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A COMPARATIVE PHYTO-PHARMACOGNOSTICAL STUDY OF GUDUCHI [TINOSPORA CORDIFOLIA (THUNB.) MIERS]; GROWING ON NIMBA (AZADIRACHTA INDICA L.) AND SAPTPARNA (ALSTONEA SCHOLARIS L.); ALONG WITH THEIR IN VITRO ANTI-MICROBIAL ACTIVITY OF SATVA (SEDIMENTED STARCHY AQUEOUS EXTRACT) ON S. AUREUS

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ABSTRACT

T. cordifolia is used for the treatment of Fever, Dyspepsia, Dysentery, Gonorrhoea, Urinary diseases, Gout, Viral hepatitis, Anaemia, etc. For centuries, it has been considered ethnobotanical; the medicine growing surrounding *Nimba (Azadirachta indica L.)* is used to enhance its potential therapeutic benefits. The host plant is a crucial component of this study, as climbers exhibit environmental effects. The study was carried out with the objective to study the variation in phytoconstituents and antimicrobial activity among *T. cordifolia* aqueous extract obtained from *growing* on *Nimba (Azadirachta indica* L.*)* and *Saptaparna (Alstonia scholaris* L.*)***.** In order to authenticate and develop the standards for this popular single drug; WHO guidelines were followed, provided by CCRAS. For the standardisation of this drug pharmacognostical and phytochemical parameters were carried out. The Standardization of herbal drugs and their bio-constituents are of paramount importance in justifying their acceptability by modern scientific methods. In this study, *Giloya* which was grown on *Saptaparna* shows significant results. While *Nimba Giloya* which is widely prevalent doesn't perform well on scientific parameters. On the other hand, *Giloya* which was grown without any host plant was also equally highly effective against S.aureus. This field requires more research based on scientific parameters.

Keywords: Ayurveda, WHO, Antimicrobial, Environment.

INTRODUCTION

Ayurveda - a biological science. The term *Ayurveda* consists of two words viz. "*Ayuh*" and "*Veda*". *Ayuh* means life and *Veda* - science or knowledge of. It may be interpreted as a science in which the knowledge of life exists, or which helps a man to enjoy a longer duration of life. In *Bhava Prakasha*, *Giloya* is considered to have medicinal properties like in Rasa it is *Katu (Pungent)*, *Tikta* (bitter), and *Kashaya* (astringent). The study will carry out with the objective to study the variation in phytoconstituents among *T. cordifolia Growing on Nimba (Azadirachta indica* L.*) and Saptaparna (Alstonia scholaris* L.**);** In order to authenticate and develop the standards for this popular single drug WHO guidelines will follow; provided by CCRAS. For the standardisation of this drug, pharmacognostical and phytochemical parameters will be carried out such as microscopic, macroscopic, extract microscopy, protein, sugar, tannins, resins, alkaloids, HPTLC, etc. Some of the parameters like foreign matter, moisture content, ash values, pH values, and extractability in hexane, alcohol, and water will also be carried out. The Standardization of herbal drugs and their bio-constituents are of paramount importance in justifying their acceptability by modern scientific methods.

Botanical description

Chief Plant:

Tinospora cordifolia is a large glabrous climbing shrub belonging to the common moonseed family Menispermaceae, often attending a great height and sending down long thread-like aerial roots. The fresh or tender stems are greenish with smooth surfaces and swelling at nodes. Branches bear smooth heart-shaped leaves. The flowers are small, unisexual greenish-yellow. Fruits are pea-shaped, shiny, drooping, and become red when fully grown. Seeds are solitary in each fruitlet, kidney-shaped, deeply grooved ventrally, and curved. Flowers grow during May- June, and fruits appear in September- October. *Giloya* prefers to grow India's tropical as well as subtropical regions[1,2]. T.cordifolia is a rich source of many phytoconstituents like Alkaloids (Berberine, tinosporin etc.), Glycosides (Tinocordioside, Cordifolioside A etc.), Steroids(Giloinsterol), Sesquiterpenoids(Tinocordifolin), Diterpenoid Lactones(Tinosporon), Aliphatic compounds(Octacosanol), Other compounds(Giloinin, Giloin etc.)[3] which shows many Pharmacological activities i.e. Nephroprotective activity[4], Cardioprotective activity[5], Antiulcer activity[6], Anti-inflammatory activity[7], Immunomodulatory activity[8], Antioxidant activity[9], Hypolipidemic activity[10], Antipyretic activity[11], Anti-malaria (HMS) Activity[12], Anti-hyperglycaemic activity[13]etc.

Host plants:

Azadirachta indica is a medium to large, deep-rooted, evergreen tree. Leaves are dark green in colour. Leaf margins are toothed. Flowers are white and fragrant that arise from the junction of the stem and petiole. The fruit is a smooth olive-like drupe. The fruit skin is thin and turns yellow when ripe. Flowers grow during January-May, and fruits appear in May-August. *Nimba* is distributed throughout india[14]. Azadirachta indica is a rich source of many phytoconstituents like Azadirachtin A, azadirachtin B, azadirachtin D, Azadirachtin H, and 11β-H epimer, Azadirachtin I, Quercetin, and β-sitosterol, glutamic acid, tyrosine, Nimbain, Nimbainin, Nimbaidin, Nimbaosterol, etc.[15] which shows many Pharmacological activities i.e Antifungal activity[16], Analgesic activity[17], Antiviral activity[18], Antibacterial activity[19], Antihyperglycemic activity [20], Hepatoprotective activity [21], etc.

Alstonia scholaris A large tree with 6-8 m. height, whorled branches, and bitter milky juice. Grayish bark, rough, lenticellate abounding. Leaves are in whorls, glabrous. Flowers are small, greenish, or greenish-yellow fragrant, borne in an umbel, corolla tube short. Fruits are slender follicles, in pendulous Clusters. Seeds are rough and with tufts of very fine

silky brownish hairs at each end. Flowers grow during November - January and fruits appear in May – July. The tree is native to the Asian subcontinent. The tree is found widely in the Sub-Himalayan tract, West Bengal, Bihar, Peninsular India, and Andamans. It is also found in moderately dry places of Karnataka like Shimoga district[22]. A. scholaris is a rich source of many phytoconstituents like a-amyrine acetate, lupeol acetate, echitamine, picrinine, venoterpine glucoside, akuammidine, strictamine, tetrahydroalstonine, A3-carene, citral, citranellol, geraniol, linalool, a-pinene, terpinolene, angustilobine B, losbanine, alschomine, isoalschomine, Alston amine, picrinine, picralinal, akuammigine, tubotaiwine, akuammicine etc[23-24]. These Phytochmical shows pharmacological activities like Antibacterial[25], Anti-diabetic-Antihyperlipidemic[26],Analgesic-anti-inflammatory activity[27] Ameliorating[28], Hepatoprotective activity[29] ,Anti-arthritic-antioxidant activity [30] etc.

Ayurvedic properties of the selected medicinal plants :

BOTANICAL NAME	Family	Rasa	Guna	Veerya	Vipaka	Doshakarma
***Tinospora cordifolia* (Thunb.) Miers**[31]	Menispermaceae	Madhura, Tikta, kashaya	Laghu	Ushna	Katu	Tridoshanashaka
***Azadirachta indica* L.**[32]	Meliaceae	Tikta	Snigdha Laghu Ushna	Sheeta	Katu	Pittashamaka
***Alstonea scholaris* L.**[33]	Apocynaceae	Tikta, Kashaya	Laghu Snigdha	Ushna	Katu	Tridoshashamaka

AIMS AND OBJECTIVES

- To do a comparative phyto-pharmacognostical study of *Giloya (Tinospora cordifolia)*, growing on *Nimba (Azadirachta indica L.)* and *Saptaparna (Alstonea scholaris)* from a recognized pharmacognosy/Pharmacology laboratory.
- To compare a Pharmaceutical preparation and analysis of *Satva* (sedimented starchy aqueous extract) in different host plants.
- To Establish the role of host plants in *Tinospora cordifolia* as phytochemical parameters.

MATERIAL & METHOD

Fresh stems of *Tinospora cordifolia* were grown and collected from the Botanical garden of Dayanand Ayurvedic College and identified by the experts of the PG Department of Dravyaguna, Dayanand ayurvedic college, Jalandhar (Punjab).

- Pharmacognostical and phyto-chemical methods Organoleptic characters, Pharmacognostical Methods, Microscopic study, Extract microscopy, Physio – Chemical Methods, Qualitative and quantitative analysis, Determination of ph Values,and HPTLC were used to standardise the *Satva* obtained from three samples of *T.cordifolia* from a recognized pharmacognosy lab and college.
- An antibacterial study from a recognized & affiliated institute of all three *Satva* (Sedimented starchy aqueous extract).

DRY PROCESS / SATVA FORMATION PROCESS[34]

The physical impurities and outer loose skin were removed, and the stem was washed thoroughly with water. Necessary equipment such as stainless steel (SS) vessel, SS ladle, cotton cloth, measuring jar, SS spoo,n etc., were arranged prior tothe beginning of the pharmaceutical procedure. Stem was well rinsed with water to remove dust, foreign particles adhered to the drug. The cover of the *Giloya* stem was removed so as to avoid interference during preparation of Sattva. The

removed cover of the stem was weighed. Stem of *Giloya* is then cut into small pieces and pounded in *Khalwa* till fibres of stem get separated and the material becomes sticky. These fibres are placed in a vessel and 21 times water was added into it and rubbed well with hands thoroughly and kept overnight for soaking. Next day the mixture was again well rubbed until the stickiness disappeared into the same water. Then fibres are removed, and the remaining material was strained through clean cloth. Sedimentation: The strained material was collected in a flat bottom stainless steel container and allowed for the sedimentation.

Decantation and Washing: The fine particles in the mixture were settled in the bottom of the container, the upper liquid portion was decanted carefully. After decantation the sediments obtained were again mixed with little quantity of water and allowed again for sedimentation and liquid was removed by decantation process. By repeated washing and decantation 7 times. Then clear white starch was obtained.

Drying: Obtained starch was taken in a plate and dried in sunlight to get *Satva*

RESULTS

ORGANOLEPTIC STUDY

Observed organoleptic characters of all the three samples of *Giloya* Satva

Characteristic	***Giloya***	***Nimba Giloya***	***Saptaparna Giloya***
Colour	Off white	Grey off white	Grey off white
Smell	Not specific	Not specific	Not specific
Taste	Slightly bitter	Slightly bitter	Slightly bitter
Touch	Smooth	Smooth	Smooth
Appearance	Powder	Powder	Powder

PHYSIOCHEMICAL SCREENING

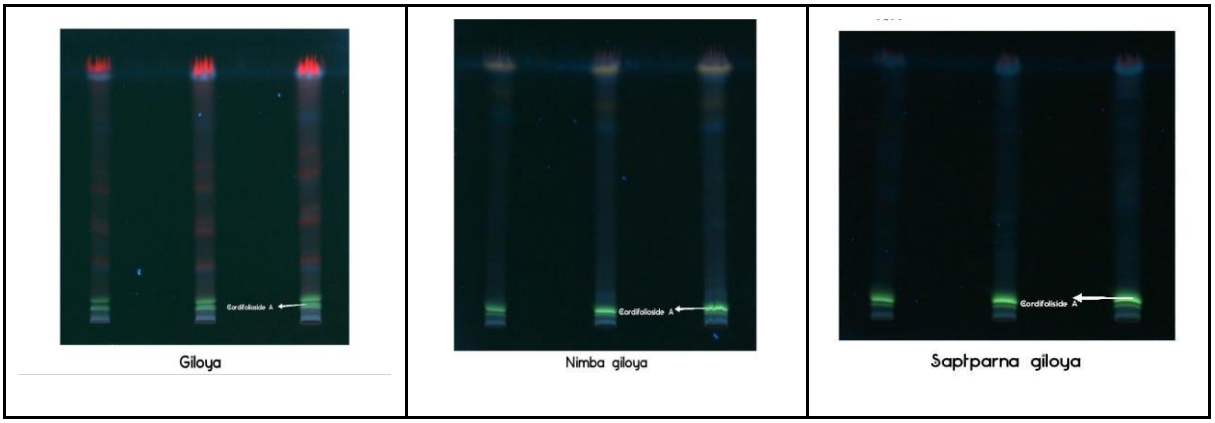
S.no.	**Parameters**	***Giloya***	***Nimba Giloya***	***Saptaparna Giloya***	**API Parameters**
1	pH	5.89	4.76	6.54	–
2	Moisture content	5.98 %w/w	7.98 %w/w	7.06 %w/w	–
3	Total ash	26.66 %w/w	4.58 %w/w	17.08 %w/w	Not more than 16
4	Acid insoluble extract	18.33 %w/w	0.20 %w/w	11.69 %w/w	Not less than 3
5	Water soluble extract	20.14 %w/w	2.02 %w/w	4.98 %w/w	Not more than 11
6	Alcohol soluble ash	2.58 %w/w	1.99 %w/w	0.79 %w/w	Not more than 3

PHYTOCHEMICAL SCREENING

S.No.	**Parameters**	***Giloya***	***Nimba Giloya***	***Saptaparna Giloya***	**Test used**
1	Carbohydrate	Present	Present	Present	Molisch's test
2	Tannin	Absent	Absent	Absent	Lead acetate
3	Alkaloid	Present	Present	Present	Dragendroff's reagent test
4	Starch	Present	Present	Present	Iodine test
5	Flavonoids	Present	Absent	Absent	Shinoda test
6	Steroids	Absent	Absent	Absent	Salkowski's test

7	Saponins	Present	Present	Present	Foam test

HPTLC



Result of HPTLC of Saptaparna Giloya Extract

Saptaparna Giloya			
Wavelength	**Track**	**Rf range**	**No. of peaks**
Wavelength 254	Track I	0.04-0.92	1-10
	Track II	0.03-0.82	1-11
	Track III	0.03-0.91	1-14

ANTIBACTERIAL STUDY

Procedure used- Kirby-Bauer Agar Well diffusion method.

Zone of inhibition(mm)					
Antimicrobial Activity (Values are mean of triplicate) S.aureus	As per standard antimicrobial sensitivity protocol of pharmacopoeia	Standard	Test Sample (in DMSO)		
		Positive control	50%/ 5mg/ml	100%/ 10mg/ml	DMSO Negative control
	Giloya	24	14	18	8
	Nimb Giloya	24	10	16	8
	Saptaparna Giloya	24	12	18	8

DISCUSSION

The pH of *Satva* of *Giloya* is 5.89, *Nimba Giloya and Saptaparna Giloya is 4.76 and 6.54.* It practically means the quantitative indication of the acidity or base of a solution. Results showing that the Extract of *Nimba Giloya is* more acidic in nature than others.

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form. crude drugs normally leave ash usually consisting of carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium. Total ash is a measure of the mineral oxide content of activated carbon on a Weight basis. The higher the Ash Value indicates the Higher the traces of organic matter. Here the study found that the Total Ash range should not be more than 16 according to API. Total Ash was comparatively higher than the range in Extract of *Giloya* (26.66%) and *Saptaparna Giloya(*17.08%);

showing higher traces of organic matter due to its ecological conditions and polluted area.

The moisture content of *Satva* of *Giloya* is 5.98, *Nimba Giloya* and *Saptaparna Giloya* is 7.98 and 7.06 respectively. It may be due to the environmental conditions of the samples.

Alcohol soluble extractive value should be less than 3. All the samples were found within limits. Comparatively, *Saptaparna Giloya* shows a lesser value (0.79). It indicates *Saptaparna Giloya* extract does not have active components which bind with alcohol.

The water soluble extractive range is not more than 11. The highest was noted in *Giloya satva (20.14).* Giloya extract has a higher starch amount which is hygroscopic than containing cells in higher amounts.

Acid insoluble ash was found higher in *Giloya* and *Saptaparna Giloya* because of the higher trace value in total ash.

The Phytochemical study reveals that the *Giloya* plant shows the presence of phytochemical constituents like carbohydrates, Alkaloids, Flavonoids, Starch, and Saponins. The *Nimba Giloya* plant shows the presence of phytochemical constituents like carbohydrates, Alkaloids, Starch, and Saponins. The *Saptaparna giloya* plant shows the presence of phytochemical constituents like carbohydrates, Alkaloids, Starch, and Saponins.

HPTLC of *Giloya* Stem Shows that *T. cordifolia* stem contains not less than 0.02% w/w of Cordifolioside A; rf range between 0.03 to 0.04 and the percentage of tinosporaside ranges from 0.03 to 0.04. All the sample range was found in the limit. However, the *Saptaparna Giloya* finds maximum peaks at the wavelength 254 which shows maximum phyto markers like codiofolioside, tisoporaside, Berberin, and others are unknown phytochemicals. Mostly track 2 and Track 3 findings showed a high number of peaks.

Antibacterial Results Show that 50%/ 5 mg/ml Test Sample (in DMSO) the inhibitory zone is 14mm,10mm, and 12 mm for *Giloya, Nimba Giloya*, and *Saptaparna Giloya* respectively. While 100%/ 10mg/ml Test Sample (in DMSO) the inhibitory zone is 18mm,16 mm, and 18 mm for *Giloya, Nimb Giloya*, and *Saptaparna Giloya* respectively. All samples exhibit antibacterial activity, according to the data. When compared to other samples and used sparingly, *Nimba Giloya* exhibits reduced activity. While *Nimba Giloya* showed less inhibition than the positive control at higher extract concentrations, *Giloya* and *Saptaparna Giloya* both demonstrated extremely significant results for the suppression of bacterial growth. So, the final result is to conclude that the antibacterial potential of *Saptaparna Giloya* is highly significant due to its high amount of active chemical constituents.

CONCLUSION

The antibacterial activity of the sedimented starchy aqueous extract or Satva from the stem of *Tinospora cordifolia* was studied using the disc- diffusion method against S. Aureus which is a gram-positive bacterium and responsible for various life-threatening diseases. Results suggest that the extract possesses a significant result against tested bacteria. However, the present study justifies the claimed uses of *Satva* in the *Ayurveda* medical system. Still, there is a need to study drug trials using different methodologies and it is imperative to explore scientific data and phyto-constituents of drugs. This dissertation cites the empirical study of the medicinal effects of host plants, particularly antibacterial and phyto-physicochemical on *Giloya* stem. In this study, *Giloya* which was grown on *Saptaparna* shows significant results. While *Nimba Giloya* which is widely prevalent doesn't perform well on scientific parameters. On the other hand, *Giloya* which was grown without any host plant was also equally highly effective against S.aureus. This field requires more research based on scientific parameters.

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