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Phytochemical and Antifungal properties of *Tinospora cordifolia* (Wild) Hook F. Thompson Against Fruit Rot Pathogens

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Abstract: *Many studies reveal that the plants are reservoirs of 'active antimicrobial compounds'.The antimicrobial activity of plants is due to the presence of different bioactive compounds of various types such as Flavanoids,triterpenoids and some essential oils like Thymol and natural phenolic compounds. Many investigations shows the usefulness of all these compounds in human welfare particularly to counteract many kinds of human disorders.This study throws light on the phytochemical and antifungal properties of T.cordifolia. In this study preliminary qualitative analysis of leaf and Stem extract of T.cordifolia. This is done by using three types of Solvents as Water, Ethanol, Methanol. Data indicates the presence of flavonoids,alkaloids,phenolic compounds,glycosides phytosterols and tannins.Most of the phytochemical compounds were found in methanolic and water solvents as compared to ethanol. The presence of these secondary metabolites and their potential suggests their future usefulness to control human as well as plant's antimicrobial attacks.*

Keywords: Active antimicrobial compound, phytochemicals, Tinospora cordifolia, solvent extracts

I. INTRODUCTION

Fruits are susceptible to attack by a variety of microorganisms during storage,transportation and marketing. In India, where bulk storage facilities are neither available nor affordable such losses are even greater particularly during wet and humid periods(Sill,1985).

Postharvest diseases due to fungal pathogens posing a major problem to the agriculture industry where they account to about 50% losses in fruits stored in poor storage conditions. Now a days fungal diesases are controlled by chemical fungicides. But their continuous use complemented with high cost, residue in plants which create toxic effects to the environment and human health.

Therefore, there is a need for the development of safe, eco-friendly and effective strategy to control the postharvest fungal pathogens.

In our ancient world, the consumption of plants as medicine was the only source against various diseases. Fossil records date human use of plants as medicine at least to the middle palaeolithic age, some 60,000 years ago (Fabricant and Fransworth,2001).At that time the use of plants as was based on visual observation of some local practioners, and their traditional knowledge pass on generation to generation.

According to WHO,almost 65% of the World's population have incorporated traditional system of medicine in their modality of health care(Fransworth et.al;1985).Now the medicinal plant sector has gained both scientific and social support, the herbal industries are playing a major source of capitalization in both developed and developing countries.

Extracts obtained from many plants have recently gained popularity and scientific interest for their antifungal activities (Lee et.al.,2007;Verastegui et.al.2008;Santas et.al.,2010). Reports on the antimicrobial properties of plant extracts containing different classes of phenolic compounds represent a rich source of preservatives thet have been explored for a long time as postharvest alternative control measures to fungicides(Lattanzio,2003;Schena et.al.2008).

Other research workers (Amadioha and Obi,1999;Amadioha,2000 and Okigbo,2009) studied the significance of fungicides of plant origin as possible means of fungal disease control in fruits and vegetables as they are easily biodegradable and nontoxic to human health.

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Zhang and Zhang(2005) have also shown that the plant extracts have great potential to be an alternative to synthetic fungicides. This investigation is therefore targeted at the phytochemical and antifungal activities of one of the most important and popular medicinal plant, *Tinospora cordifolia* against postharvest fruit rot pathogens.

Taxonomic Classification of *Tinospora cordifolia*(Wild) Miers ex. Hook and Thompson

- Kingdom-Plantae
- Subkingdom:Trachaeophyta
- Superdivision: Spermatophyta
- Division :Magnoliophyta
- Class:Magnoliopsida
- Subclass: Polypetalae
- Series:Thalmiflorae
- Order: Rannunculales
- Family:Menis.permaceae
- Tribe: Tinosporaceae
- Genus: *Tinospora*
- Species: *cordifolia*

Selected Vernacular Names

- Sanskrit-Guduchi, Madhuparni, Amrita, Tantrica
- Hindi-Giloya,Guduchi ;Marathi-Gulvel
- Gujrati- Galo; Kannada- Amrita balli, Madhupa

Plant Description

Tinospora cordifolia(Willd)Miers, is one of the important of deciduous plant belongs to the family Menispermaceae found throughout the India,Srilanka, Bangladesh and China.It is endemic to the tropical region of the India, growing to temperature range of 25^0C to 45^0C at an altitude of 500 meters.(9). It is a perennial twine usually found with scrumptious stem and papery bark in India. It often attains a great height and mostly climbs up the trunks of large tree. Roots are long filiform, and fleshy. Bark is thin grey or creamy white in colour with deep spotted clefts and large lenticels. Leaves are heart shaped, membranous, juicy and cordate. The leaf blade is broadly ovate to roundish, cordate, cordate,5 to 12cm in diameter with smooth surfaces. It has greenish flowers which are unisexual and bloom in summer. Male flowers are small, yellow or green coloured occur in clusters whereas female flower occur singly. Fruits are spherical or rounded in shape,fleshy,shiny green which turn red after ripening. Seed is curved.

Phytochemical Analysis of Different Parts of *Tinospora cordifolia*

Leaves are rich in protein, calcium and phosphorus.(5,6). Methanolic extracts of leaves is rich in flavonoids, alkaloids and glycosides(7). Herbal extracts of various forms like infusion, decoction, tinctures, syrups and maceration etc. are commonly used sunce ancient time for medicinal purpose. Cold infusion of Giloy prepared by using stem is given in chronic conditions of fever. The juice extract from the stem of Giloy is highly effective for the treatment of gout as it helps to neutralise the increased uric acid levels in the body. Strong decoction of giloy with basil leaves offer resistance against swine flu. (Promilla et.al.2017)

The phytochemicals mainly synthesized in all plant parts like stem, leaves, root, bark, flower and fruits. Isolation of these compounds from plants is mainly depending upon the solvents which used for extraction(3).This emphasize there is an urgent need to try as much solvents as possible in qualitative phytochemical screening of plants(4). In the present investigations, three different solvents are used to obtain extract of leaves and stem of *Tinospora cordifolia* .
These extracts were used for preliminary phytochemical analysis by using standard chemical methods.

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II. MATERIAL AND METHODS

2.1 Collection of Plant Material

Fresh stem and Leaves of *T.cordifolia* are collected from wild areas of Parbhani District of Maharashtra. Collected plant material washed under the tap water to eradicate dust and microbes. The plant material then air dried under shade at room temperature for 15 days. The plant material then crushed well into fine powder in an electronic grinder and kept into air tight polythene bags for further use and stored at room temperature.

2.2 Preparation of Plant Extract

The extracts of selected sample powder were prepared by soaking 50gm of dried powder in 100ml.of each methanol, ethanol and water. The solution left at room temperature for 72 hours and then filtered with the help of of filter paper. The filtrate of the selected plant sample were taken and used for further phytochemical screening.

2.3 Phytochemical Screening:

A. Detection of Alkaloids

A small amount of extract was treated with 2ml.of Wagners reagent(1.27g of iodine and 2gm of Potassium iodied in 100ml of water) and observed till the formation of reddish brown precipitation which indicates the presence of Alkaloids.

B. Test for Carbohydrates

A few drops of Bennedicts reagent were added to 2ml of various extracts, boiled in waterbath for for 5min.cooled and observed for a reddish brown precipitate(19)

C. Detection of Glycosides

Extracts were hydrolysed with dil. HCL, and then subjected to test for glycosides.

Modified Borntragers Test

Extracts were treated with Ferric chloride solution and immersed in boiling water for about 5minutes. The mixture was cooled and extracted with equal volume of Benzene. The Benzene layer was separated and treated with Ammonia solution. Formation of rose-pink colour in the ammonical layer indicate the presence of anthranol glycosides.

D. Detection of Saponin

2ml of extract was added 12ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam.

E. Detection of Phenol

A fraction of the extracts was treated with aqueous 5% ferric chloride and noticed for formation of deep blue or black colour.(22)

F. Detection of Flavonoids (Alkaline Reagent Test)

Few drops of 20% sodium hydroxide solution were added to 2ml of extracts. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, revealed the presence of flavonoids(22,23).

G. Detection of aminoacids and Proteins (1% Ninhydrin Solution)

2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in boiling water bath for 1-2 minutes and observed for the formation of Purple colour(24).

H. Detection for Tannins (Braymers Test)

2ml of extract was treated with 10% ferric chloride solution and observed for formation of blue or greenish colour solution(19).

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I. Detection of Terpenoids (Salkowkis Test)

2ml of each extract were treated with 1ml of Chloform followed by a few drops of concentrated Sulphuric acid. A reddish brown precipitate is formed immediately indicated the presence of terpenoids(25).

III. RESULTS AND DISCUSSION

Table 1: Phytochemical Screening of *Tinospora cordifolia* leaves and stem extracts.

Phytochemical Test	Leaf extracts			Stem Extracts		
	M	E	A	M	E	A
Alkaloids	+	+	+	+	+	-
Carbohydrates	+	+	+	+	-	+
Glycosides	+	+	+	+	-	-
Saponin	+	-	+	-	-	+
Phenol	+	+	+	+	+	-
Flavonoids	+	+	-	+	+	-
Amino acids	+	+	-	-	-	-
Tannins	-	+	+	-	+	-
Terpenoids	+	+	-	-	+	-

In Table-1, where, M-Methanolic extract; E- Ethanolic Extract;
Aquaeous extract; +sign indicates presence and -sign indicates absence of respective phytochemical.

There is a consensus that the antifungal effect of plants could be associated with the quality or/and quantity of their secondary metabolites(34). Indeed, correlation analysis revealed that inhibition of mycelial growth and spore germination were very strongly corerelated with polyphenol and flavonoid levels.

As reported by E.I.Matti et.al.(34), plant extracts with higher antimicrobial ability had higher phenolic content. Alternatively, Assiri et.al(35) have demonstrated that bioactive lipids including fatty acids and hydrophobic vitamins are implicated in the antimicrobial properties of plant extracts.

All these compounds can work by inhibiting metabolic enzymes, interefering with cell wall synthesis and electron transport, altering cell permeability inhibiting nutrient absorption, and interfering with other cellular metabolic pathways(36).

In fact, the antioxidants have been reported to play a major role in increasing the effectiveness of treatments against plant fungal pathogens when combined as adjuvants with fungicides(37).

Their effect may be due to an increase in membrane permeability, subsequently allowing a greater diffusion of fungicides in cells, or reduced oxidation of intracellular fungicides resulting in higher toxicity for fungi(38).

Available literature indicates that the antimicrobial activity of plant is due to the presence of different bioactive compounds in various types of extracts such as flavanoids, triterpenoids and some essential oils like Thymol and natural phenolic compounds that are classified as **Active antimicrobial compounds** (Hasan et.al.1994).

Successful anticipation of various herbal chemical compounds from plant is largely reliant on the type of solvent that were used in the extraction procedure. The customary practioners in our medicinal system suggested water primarly as a good solvent for extraction which has better solubility of bioactive compounds. Later investigations verified that in comparison to water extracts, organic solvents extracts exhibits strong results (Krishana et.al.1997;Singh I. and Singh V.P.2000; Natrajan et.al.2003;Natrajan D.et.al.2005).

Flavanoids are least soluble in water which is the primarily phenolic compound in plants and responsible for several therapeutic activity of plants(Do Boer et.al.2005).

Several studies have been conducted to shed light on the mechanism of action of several active compounds of plant extracts including essential oils(Chang et.al.2001).The crude sap, volatile and essential oil extracted from whole plant or specialized plant parts like root, stem, leaves, flowers, fruits and seeds are widely used in preparing the antimicrobial compounds which are significantly used against the different plant pathogens or diseases (Gurjar, M.S.et.al. 2012).

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3.1 Mode of action of Phytochemicals

Sr. No.	Class	Subclass	Mechanism
1.	Phenolics	Simple phenols	Membrane disruption
2.	Phenolic acids	Phenolic acids	Bind to adhesions, complex with cell wall, inactivate enzymes
3.	Terpenoid	-	Membrane disruption
4.	Alkaloids	-	Intercalate into cell wall
5.	Tannins	-	Bind to proteins, enzyme inhibition, substrate deprivation
6.	Flavanoids	-	Bind to adhesions, complex with cell wall, inactivate enzymes.
7.	Coumarins	-	Interaction with eukaryotic DNA
8.	Lectins and Polypeptide	-	Form disulphide bridges

However, such mechanisms are still unclear although some studies suggested that these compounds penetrate inside the cell where they interfere with cellular metabolism (Marino et.al.2001). The use of plant products or preparations for control of postharvest diseases caused by fungi has not received proper attention. Most of such studies, were preliminary but indicated the possible use of preparation of various plants in actual disease control(Ark and Thompson,1959;Dixit et.al.1983).

REFERENCES

[1]. Indian medicinal plant, P.C.Trivedi, 2009.Avishkar Publishers Distributors.pp.1-2
[2]. Sill, W.H.(1985). Plant protection. Kalyani Publishers, New Delhi, India.
[3]. Lee, S.H.,Chang,K.S.,Su M.S.,Huang Y.S. and Jang H.D.(2007). Effects of some Chinese medicinal plant extracts on different fungi.Food Control.18: 1547-1554.
[4]. Verstagui, A.; Verde, J.;Garcia, S.; Heredia, N.; Orandy, A and Rivas, C.(2008).Species of Agave with antimicrobial activity against selected pathogenic bacteria and fungi. World Journal of Microbiology and Biotechnology 24:1249-1252.
[5]. Santas,J.; Almanjano,M.P.; and Carbo, R.(2010). Antimicrobial and antioxidant activity of crude Onion(Allium cepa L.) extracts. International Journal of Food Science and Technology 45, 403-409.
[6]. Lattanzio,V.(2003). Bioactive polyphenols:Their role in quality and storability of fruits and vegetables. Journal of Applied Botany;77,128-146.
[7]. Schena, L., Nigro, F.and Ippolito,A.(2008). Natural antimicrobial to improve storage and shelf life of fresh fruit, vegetables and cut flowers. In: Microbial biotechnology in Horticulture, vol.2.Science Publishers, Enfield, NH, Ray, R.C. and Ward,O.P.(Eds),USA,pp.259-303.
[8]. Amadioha, A.C. and Obi, V.I.(1999). Control of Anthracnose disease of Cowpea by *Cymbapogon cunitus* and *Ocimum gratissium* Acto phytopathology and Entomology 85:89.
[9]. Amadioha, A.C.(2000).Fungitoxic effects of some leaf extracts against Rhizopus oryzae causing tuber rot of Potato.Archieves of phytopathol, Planzo 34:19
[10]. Okigbo, R.N.(2009). Variation in phytochemical properties of selected fungicidal aqueous extracts of some plant leaves in Kogi State, Nigeria.American-Eurasian Journal of Sustainable agriculture 3(3): 407-409.
[11]. Zhang,H. and X.Zhang(2005). Biological control of postharvest blue mold of oranges by Cryptocroccus laurentii(Kufferath) Skinner. Biocontrol 50: 331-342.
[12]. Meshram A., Bhagyant S.S., Gautam S., Shrivastava N. Potential role of *Tinospora cordifolia* in pharmaceutical sciences.2013;2(6):4615-4625.
[13]. Singh S.S., Pandey S.C., Srivastava S., Gupta V.S, Patro B., Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian Journal of Pharmacology.2003; 35:83-91.
[14]. Sinha K, Mishra N. P., Singh J., Khanuja SPS. Tinospora cordifolia(Guduchi), a reservoir plant for theraupetic applications: A review. Indian Journal of Traditional knowledge. 2004;3:257-70.

IJARSCT
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International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
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[15]. Soni H.P., Nayak G, Patel S.S., Mishra K, Singh R.P. Pharmacognostic studies of the leaves of *Tinospora cordifolia*.IJPI Journal of Pharmacognosy and Herbal formulations.2011; 1:1-6.

[16]. Promilla, Sushila Singh and Parvesh Devi 2017.Pharmacological Potential of *Tinospora cordifolia* (Wild). Miers ex.hook and Thomas(Giloy): A review. Z.Journal of Pharmacognosy and Phytochemistry.6(6): 1644-1647.

[17]. Jyoti Rani, Lalita Singh, Hoshiyar Singh, Manish Kapoor and Gangadeep Singh. Preliminary phytochemical analysis of different solvent extracts from Leaf and stem of *Tinospora cordifolia*. International Journal of Phytotherapy.2015.Vol.5 (3) 124-128.

[18]. M.F.A. E.I-Matti, S.A.Mahgoub, S.M. Labib, A.M.A.Al.Gaby, and M.F. Ramadan Phenolic extracts of Clove(*Syzigium aromaticum*) with novel antioxidant and antibacterial activities. European Journal of Integrative medicine, vol.8 no.4 pp.494-504,2016.

[19]. A.M.A. Assiri, K.Elbanna, A. AI-Thubiani, and M.F.Ramadan,.Cold pressed Oregano(*Origanum vulgare*) oil: a rich source of bioactive lipids with novel antioxidant and antimicrobial properties. European food Research and Technology, vol.242,no.7,pp.1013-1023,2016.

[20]. M.M.Cowan, Plant Products as antimicrobial agents, In Clinical Microbiology Reviews, vol.12, no.4,pp.564-582, American Society of Microbiology(ASM).1999.

[21]. A.M.M.Shomeet, A.M.I.E.I.-Samadisy, R.M.A. E.I. Kholy and I.S. Ibrahim, Effect of fungicides, antioxidants and their mixtures on mycelial growth of some fungi. Middle East Journal of Applied Sciences, vol.8 no.2 pp.645-655, 2018.

[22]. G.Simonetti, A.Villa, and N.Simonetti, Enhanced contact activity of Flucanzole in association with antioxidants against fluconazole-resistant organisms. Journal of Antimicrobial chemotherapy, vol.50.no.2, pp257-259,2002.

[23]. Eimad Dine Tariq Bouhlall, Mgal Derouich, Reda Meziani and Adil Essarioui. Antifungal potential of phytochemicals against Mauginella scaettae, the plant pathogen causing inflorescence rot of Datepalm. Scientificia, vol.2021.Article. ID 1896015, 8 pages 2021.Research Article/ Open access vol.2021/ Article ID1896015. https://doi.org/10.1155/2021/1896015.

[24]. Hasan A., Farman M.,Ahmed I.Flavanoid glycosides from Indigofera hebepetaela.Phytochemisrry 1994;33:275-276.

[25]. Krishna K.T.,Ranjini C.E.,Sasidharan V.K.Antibacterial and antifungal activity of secondary metabolites from some medicinal and other common plant species.J. Life Sciences 1997;2:14-19.

[26]. Singh I.and Singh V.P. Antifungal properties of aqueous and organic solution extracts of seed plants against *Aspergillus flavus* and *Aspergillus niger* , Phytomorphology.2000;50:151-157.

[27]. Natrajan E.,Senthilkumar S.,Xavier F.T.,Kalaiselvi V.Antibacterial activities of leaf extracts of *Alangium salvifelium* J.Trop. Med.Plants 2003;4:9-13.

[28]. Natrajan D.,Brito J.S.,Srinivasan K.,Nagmurgan N.,Mohansundari C., Perumal G. Antibacterial activity of *Euphorbia fusiformis* a rare medicinal herb.J.Ethnopharmacol, 2005; 102:123-126.

[29]. De Boer HJ, Kool A., Broberg A., Mziray W.R.,Hedberg I.,Levenfors J.J.Antifungal and antibacterial activity of some herbal remedies from Tanzania.J.Ethnopharmacol, 2005;96-461-469.

[30]. Chang S.T.,Chen P.F., Chang S.C. Antibacterial activity of leaf essential oils and their constituents from *Cinnamon osmopholeum* J.Ethnopharmacol.2001;77:123-127.

[31]. Marino M., Bersani C.,Comi G.Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae.2001.Int.J.Food.Microbiol.67:187-195.

[32]. Ark, P.A. and Thompson, J.P.(1959). Antibiotic properties of seeds of wheat and Barley.Plant disease Repotr.43:276-282.

[33]. Dixit,S., Dubey, N.K., Tripathi, N.N.and Dixit,S.N(1983).Cedrus Oil- promising storage fungitoxicant. Journ.Stored Prod.Res.19:159-162.

[34]. Gurjar, M.S., Shahid Ali, Masood Akhtar, Kangabam S.S.2012.Scientific Research publishing. Visited at Google Scholar.