Continuous shaking was done for 6 hours and the mixer was kept overnight and next day filtration was done. The filtrate was collected in a evaporating dish. The filtrate was kept on water bath and evaporated until a concentrated extract was obtained.

Concentrated extract was taken into a pre-weighed evaporating dish. Then the extract was kept in hot air oven for drying until constant weight is obtained.

After cooling, weigh of evaporating dish with the dried extract was recorded. The alcohol-soluble extractive value was calculated, by using the formula:

$$\boldsymbol{Alcohol - Soluble\ Extractive\ Value}\ (\%) = \frac{\boldsymbol{initial\ wt. - mass\ of\ alcohol\ extraction\ residue}}{\boldsymbol{initial\ mass}} \times \mathbf{100}$$

Water Soluble extractive value

5 grams of the powdered sample was weighed accurately using the analytical balance and taken into a conical flask. 100 mL of water was added to the flask containing the sample. Continuous shaking was done for 6 hours and the mixer was kept overnight and next day filtration was done. The filtrate

was collected in an evaporating dish. The filtrate was kept on water bath and evaporated until a concentrated extract was obtained.

Concentrated extract was taken into a pre-weighed evaporating dish. Then the extract was kept in hot air oven for drying until constant weight is obtained.

After cooling, weigh of evaporating dish with the dried extract was recorded. The water -soluble extractive value was calculated, by using the formula:

$$\boldsymbol{Water-Soluble\ Extractive\ Value}\ (\%) = \frac{\boldsymbol{initial\ mass - mass\ of\ water\ extraction\ residue}}{\boldsymbol{initial\ mass}} \times \mathbf{100}$$

Sulphated Ash:

Ash prepared was taken and few drops of concentrated sulphuric acid was added then heated gently over hot plate to remove any remaining carbon content init. Again, the crucible was kept in muffle furnace for 6 hours to remove all the organic content. After cooling the weight of sulphated ash was taken and percentage of the Sulphate ash value was noted:

$$\boldsymbol{Sulphated\ Ash\ Value}\ (\%) = \frac{\boldsymbol{W2 - W1}}{\boldsymbol{W3 - W1}} \times \mathbf{100}$$

W1 = Wt. of the empty crucible.

W2 = Wt. of the crucible with the sample before ashing.

W3 = Wt. of the crucible with the sulphated ash after ashing

Microbial and Pathogen test:

Sample of *T. cordifolia* Male and *T. cordifolia* Female was prepared (stored at room temperature) and then studied to detect microbial contamination at regular intervals. Microbiological study has been carried out in Microbiology Laboratory, Herbal health research consortium Pvt. Ltd., Punjab. Mainly two studies have been carried out Total Microbial plate count and Total yeast and Moulds to check the presence of any microbes in Guduchi. In pathogen test *E. coli, S.aureus, P.aeruginosa, S.typhii* were examined. The fungal species were collected on Sabouraud Dextrose Agar (SDA) slants and stored at 4°C prior to use, while the bacteria were collected on solidified MacConkey agar. Aseptic techniques were followed throughout the study.

Heavy metal test:

Metals analyzed in both *T. cordifolia* samples included arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). The samples were prepared using the wet digestion technique. Five grams of air-dried powder were placed in a 250 mL conical flask and heated on a hot plate until sufficient water evaporated, leading to partial carbonization. The flask was then removed and allowed to cool. A 1:1 mixture of HNO3 and H2O was prepared, and approximately 5 mL of this mixture was added to the ashed sample and warmed for about 5 minutes. The mixture was then filtered using Whatman filter paper (Millipore) for analysis by Atomic Absorption Spectroscopy (AAS). The atomic absorption spectrophotometer was calibrated using stock standard solutions, which were subsequently used for metal analysis.

Aflatoxins Analysis:

Twenty grams of each *T. cordifolia* sample were mixed with 70% methanol for 3 minutes and then left undisturbed at room temperature for 5-10 minutes. The cleared top layer was filtered and diluted 1:3 with a washing buffer. Aflatoxin was tested using the Screen EZ-Aflatoxin ELISA Test Kit, which is based on the Direct Competitive enzyme-linked immunosorbent assay.

Pesticide residue:

Pesticide residues were assessed by extracting 2 g of each sample using a Soxhlet apparatus with 150 mL of hexane. The hexane extract contained traces of oil and water. After removing the oil, the extract was concentrated using a rotary evaporator under reduced pressure, then transferred to a clean-up column (Naithani and Kakkar 2006). The elute was carefully collected and concentrated to 5 ml with hexane.

Aliquots of the concentrated extract were loaded into a pre-calibrated gas chromatograph (GC) equipped with a 63Ni electron-capture detector. The temperatures were set to 195°C for the column, 200°C for the injector, and 220°C for the detector. Purified nitrogen gas was used as the carrier gas at a flow rate of 60 mL/min. The detection limits for the analyzed organochlorine pesticides ranged from 0.1 to 0.5 ppb. Procedural blanks were periodically used to ensure accuracy.

Result and Discussion:

Physio-chemical analysis for Male and Female *T. cordifolia*

Table 1: Physio-chemical analysis of Male *T. cordifolia* and Female *T. cordifolia*

S.No.	Tests	Male *T. cordifolia*	Female *T. cordifolia*
1	Loss on Drying (%w/w)	4.09 ± 0.05	5.24 ± 0.04
2	Total Ash Content (%w/w)	6.81 ± 0.03	9.81 ± 0.03
5	Sulphated Ash(%w/w)	9.93 ± 0.04	11.64 ± 0.03
4	Alcohol soluble extractive value (%)	8.45 ± 0.27	7.66 ± 0.3
5	Water soluble extractive value (%)	18.33 ± 0.03	18.32 ± 0.02
6	pH	6.42 ± 0.01	6.44 ± 0.21

Microbial test for Male and Female *T. cordifolia*

Table 2: Microbial test for Male *T. cordifolia*

S.No.	Tests	Result	Specifications
1	Total Microbial Plate Count	500 cfu/g	$1x10^5$ cfu/g
2	Total Yeast and Moulds	90 cfu/g	$1x10^3$cfu/g

Table 3: Microbial test for Female *T. cordifolia*

S.No.	Tests	Result	Specifications
1	Total Microbial Plate Count	400 cfu/g	$1x10^5$ cfu/g
2	Total Yeast and Moulds	60 cfu/g	$1x10^3$cfu/g

Pathogen test and Heavy metal Analysis of Male and Female *T. cordifolia*

Table 4: Pathogen test in Male and *T. cordifolia*

S.No.	Tests	Result	Specific Requirement
1	*Escherichia coli*	Ab	Ab
2	*Staphylococcus aureus*	Ab	Ab
3	*Pseudomonas aeruginosa*	Ab	Ab
4	*Salmonella typhii*	Ab	Ab

*Ab= Absent

Table 5: Pathogen test in Female *T. cordifolia*

S.No.	Tests	Result	Specific Requirement

1	*Escherichia coli*	Ab	Ab
2	*Staphylococcus aureus*	Ab	Ab
3	*Pseudomonas aeruginosa*	Ab	Ab
4	*Salmonella typhii*	Ab	Ab

*Ab= Absent

Table 6: Heavy metal analysis in Male *T. cordifolia*

S.No.	Tests	Result	Specific Requirement
1	Arsenic	N	3 ppm
2	Cadmium	N	0.3 ppm
3	Mercury	N	1 ppm
4	Lead	N	10ppm

*N= Not detected

Table7: Heavy metal analysis in Female *T. cordifolia*

S.No.	Tests	Result	Specific Requirement
1	Arsenic	N	3 ppm
2	Cadmium	N	0.3 ppm
3	Mercury	N	1 ppm
4	Lead	N	10ppm

*N= Not detected

Aflatoxin and Pesticide Residue in Male and Female *T. cordifolia*

Table 8: Aflatoxin and Pesticide Residue in Male *T. cordifolia*

Test	Result	Specific Requirements
Aflatoxins	N	N
Pesticide residue	N	N

*N= Not detected

Table 9: Aflatoxin and Pesticide Residue in Female *T. cordifolia*

Test	Result	Specific Requirements
Aflatoxins	N	N
Pesticide residue	N	N

*N= Not detected

Comparison between Male and Female *T. cordifolia* plant: Table below shows the comparative study between Male and Female *T. cordifolia* Plant.

Table 10: Comparison between Male and Female *T. cordifolia* plant

S.No.	Tests	Male *T. cordifolia*	Female *T. cordifolia*	Specific Requirements
1.	Loss on drying (%w/w)	4.09 ± 0.05	5.24 ± 0.04	75 %
2.	Total Ash value (%w/w)	6.81 ± 0.03	9.81 ± 0.03	NMT 16 %
3.	Alcohol soluble extractive value (%)	9.93 ± 0.04	11.64 ± 0.03	NLT 3 %
4.	Water soluble extractive value (%)	8.45 ± 0.27	7.66 ± 0.3	NLT 11 %

5.	**Sulphated Ash (%w/w)**	18.33 ± 0.03	18.32 ± 0.02	–
6.	**pH**	6.42 ± 0.01	6.44 ± 0.21	–
7.	**Microbial test**			
	Total Microbial Plate Count	500 cfu/g	400 cfu/g	$1x10^5$ cfu/g
	Total Yeast and Moulds	90 cfu/g	60 cfu/g	1x103 cfu/g
8.	**Pathogen test**			
	Escherichia coli	Ab	Ab	Ab
	Staphylococcus aureus	Ab	Ab	Ab
	Pseudomonas aeruginosa	Ab	Ab	Ab
	Salmonella typhii	Ab	Ab	Ab
9.	**Heavy Metal test**			
	Arsenic	N	N	<10.0
	Cadmium	N	N	<3.0
	Mercury	N	N	<0.3
	Lead	N	N	1.0
10.	**Aflatoxins**	N	N	N
11.	**Pesticide residue**	N	N	N

*N= Not detected, Ab= Absent

Evaluation Parameters of *T. cordifolia Kwatha*

Table 12: Evaluation Parameters of *T. cordifolia Kwatha*

S.No.	Parameters	Result (Average)	
		Male	**Female**
1.	**Colour**	Brownish green	Brownish green
2.	**Odour**	C	C
3.	**Taste**	B	B
4.	**pH**	6.42	6.44
5.	**Total Solid Content (%)**	5.96	6.13
6.	**Viscosity**	0.97	0.96
7.	**Specific Gravity**	1.022	1.020
8.	**Refractive index**	0.37	0.36

*'C= Characteristic, B= Bitter'

Evaluation Parameters of *T. cordifolia Kwatha*

Table 13: Evaluation Parameters of *T. cordifolia Kwatha*

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1.	**Colour**	Brownish green	Brownish green
2.	**Odour**	C	C
3.	**Taste**	B	B
4.	**pH**	6.42	6.44
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8.	**Refractive index**	0.37	0.36

*C= Characteristic, B= Bitter

Both male and female *T. cordifolia* plants exhibit nearly identical results, showing minimal discernible differences. The refractive index, specific gravity, and viscosity values of male and female *T. cordifolia* are remarkably similar, indicating a high degree of consistency between the two genders. These findings suggest that there are no significant distinctions between male and female *T. cordifolia* in terms of these physical properties.

Conclusion

T. cordifolia does have separate male and female plants, the medicinal properties and uses of both are generally similar. While there might be some subtle botanical differences between male and female plants, they are not typically relevant in the context of medicinal use. The pH of both plants was weakly acidic, measured at 6.42 and 6.44, showing no significant variation between them. The high drying loss in the female varieties indicates a higher moisture content and hygroscopicity. The ash value of a substance reflects its inorganic component content; thus, a higher ash value indicates a greater presence of inorganic chemicals. The female variety has a significantly higher ash value at 9.81% (w/w) compared to the male variety's 6.81% (w/w). Female *T. cordifolia* have more alcoholic extractive value indicates its solubility in ethanol and methanol extract as compared to Male Plant. The alcoholic soluble extractive value of female variety is 11.64% and male variety is 9.93%. The organoleptic characters are the same in both of the varieties of *T. cordifolia*. No heavy metal, pathogen and microbes are detected in both of the varieties of *T. cordifolia* and all the parameters are detected in under standard values. The stems, leaves, and roots of both male and female plants contain active compounds such as alkaloids, glycosides, and polysaccharides, which contribute to their therapeutic effects. Also, *Kwatha* prepared by Male *T. cordifolia* and female *T. cordifolia* exhibits similar properties.

In examining male and female *T. cordifolia* plants qualitatively, identified slight differences between the two genders. Moreover, male and female plants demonstrate nearly identical chemical composition, nutrient content, and concentrations of active components. This underscores the similarity in chemical profiles and effectiveness between male and female *T. cordifolia* specimens.

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