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TINOSPORA CORDIFOLIA: PHYTOCHEMICAL SCREENING & ANTHELMINTIC STUDY OF IT'S DIFFERENT EXTRACTS

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Abstract:

The present study attempts to evaluate the phytochemicals parameters of Tinospora cordifolia leaves belong to family Menispermaceae that found in throughout India. The plant is used in the treatment of heart disease, leprosy, helmenthiasis, cancer, bone fracture, pain, skin disease, poisonous insect, snake bite and eye disorders. Its stem is highly nutritive and digestive purposes. Tinospora cordifolia is also used as a blood purifier that removes defective and damaged red blood cells from peripheral blood circulation by stimulating liver and spleen. Aim: The aim of the study is to investigate Phytochemical screening and anthelmintic study of Tinospora cordifolia by Petroleum ether and aqueous extract. Result: The percentage yield of Petroleum ether and aqueous extract were found to 7.82%w/w and 9.54 %w/w (Table-1). The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, glycosides, saponins, anthraquinone, terpenoids, phlobatanin like compounds (Table-2) but do not show the presence of carbohydrate in Petroleum ether, phenolic, flavonoid and lignin. The Powder analysis and fluorescence was observed in ultra-short, ultra-long and visible. The results were shown in (table-3 and 4). Results: The results (Table-5) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Petroleum ether extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

KEY WORD: *Tinospora cordifolia, Phytochemical screening, Albendazole, Anthelmintic Activity,*

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INTRODUCTION:

India has an ancient heritage of traditional medicine. Meteria Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various systems including Ayurveda, Siddha & Unani. An herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the use of herbal medicine & to determine their potential as a source for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way [1]. Human beings have used plants for medicinal purposes for centuries. Traditional forms of medicine have existed and still exist in many countries of the world including countries in the Indian sub-continent like India, Pakistan and Bangladesh [2]. The writings indicate that therapeutic use of plants is as old as 4000–5000 B.C. and Chinese were the first to use the natural herbal preparations as medicines [3].

Plants are one of the most important sources of medicines. Today the large number of drugs derived from plants, like morphine from Papaver somniferum, Aswagandha from Withania somnifera, Ephedrine from Ephedra vulgaris, Atropine from Atropa belladonna, Reserpine from Rauwolfia serpentina etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [4, 5]. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day-to-day practice.

The future of higher plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is also very promising. Natural products have provided us some of the important life-saving drugs used in the armamentarium of modern medicine. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and 15% have been investigated phytochemically. This shows a need for planned activity guided Phyto-pharmacological evaluation of herbal drugs. This article intends to provide an overview of the chemical constituents present in various parts of *Tinospora cordifolia* and their pharmacological actions.

Tinospora cordifolia that belongs to family Menispermaceae is one of the most widely used shrub from the ancient medicinal history of Ayurveda as a medicine.

PLANT DESCRIPTION:

Tinospora cordifolia is a glabrous, succulent, woody, large, deciduous, extensively-spreading, climbing shrub with a number of coiling branches and several elongated twining branches. *Tinospora cordifolia* is a native to India. *Tinospora cordifolia* is a twinning, fast growing shrub with grey-green branches becoming brown with age and tuberous roots, heart shaped, juicy, cordate and membranous leaves. It thrives well in the tropical region, often attains a great height and climbs up the trunk of large trees. *Tinospora cordifolia* grows to about 3–4 feet in height and is about 1 feet in width. The climbing plant is seen to bear lots of spreading slender branches which grab on to the nearby objects for support. Tinospora cordifolia is popularly known as Guduchi, is and is found in dry forests. *Tinospora cordifolia*, which is often found climbing up to the trunks of large neem trees and can grow in different types of soils, ranging from acidic to alkaline with moderate moisture. Stems of the *T. cordifolia* are succulent, having long filiform fleshy aerial roots, arising from the branches, varies in thicknesses, from 0.6 to 5 cm in diameter.

Leaves of *Tinospora cordifolia* are simple, alternate, entire and exstipulate with long petioles up to 15 cm long which are roundish with 2–9 cm long leaflet branches and pulvinate leaves at apex and at basal region, both at the base and apex with the basal one longer and twisted partially and half way around. The leaves are membranous. It gets its name heart-leaved moonseed by its heart-shaped leaves and its reddish fruit. Lamina is broadly ovate, 10–20 cm (4–8 in) long or 8–15 cm (3–6 in) broad, 7 nerved and deeply cordate at the base and membranous [6-7] pubescent above, whitish tomentose with a prominent reticulum beneath. Leaves are chordate in shape showing multicoated reticulate venation.

Flowers of *Tinospora cordifolia* are unisexual, recemes, small on separate plants and appearing when the plant is leafless, greenish-yellow (when plant is leaf less.) on axillary position, 2-9 cm long raceme on leaflet branches, and terminal racemes. Male flowers are clustered, but female flowers are usually solitary. It has six sepals in two series of three each. The outer ones are smaller than the inner. It has six petals which are smaller than sepals, whorls/obovate, and membranous. The sepals and petals are 6 in number and are usually free or grouped in 2 or 3 numbers. The female and male flowers are seen in different plants. On the flowering season, the

plants bear no leaves and the flowers bear yellowing green colour and the flowers are positioned at the apex and terminal racemes. The differentiations in the sexes are seen in the form that the male flowers are usually clustered and the female flowers are solitary in positioning. Flowering occurs during March to June they are orange-red in colour, fleshy, aggregate of 1-3 and ovoid, smooth, drupelets on thick stalk. Flowers grow during the summer and fruits during the winter [8 -13].

Fruits drupelets are single-seeded and aggregate in clusters of one to three. They are ovoid smooth on thick stalks with sub terminal style scars, scarlet or orange colored fleshy, aggregate of 1-3 and ovoid, smooth, drupelets on thick stalk with a sub terminal style scar. Fruits develop during winter [14-16] Fruits are fleshy, pea shaped, shiny, turn red when boiled [17-18] Fruits are drupes, turn red when ripe. It thrives easily in the tropical region, often attains a great height.

Young stems are green in colour with smooth surfaces and swelling at nodes [18], while circular lenticels impart warty protuberances in the light brown-colored older stems. The stem is gray and creamy white, deeply cleft spirally and longitudinally, with the space between spotted with large rosette like lenticels. filiform, fleshy and climbing in nature; [6]. The stem structure is fibrous and the transverse section exhibits a yellowish wood with radially arranged wedge shaped wood bundles, containing large vessels, separated by narrow medullary rays [10].

Powder of the stem is creamish brown or dark brown, having characteristic odour, bitter taste and is used in dyspepsia, fever, and urinary diseases [19]. It is highly nutritive and digestive.

The roots which are present in this plant are seen in both underground and aerial form. Roots of *Tinospora cordifolia* is a long thread-like[20], aerial, squairshin, sometimes continuously lengthening touch the ground [21] and aerial roots are characterized by tetra to penta arch primary structure [16,22]. However, cortex of root is divided in to outer thick walled and inner parenchymatous.[6]

The seeds are curved shape and pea sized. Hence this family is Menispermaceae and is also named as moonseed family also. As seeds are curved in shape, embryo also turned in to curve shape automatically and are transverse dehiscent in nature and Fruits are fleshy and single seeded. endocarp is variously ornamented, which provides critical taxonomic characters. Moreover, the endocarp is variously ornamented and provides important taxonomic characters. [6, 22].

The wood is white, soft and porous, white in colour and the freshly cut surface assumes a yellow tint when exposed to air. The bark is creamy white to grey in color with deeply left [6]

BOTANICAL CLASSIFICATION:

The botanical classification of this medicinal herb is given below;
Kingdom: Plantae.
Division : Magnoliophyta.
Class : Magnoliopsida.
Order : Ranunculales.
Family : Menispermaceae.
Genus : Tinospora
Species : T. cordifolia.

Common Names [23]

Latin : *Tinospora cordifolia* (Willd.) Hook.f. & Thomson
English : Tinospora Gulancha /, heartleaf moonseed, moon creaper,Bile killer
Sanskrit : Guduchi, Madhuparni, Amrita,Chinnaruha, Vatsadaani, Tantrika,Jivantika,Amrita druma Kundalini and Chakralakshanika
Hindi **:** Giloya, Guduchi (Hindi),Gulancha, Amrita,Gurcha,Gulneha,Gulbel,Guloh.
Bengali : Gulancha, giloe,Golancha
Telugu : Tippaatigo,Dussiramu,Thippateega,Amruta.
Tamil : Shindilakodi, Amritavalli, Chintilikkoti,Chindil,Seendal,Seendil kodi,Silam,Kunali
Marathi : Shindilakodi,Gulvel,Amrita, Amritavalli
Gujarati : Galo,Gulvel
Kannada : Amritaballi, Amrudavalli,Agnibali.
Malayalum:Amrutavalli,Chintilikkoti,Chitamrith,Amrtu,Amritavalli,Amrthu
Punjabi:Gilo,Gilo-Gulanch,Garham,Palo
Oriya:Gilochi,Gulancha
Urdu:Gurch,Guluncha

Parts used:
Stems -The stem is bitter, stomachic, diuretic stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice (Meshram *et al.*, 2013).
Roots - The root and stem of T. cordifolia are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting (Meshram *et al.*, 2013).

Leaves - Juice or decoction of leaves is administered orally with honey in fever (Shah, 1984).

Occurrence and distribution:
The species *Tinospora Cordifolia* (wild) Miers ex Hook. F and Thoms belonging to the family Menispermaceae is a large deciduous climbing shrub found throughout India and also in Srilanka, Bangladesh and China [24].

It is distributed throughout the tropical Indian subcontinent and China as well as in Srilanka and Burma. There is evidence that the plant is found in some tropical regions of Australia and Africa as well. In India, it grows right from the Kumaon Mountains to Kanyakumari. It is a very common plant of dry and deciduous forests. It can grow at any temperature and has a long life. It is also found in other tropical and subtropical countries such as Srilanka South Asia, Indonesia, Philippines, Thailand, China and in Srilanka worldwide and Myanmar [24,25]. The plant is distributed throughout the tropical region of India up to 1,200 m above sea level from Kumaon to Assam, in north extending through West Bengal, Bihar, Deccan, Konkan, Karnataka and Kerala.

Habitat
Tinospora cordifolia prefers wide range of soil, acid to alkaline and it needs moderate level of soil moisture. Found throughout tropical India ascending to an altitude of 1000 feet. It grows well in almost any type of soils and under varying climatic conditions.

Nutritive Composition
It contains high fiber content approx (15.9%), proteins (4.5%-11.2%), sufficient carbohydrate (61.66%) and low fat (3.1%). Its nutritional value is 292.54 calories per 100 g. It has high potassium (0.845%), high chromium (0.006%), sufficient iron (0.28%) and sufficient calcium (0.131%). [26]

CHEMICAL CONSTITUENT-
Tinospora contains diverse phytochemicals, including alkaloids, phytosterols, glycosides, and mixed other chemical compounds. The different compounds are
Columbin, tinosporaside, jatrorhizine, palmatine, ber berine, tembeterine, tinocordifolioside, phenylpropen e disaccharides, choline, tinosporic
acid, tinosporal, tinosporon, and tinosporide have been isolated from *Tinospora cordifolia*. Nutritive Composition of Tinospora cordifolia: it contains high fibre (15.9%), sufficient protein (4.5%-11.2%), sufficient carbohydrate (61.66%), and low fat (3.1%). Its nutritive value is 292.54 calories per 100 g. It has high potassium (0.845%), high chromium (0.006%), sufficient iron (0.28%) and sufficient calcium (0.131%), important in various regulatory functions [26].

The different classes of compounds which are found in this plant are classed in groups like alkaloids, steroids, terpenoids, polysaccharides, and different aromatic and aliphatic compounds that are present in their phytoactive form that are responsible for the wide range of medicinal and therapeutic properties. The presence of these compounds is found in various plant parts but highly concentrated in the stem, leaves and root part of the plant. [14]

The main compound of this plant is berberine and furanolactone and furthermore compounds like tinosporone, tinosporic acid, cordifolisides , gilenin, crude giloininand, arabinogalactan polysaccharide, picrotene, bergenin, gilosterol, tinosporol, tinosporidine, sitosterol, cordifol, heptacosanol, octacosonal, tinosporide, columbin, chasmanthin, palmarin, palmatosides C and F, amritosides, cordioside, ecdysterone, makisterone A, hydroxyecdysone, magnoflorine, tembetarine, syringine, glucan polysaccharide, syringine apiosylglycoside, isocolumbin, palmatine, tetrahydropalmaitine, jatrorrhizine are few of the compounds that have been iolated from the plant. The presence of three compounds like cycloeuphordenol, Cyclohexyl–11–heneicosanone and 2–Hydroxy–4–methoxy– benzaldehyde has been isolated from the plant giloin. It also contains tinosporaside, palmatine, berberine, It also contain tembeterine, tinocordifolioside, phenylpropene disaccharides, have been isolated from Tinospora cordifolia.

As it stated above, a variety of constituents have been isolated from different parts of *Tinospora cordifolia*. They belong to different classes such as diterpenoid lactones, steroids, aliphatic compounds, some constituents are as follows [16].

It contain many alkaloid (from stem, root) as Tinosporin, Palmetine, Isocolumbin, Aporphine alkaloids, Jatrorrhizine, Tetrahydropalmatine, It contain many Diterpenoid Lactones (from whole plant) as Furanolactone, Clerodane derivatives [(5R,10R)-4R 8Rdihydroxy- 2S-3R:15,16- diepoxy-cleroda-13 (16), 14-dieno-17,12S:18,1Sdilactone],Tinosporon, Tinosporides, Jateorine, Columbin. It contain many Glycosides (from stem) as 18-norclerodane glucoside, Furanoid diterpene glucoside, Tinocordiside, Tinocordifolioside,Cordioside, Cordifolioside

Syringin, Syringinapiosylglycoside, Pregnane glycoside, Palmatosides, Cordifolioside A, B, C, D and E It contain many steroid(from shoot) as β–sitosterol, δ-sitosterol, 20 β-hydroxyecdysone, Ecdysterone, Makisterone A, Giloinsterol. It contains many Sesquiterpenoid (from stem as Tinocordifolin). It contain many aliphatic (from whole plant) as Octacosanol, Heptacosanol Nonacosan-15-one dichloromethane.It also contain others(from root and whole plant) as 3,(a,4-di hydroxy-3- methoxy-benzyl)-4-(4- compounds hydroxy-3- methoxy-benzyl)- tetrahy drofuran, Jatrorrhizine, Tinosporidine, Cordifol, Cordifelone, Giloinin, Giloin, N-transferuloyltyramine as diacetate, Tinosporic acid.

TRADITIONAL MEDICINE

In Ayurveda, *Tinospora cordifolia* has been used over centuries to treat numerous diseases, such as jaundice, and rheumatoid arthritis, among others. Due to inadequate design or other limitations of clinical research on Tinospora, there is no high-quality scientific evidence that it affects any disease, and it is not in use as a prescription drug. Indian systems of medicine and used in medicines since times immemorial. The drug is well known Indian bitter and prescribed in fevers, dyspepsia, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart disease, leprosy, and helmenthiasis. The starch obtained from the stem is highly nutritive and digestive and used in many diseases [14]. *Tinospora cordifolia* is also used as a blood purifier which removes defective and damaged red blood cells from peripheral blood circulation by stimulating liver and spleen. *Tinospora cordifolia* has great importance in traditional ayurvedic medicine used for in the treatment of cancer, bone fracture, pain, skin disease, poisonous insect, snake bite, eye disorders [27].

Medicinal and therapeutic properties

The plant has been titled to many properties that have been used from time immemorial and few of them include curative properties against gout, urinary and upper respiratory infections and preventive measures against bleeding piles, itching and erysipelas. The plant is known for its potent aphrodisiac nature and its rejuvenating nature [28].

1.Plant

The plants show anti–diabetic properties due to the presence of tannins, alkaloids, flavonoids, glycosides, saponins and steroids. The plant has seen to have effect on both the sexual arousal and the sexual performance of the biological systems and these drugs have stimulatory effect on the copulatory behavior and thereby entitled to aphrodisiac activity. The plant also bears anti–oxidant, anti–inflammatory, anti–tuberculosis; wound healing, immunomodulatory and immunoprotective, anti-allergic, anti-arthritis, hepatoprotective, anti–osteoporotic, anti–cancer, anti–tumor, anti– malaria, cardio protective nature [29-31].

2. Bark

Dry barks of *T. cordifolia* has anti-spasmodic, anti-pyretic, anti-allergic and anti-leprotic properties [32-34]

3. Root

The aqueous extract of *T. cordifolia* root has anti-oxidant property. It is successfully experimented on diabetic male albino rats [29]. The plant is commonly used as traditional ayurvedic medicine and has several therapeutic properties [30-31] such as rheumatism, anemia, inflammationanti-periodic, radioprotective properties, etc. [32, 25] .The root of Giloya (T. cordifolia) is used as potent emetic and for bowel obstruction. Giloya is useful in the treatment of helminthiasis, heart diseases, leprosy, support the immune system, the body's resistance to infections, supports standard white blood cell structure, function, and levels [33]. It also helps in digestive ailments such as hyperacidity, colitis, worm infestations, loss of appetite, abdominal pain, excessive thirst, and vomiting, and even liver disorders like hepatitis [34-35].This pharmacological activities of the plant is due to its chemical constituents like diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, essential oils, a mixture of fatty acids, and polysaccharides and is present in a different part of the plant body, including root, stem, and whole part.

MEDICINAL APPLICATIONS

1. Oral administration of the juice of stem with honey can also be used for treatment of asthma [14].
2. Plant stem is used in general debility, dyspepsia, fever and urinary diseases [36]. It is used in the treatment of jaundice because it reduces body heat [36].
3. For treatment of cancer, the powder of root and stem is used along with milk [37].
4. It has been observed that polyherbal formulation of *T.cordifolia* possesses favourable effect in patient with HIV infection [38].

5. Since *T. cordifolia* is a good antioxidant, it is given with L-DOPA during Parkinson's disease. L-DOPA produces free radicals during the formation of dopamine. Thus *T. cordifolia* neutralizes the side effect of drug [38].
6. In Urinary disorder, the juice of the roots is very much effective.
7. Diabetes mellitus is a worldwide chronic disease of humans related with the elevated blood sugar level due to insulin deficiency.[39].
8. Guduchi is also used for soothing inflamed and injured mucous membranes in the digestive tract. It protects the stomach and duodenum by increasing the production of mucin.
9. It is regarded as one of the best psychotropic drugs in India.
10. It also works as immunomodulators in diseases like obstructive jaundice, hepatic fibrosis, peritonitis and sepsis.

Leaves- Juice or decoction of leaves is administered orally with honey in fever The leaves are beaten with honey and applied to ulcers and also treatment of gout and erysipelas (bacterial skin infection) [14, 16, 40]

Stem: The stem is bitter stomachic, stimulates bile secretion, diuretic, enriches the blood, useful in skin diseases and cures jaundice; The juice is useful in diabetes, vaginal and urethral discharges, low fevers and enlarged spleen. Stem as an infusion used as a vermifuge, jaundice, against intestinal worms. Stem as decoction used for washing sore eyes and syphilitic sores, antipyretic, antimalarial. Stem used for chronic diarrhoea and some form of obstinate chronic dysentery, deal with intestinal problems and improves digestion.

Root: The root is powerful emetic and used for visceral obstruction; its watery extract is used in leprosy.

Stem and Root: As an antidote to snake bite and scorpion sting.

Bark: Anti-allergic, Anti-spasmodic, Anti-pyretic, Anti-leprotic. [41-43]

Fruit: Dried and powdered fruit, mixed with ghee or honey, is used as a tonic and also in the treatment of jaundice and rheumatism.

Whole plant: The whole plant is used in scabies in swine, diarrhoea and stomach trouble. Urinary diseases, syphilis, skin diseases, bronchitis, promote longevity and increase body'sresistance. Stimulate the immune system [44-45].

Traditional Uses

1. Giloe is a tonic and has alterative, diuretic, and aphrodisiac properties.
2. It is a febrifuge used in malarial and chronic fever.
3. It is also a liver tonic.
4. The plant is used in general debility, loss of appetite, fevers, urinary disorders, diabetes, rheumatism, and dyspepsia.
5. *Tinospora* cordifolia extracts are extensively used in various herbal preparations the treatment of different ailments for its anti-periodic, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic properties [30].

PHARMACOLOGICAL ACTION

1. The alcoholic extract of the dried stem has Anti-inflammatory action.
2. Tinospora cordifolia is traditionally used for asthma, in treatment of cough.
3. Alcoholic and aqueous extract of *Tinospora cordifolia* has immunomodulatory action due to the presence of polysaccharides present in it.
4. The stem of *Tinospora cordifolia* is used in general debility, dyspepsia, fever, urinary diseases. The extract of stem is used in skin diseases.
5. The roots posses Anti-stress and Anti-oxidant activity.
6. *Tinospora cordifolia* also has cardio protective activity.

Parts used

Stems-The stem is bitter, stomachic, diuretic [46] stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice.

Roots- The root and stem of *T. cordifolia* are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting [47-48].

MATERIALS AND METHODS:

The different Mayer's, Hager's, Barfoed's, Benedict's and millon's reagent, Wagner's, Dragendorff's, Fehling's A & B, α-naphthol, Ferric chloride,Conc. Sulphuric acid, Pyridine, Sodium nitropruside, Acetic anhydride, were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Ethanol were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer. Other chemicals were prepared in the laboratory as, 10 % Lead acetate, 10 % Ammonium hydroxide solution, 10 % Ammonia. And to carry out the anthelmintic study of different extracts, we have taken following chemicals as Saline water (Claris Lifesciences

Ltd.,Ahmedabad). Albendazole (Alkem Ltd.) is used as reference standards.

EXPERIMENTAL WORK:

Plants collection, Identification and processing:

The plant *Tinospora cordifolia* was collected from adjoining area of Barpali (Dist-Bargarh, Odissa) in the month of April-2021. The plant was identified by Botanist Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D, P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust if any; initially it was dried in sunlight for an hour and shade dried completely. Also all the foreign matters like dead or destructed part were removed precautionary. The plant *Tinospora cordifolia* was cut into small pieces by means of wood grinder and knives.

Fig-1 Aerial parts of plant *Tinospora cordifolia*

Preparation of the extracts:

1. Petroleum ether Extract
2. Aqueous Extract

(a) Petroleum ether extract

The shade dried course powder of *Tinospora cordifolia* (200 gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with Ethanol extract for 8 hours at 50^{O}C for five days. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely and to obtain a semisolid product. The dried product was kept in the desiccators till experimentation. Then the extract was weighed and it's percentage yield was calculated in terms of air-dried powdered crude material.

b) Aqueous extract

The shade dried course powder of *Tinospora cordifolia* (200 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water for 8 hours at 50^{O}C for five days. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely and to obtain a semisolid product. The dried product was kept in the desiccators till experimentation with the use of anti-microbial agents as it has the presence of water which has the chance of microbial contamination. The obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude material. The yield and % yield of both Petroleum ether and aqueous extracts of *Tinospora cordifolia* was reported.

Table-1 % Yield values of Petroleum ether and aqueous extracts of *Tinospora cordifolia.*

Sl. No.	Extracts	% Yield (w/w)	Colour of extract
1.	Petroleum ether	7.82%	Brown Black
2.	Aqueous	9.54%	Yellowish Green

QUALITATIVE PHYTOCHEMICAL ANALYSIS [49-51]

Both Petroleum ether and aqueous extracts obtained by the powdered rhizome of *Tinospora cordifolia* was subjected to various qualitative tests for the identification of different phytoconstituents present in it. The constituents present in different extracts of *Tinospora cordifolia* are summarized.

Table.No-2 Phytochemical analysis of Petroleum ether & aqueous extracts of *Tinospora cordifolia.*

Phytochemical test	**Petroleum ether Extract**	**Aqueous Extract**
Alkaloid test		
Mayer's test	Absent	Present
Wagner's test	Present	Absent
Hager's test	Absent	Present
Dragendorff's test	Present	Absent
Carbohydrates		
Molish's test	Present	Absent
Fehling's test	Absent	Absent
Benidict's test	Present	Present
Borntrager's test	Absent	Present
Saponins		
Foam test	Present	Present
Proteins & amino acid		
Xanthoprotic test	Absent	Present
Biuret's test	Present	Absent
Ninhydrin test	Absent	Present
Phenolic compounds & flavonoids		
Ferric chloride test	Present	Absent
Lead acetate test	Absent	Present
Alkaline test	Present	Present
Phytosterol :		
Libermann-Burchard's test	Absent	Present
Glycosides		
Modified Bront.	Absent	Absent
Cardiac Glucoside	Absent	Present
Terpinoids		
Salkowski test	Absent	Absent
Cu. Acet. test	Present	Present
Lieberman test	Absent	Absent
Tannin		
Alkaline reagent	Present	Present
Lignin		
Phloroglucinol	Absent	Present
Toluene	Present	Absent
Phlobatannin Anthraquinone		
Nitric acid	Present	Present
Benzene	Absent	Absent

Table No – 3 Powder analyses with chemical reagent

Reagents	Colour of powder
Powder as such	Dark Green
Powder + conc. HCI	Greenish Black
Powder + conc. HNO_3	Reddish Brown
Powder + conc. H_2SO_4	Greenish Black
Powder + glacial acetic acid	Yellowish Brown
Powder + dil. HCI	Dark Green
Powder + NaOH sol.	Greenish Black
Powder + $FeCl_3$	No change
Powder + picric acid	No change
Powder + ammonia	Greenish Black
Powder +Iodine	Brown

Table No – 4 Fluorescence analysis of powder drug

Reagent	Ultra Short	Ultra Long	Visible
Powder as such	Dark Green	Dark Green	Dark Green
Powder + 1N NaOH in methanol	Dark Green	Black	Dark Green
Powder + NaOH in water	Black	Black	Dark Green
Powder +H_2SO_4	Black	Black	Dark Green
Powder +HNO_3	Black	Greenish Black	Reddish Brown
Powder + pet.ether	Green	Dark Green	Black
Powder + chloroform	Brown	Light Green	Black
Powder + Dil HNO_3	Black	Black	Reddish Brown
Powder + $FeCl_3$	Brown	Light Green	Black
Powder + methanol	Dark Brown	Dark Green	Black

DETERMINATION OF BIOLOGICAL (ANTHELMINTIC) ACTIVITY [52]

The anthelmintic study was done by using one in-vitro species adult earthworms *Pheretima posthuma*. Earthworms were collected near the swampy water in our locality. The average size of the round worm was 5-7 cm; average size of the earthworm was 8-9 cm. These earthworms were identified and services of veterinary practioner were utilized to confirm the identity of worms. The suspensions of various extracts were prepared in 2% gum acacia solution to obtain 1, 2.5 and 5% concentrations. Solutions of

similar concentrations of the standard drug albendazole were also prepared in distilled water.

Two ml of each concentration of various extracts of *Tinospora cordifolia* and standard drug albendazole were diluted to 10 ml separately with normal saline and poured in petridishes. 2ml of 2% gum acacia solution was diluted to 10ml with normal saline to serve as control. Six earthworms of nearly equal size were placed in each Petridis at room temperature. Time was recorded at the time of releasing the earthworms to each concentration. The time taken (minutes) for the complete paralysis and death were recorded. The mean paralysis time for each sample was recorded. The anthelmintic activity was evaluated on adult Indian earthworm *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colour.

Table 5: Anthelmintic effect of *Tinospora cordifolia* extracts.

Group	Concentration of Extract (%)	Time in minutes (Mean ± SEM)	
		Paralysis time(Min)	Death time(Min)
Albendazole (standard)	10 mg/ml	14min,18 sec ±17	18min,18 sec ±42
	30 mg/ml	10min,21 sec ±12	14 min,12 sec ±10
	50 mg/ml	8 min,12 sec ±14	12min,40 sec ±11
Petroleum ether extract	15 mg/ml	22min,16 sec ±17	28min,17 sec ±48
	30 mg/ml	20min,16 sec ±12	24 min,36 sec ±12
	50 mg/ml	18 min,28 sec ±14	22 min,41 sec ±14
Aqueous extract	15 mg/ml	12min,19 sec ±17	20min,15 sec ±48
	30 mg/ml	12min,26 sec ±12	16 min,26 sec ±12
	50 mg/ml	10 min,48 sec ±14	13min,14 sec ±10
Control	-	-	-

Results are expressed as mean ± SEM from six observations, *Control worms were alive upto 24 hrs. of observation,* N/A= No Activity shown within 24 hours.

RESULTS AND DISCUSSION:

The percentage yield of Petroleum ether and aqueous extract were found to 7.82%w/w and 9.54 %w/w (Table-1). The preliminary phytochemical screening on the leaf extract was carried out by subjecting the different extracts to qualitative test for the identification of various plant constituents. It showed the presence of alkaloids, glycosides, saponins, anthraquinone, terpenoids, phlobatanin like compounds (Table-2) but do not show the presence of carbohydrate in Petroleum ether, phenolic, flavonoid and lignin. The Powder analysis and fluorescence was observed in ultra-short, ultra-long and visible. The results were shown in (table-3 and 4). The results (Table-5) depict the time taken for paralysis and death of earthworms after the treatment with the test extracts at the selected concentrations. The data revealed that the aqueous extract has a better wormicidal effect than Petroleum ether extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

CONCLUSION:

These Phytochemical analysis tests will help us for the identification and isolation of different chemical compounds for the future work. This plant has a better wormicidal effect than Petroleum ether extract with compared with the standard drug Albendazole. Further study is required to find out the novel phytoconstituents responsible for anthelmintic action against various helminthes.

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