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"PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF TINOSPORA CORDIFOLIA LEAVE"

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ABSTRACT-

The aim of this work to was to do phytochemical extraction and also evaluation of their antimicrobial and antioxidant properties for tinospora cordfolia.It is also important to see whether variations in these conditions affect Phytochemical as well. Tinospora cordifolia. is an important medicinal plant and it plays important role in treatment of different diseases. reliminary phytochemical screening of T. cordifolia showed showed the presence of carbohydrates, glycosides, flavonoids, phenols, tannins and amino acids in the crude drug. Antimicrobial resistance is the main concern worldwide to combat infectious. Over the years studies on leaf extracts Tinospora cordifolia have demonstrated the potent role its antibacterial property.

KEYWORDS- ,Phytochemical,,screening,antimicrobial,tinospora cordfolia ,leave.

INTRODUCTION-

Tinosporacordifolia commonly named as "Guduchi" in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude.In racemes or racemose panicles, the male flowers are clustered and female are solitary. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant.

Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, antispasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, antistress, anti-leprotic, anti-malarial, hepatoprotective. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. This review attempts to summarize the current status of botanical screening efforts, as well as in vivo studies of their effectiveness and toxicity. The structure and antimicrobial properties of phytochemicals are also addressed. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self- medicating with these preparations. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans (see below). A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances is commonplace. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying

great popularity in the late 1990s. Earlier in this decade, approximately one third of people surveyed in the United States used at least one "unconventional" therapy during the previous year .

PLANT PROFILE:

T. cordifolia is a perennial, climbing, deciduous, fleshy, and robust shrub with succulent stem and papery bark .The leaf of the plant is heart-shaped, membranous, alternate, long petiolate, cordate, exstipulate, and glabrous with multicoated reticulate venation. The plant stems are fibrous and the crosssection in such stems has radially located V-shaped wood bundles with canals, separated with medullary rays. The bark is gray to white in color. The plant's flower is in an axillary position, 2- to 9-cm-long raceme on leaflet branches, small, unisexual, and yellow in its color. The female is usually solitary and male flowers are clustered. The plant seed is curved. The fruit is succulent and single-seeded and red when ripe. The flower grows during the summer, while fruit develops during the winter (Reddy and Rajasekhar, 2015; Sharma et al., 2019; Shetty and Singh, 2010; Upadhyay et al., 2010; Tiwari et al., 2018).

Parts	Uses
Stem	Use to treat a various neurological disorder like pakinsons, dementia, ALS , cognitive deficit and neuro loss in spine and hypothalamus, Act as nitic oxide scavenger and inhibit NF-kBand.
Root	Anti-cancers, anti-diabetes, Anti-infection and psychiatric condition.
Shoot	Induce cell cycle arrest in G2/M phase and apoptosis through c-Myc suppression. IgA neuropathy, anti-inflammation.
Bark	The bark is used to treat fever, wounds ,cuts and injuries due to its antimicrobial and anti-inflammation properties.

Table : Ethno medicinal uses of *Tinosporacordifolia.*

leaves	Anti-allergy property, neuro-protective, anti-microbial, anti-stress, etc.
Flower	Treats to used various disorder like gouts, skin disease and anaemia.
Seeds	Anti-diabetes, immune-booster, antipyretics, anti-plastic activities, etc.

MATERIAL METHOD :

Collection of the plant material

The medicinal plant T. cordifolia was identified and collected . A young and healthy Guduchi or T. Cordifolia plant was selected for research because of its many medicinal properties that would be of great demand in the future. The plant was washed thoroughly with water to remove soil and dirt. The plant materials were separated and cut into small pieces so that they can be dried easily. The pieces of leaves, stem and root were spread on the filter paper and were shade dried for nearly two weeks. Then the pieces were ground in the grinder in order to obtain fine powder. The plant extracts were obtained with the help of incubation in orbital shaker (for 48 hours at 22 ℃ and 120 rpm) by using different solvents like aqueous, butanol, methanol, ethanol and chloroform. Then the extracts were filtered with Whatman filter paper and subjected to preliminary phytochemical analysis. The procedure for the detection of various phytochemicals in the plant solvent extracts is as follows.

Phytochemical Analysis

The qualitative phytochemical analysis was carried out to detect the presence of different phytochemicals in guduchiand ashwagandha powder. The procedures for the tests are as follows:

Test for Alkaloids -

Wagner's test:

200 µl of crude extract was taken in test tube. The few drops of Wagner's reagent were added to the inner side of test tube. A reddish brown precipitate was formed which confirmed the presence of alkaloids

Mayer's and Wagner's test:

Equal amount of extract and 1% HCl were added and heated gently. Mayer's and Wagner's reagent were added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for glycosides -

Sakowaski's Test:

Crude extract was mixed with 2ml of chloroform. Then 2ml of conc. H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e.,glycone portion of glycoside.

Keller-Kilani Test:

Crude extract was mixed with the 2ml of glacial acetic acidcontaining 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of conc. H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for flavonoids -

Alkaline reagent test:

Add 5 drops of dilute sodium hydroxide (NaOH) to 2 mL of plant extract, then add diluted hydrochloric acid (HCl). If the yellow solution turns colorless, flavonoids are present.

Ammonium hydroxide test:

Mix the plant extract with a 10% ammonium hydroxide solution in a test tube. If the solution fluoresces yellow, flavonoids are present.

Magnesium and hydrochloric acid test:

Mix 2 mL of filtrate from a plant extract with concentrated HCl and magnesium ribbon. If the mixture turns pink or red, flavonoids are present. The magnesium and hydrochloric acid reduce the benzopyrone in the flavonoid structure, which forms flavylium salts that are red or orange in color.

Test for phenols -

Ferric chloride test:

A reaction that produces a color change when added to a compound with a phenol group. The color can be blue, violet, purple, green, or red-brown.Add 5% ferric chloride to an equal volume of Tinosporacordifolia extract in a test tube. A dark green or bluish green color indicates the presence of phenols

Liebermann's test:

A small sample of the test substance is dissolved in warm sulfuric acid with a crystal of sodium nitrite. The solution is then poured into excess aqueous alkali, and a blue-green color indicates the presence of a phenol.

Screening for antimicrobial activity: The hot and cold aqueous and ethanol extracts of the T. cordifolia were used for the antimicrobial screening using the agar well diffusion method. the media was punched with 7mm diameter wells and were filled with various concentrations of the extracts 5mg/ml,10mg/ml,15mgmg/ml,20mg/ml and 25mg/ml. the plates were then incubated at 37c for 24hours.after incubation ,zone of growth inhibition for each extract was measured in millimeters by using a scale.

RESULT AND DISCUSSION:

Observation table for Phytochemical screening

Name of compounds	Name of test	Leaf				Stem			
		Aqueous ext.	Ethanol ext.	Methanol ext.	Acetone ext.	Aqueous ext	Ethanol ext.	Methanol ext.	Acetone ext.
Proteins	Millon's test	+	-	+	+	+	+	+	+
	Ninhydrin test	+	+	+	+	+	+	+	-
Carbohydrates	Fehling's test	+	+	-	-	-	+	+	+
	Benedict's test	-	-	-	-	-	-	+	-
	Iodine test	-	-	-	-	-	-	-	-
Phenols and tannins	2ml ext.+ 2% $FeCl_3$	-	-	+	-	-	+	+	-
Test for flavonoids	Shinoda test	-	-	-	-	-	+	+	-
	Alkaline reagent test	+	-	-	+	-	+	+	+
Test for saponins	Foam test	+	+	-	-	+	-	-	-
Test for glycosides	Liebermann's test	+	+	+	-	+	+	+	-
	Salkowski's test	+	+	+	-	+	+	+	-
	Keller-Kilani test	+	+	+	-	+	+	+	-
Test for steroids	Liebermann's test	-	+	+	-	-	-	+	-
	2ml ext.+ 2ml chlo	+	+	+	+	-	-	-	-

	+ conc. H_2SO_4								
	2ml ext.+ 2ml chlo + conc. H_2SO_4 + acetic acid	+	+	+	-	+	+	+	-
Test for alkaloids	2ml ext. + 2ml 1%HCl + Mayer's + Wagner's reagents	+	+	+	+	-	+	+	+
Test for phlobatanine	2ml ext.+1ml 1% HCl	-	-	-	-	-	-	-	-
Test for terpenoids	2ml ext. + 2ml chlo + conc. H_2SO_4	+	+	+	+	+	+	+	-
Abbreviation chlo: chloroform, ext.: extract, + : present, - : absent									

*Tinosporacordifolia*having various different type of activity and it can possess a various chemical compound which is isolated from all part of giloy plant.A total of seven different concentrations of *T. cordifolia* extract along with positive and negative control were assessed for antimicrobial activity against *S. mutans*. At 2% concentration, a maximum zone of inhibition of 19 mm was seen with a volume of 40 µl followed by 9 mm with 30 µl. On the contrary, no inhibition zone was found with secondary volumes such as 10 µl and 20 µl. At 4% and 5% concentrations, a maximum area of inhibition of 5 mm was observed at a volume of 40 µl while lesser quantities failed to produce any zone of inhibition. At 7% and 8% concentrations, a maximum area of inhibition of 2 mm was observed at a volume of 40 µl while no zone of inhibition was seen with lesser volumes. At 3% and 6% concentrations, no area of inhibition was observed with any of the volumes [Table 1].

Table 1

Concentrations (%)	Volumes			
	10 µL	20 µL	30 µL	40 µL
	Zones of inhibition			
2	Resistant	Resistant	9 mm	19 mm
3	Resistant	Resistant	Resistant	Resistant
4	Resistant	Resistant	Resistant	5 mm
5	Resistant	Resistant	Resistant	5 mm
6	Resistant	Resistant	Resistant	Resistant
7	Resistant	2 mm	Resistant	Resistant
8	Resistant	Resistant	2 mm	Resistant

The results with 0.2% chlorhexidine which was used as a positive control showed a maximum zone of inhibition of 28 mm with 30 µl followed by 20 mm with a volume of 40 µl, 8 mm with 20 µl, and the least of 7 mm with 10 µl, respectively [Table 2]

Table 2

Zones of inhibition with different volumes of 0.2% chlorhexidine (positive control)

Concentrations	Volumes			
	10 µL	20 µL	30 µL	40 µL
	Zones of inhibition			
Chlorhexidine (0.2%)	7 mm	8 mm	28 mm	20 mm

No zone of inhibition was seen with any of the volumes of dimethylformamide, which was used as a negative control, indicating total lack of antimicrobial activity [Table 3].

Table 3

Zones of inhibition with different volumes of dimethylformamide (negative control)

Volumes	Dimethylformamide			
	10 µL	20 µL	30 µL	40 µL
Zones of inhibition	Resistant	Resistant	Resistant	Resistant

SUMMARY AND CONCLUSION :

Bioactive compounds were successfully isolated from *T. cordifolia* stems through extraction, fractionation, and isolation. Compounds isolated from this plant exhibit significant antimicrobial activity. The isolated compound moupinamide could be responsible for the high antibacterial activity against *P. aeruginosa* bacteria. Our findings indicate that the bioactive isolate from *T. cordifolia* is a great potential source of green material with the prospect of inhibiting the biofilm of *P. aeruginosa*, which is responsible for microbial corrosion. Before using this component as a green antimicrobial biocide, the anti-biocorrosion performance on metals must be evaluated. The result clearly show that ecological condition specially that edaphic factors plays important role in Quantitative Parameter of various phytochemicals.So far the Qualitative parameters was concerned , No significant variation was noticed. One of the most striking feature observed in this study was clear cut antagonistic response so far Phenol and Flavonoids are concerned.

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