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Phytochemical Screening and Study of Antioxidant, Antibacterial, Antifungal activities for the ethanolic leaf extract of Tinospora Cordifolia

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Abstract

Natural products with medicinal importance are slowly gaining prominent objective in clinical research due to their well-known assets of no side effects as compared to drugs. Tinospora cordifolia (Tc) most commonly named as “Guduchi” is known for its huge application in healing of a variety of diseases in the traditional medicine literature. Now a day, the finding active organic components from the medicinal plants and their biological functions in infectious diseases control has been led to an active interest in the plants across the World. Our current study in this work was initially, collected the plant at historical campus Andhra University, Visakhapatnam City surroundings (Located in the region Eastern Ghats of Andhra Pradesh, India). The Herbarium has been prepared and authenticated. The shade dried leafs were being powdered and extracted with the ethanol using Soxhlet apparatus. The crude extract has been tested for GC-MS, FT-IR analysis and then tested for the detection of active components. Later, the ethanolic leaf extract has been tested for its Phytochemical, antioxidant, antimicrobial and antifungal properties. The crude extract had showed potential and antioxidant and antimicrobial properties.

Key words : Tinospora Cordifolia, Organic solvent, Ethanolic leaf extract, Phytochemicals, Antioxidant activity, Antimicrobial activity, Antifungal activity.

1. Introduction:

Natural products have been traditionally used as oral medicine or applied on skin for many diseases in the humanbeings for past centuries together. The helpful medicinal effects of plant products classically result from the combinations of plant synthesized secondary metabolites. The most significance of these bioactive constituents is alkaloids, phenolics, flavonoids and tannins[1]. Since ancient times, plant extracts have been recognized for their remarkable biological activity, which include antibacterial, antioxidant, and anticancer effects. The general consensus is that they have mild side effects. Globally, infectious diseases are the primary cause of mortality. The need for new bioactive chemicals has arisen from the infections' growing resistance to antibiotics and the unfavorable effects of some antimicrobial medicines.[2] Medicinal herbs are becoming more and more popular as a natural substitute for manufactured medications. Severe infections are brought on by a number of enterobacteriaceae members. The antibacterial efficacy of essential oils and crude plant extracts against food-borne pathogens and the etiological agents of infectious illnesses has been the subject of numerous articles reported in recent years.[2-4] Overproduction of free radicals in the body causes oxidative stress, a condition that has detrimental effects on human health. It activates procarcinogens, oxidative deteriorates lipids, proteins, and DNA, inhibits cellular and antioxidant defense systems, alters gene expression, and plays a major role in the development of human disease.[3] Antioxidants are always needed to maintain an adequate amount of oxidants in order to balance the reactive oxygen species (ROS) in the human body. They have been demonstrated to prevent oxidative damage caused by free radicals. Because phytochemicals can shield against damage caused by reactive oxygen species (ROS), they may be used to prevent and treat illnesses.[4] Many plant products, including fruits, leaves, seeds, and oils, include natural antioxidants like flavonoids, curcuminoids, coumarins, xanthones, tannins, phenolics, and terpenoids[5]

Herbaceous vine Tinospora cordifolia (Menispermaceae) is native to tropical regions of India, Myanmar, Bangladesh, and Srilanka. It is often referred by several colloquial names, including giloy, guduchi, amrita, and so on (It is locally called as Tippa Teega in the Indian state of Andhra Pradesh). Indigenous medical systems make extensive use of it.[6,7] The herb is well-known for its hepatoprotective, hypoglycemic, hypolipidemic, antipyretic, antispasmodic, and antineoplastic qualities.[8] Furthermore, it is utilized to treat anemia, diarrhea, urinary tract infections, gonorrhea, viral hepatitis, fever, loss of appetite, and

general debility in children.[9-10] The current study reveals the scientific validation of phytochemical study and therapeutic effectiveness as an antioxidant, antibacterial of T. cordifolia's ethanolic leaf extract.

2. Materials and Methods

The T. cordifolia leaves were shade-dried (Fig.1 A), crushed, and grinded into a fine powder (Fig.1 B), with a mechanical mixy Jaar. Subsequently, the powdered material was extracted with ethanol in Soxhlet apparatus as described earlier.[11,12] The respective extract fractions had centrifuged, filtered, and lyophilized. The dried residues were tested for the determination of antioxidant, antibacterial, antifungal properties.

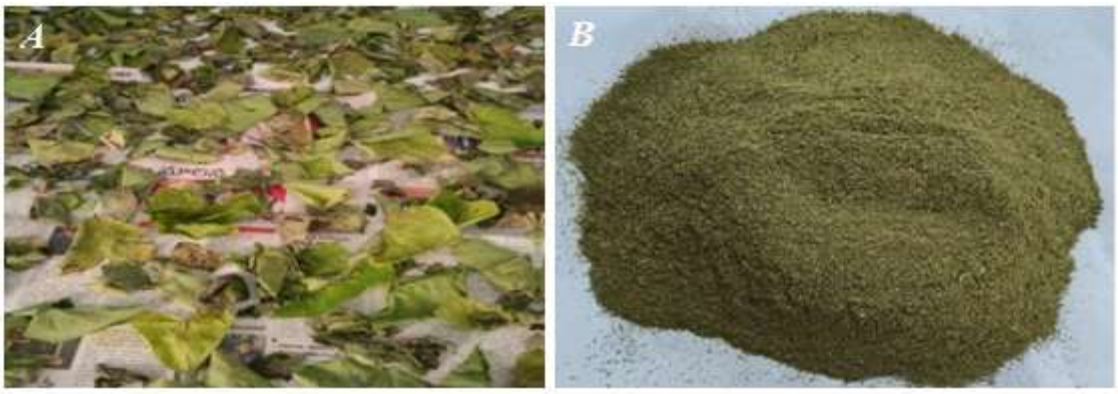

Fig.1. A) Shade dried leaves of Tc B) Leaf powder of the Tinospora cordifolia

2.1 Phytochemical Screening

Phytochemical screening of T.cordifolia leaf extracts was preformed for the qualitative determination of reducing sugars, phenolics, terpenoids, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, alkaloids using standard protocols[13,14]

2.2. UV-Visible spectral Technique. UV-Visible spectroscopy analysis for the samples was tested by diluting 1g of the extracted leaf powder with 15ml of the same solvent.[15] The extracts were scanned in the wavelength extending from 200-800 nm using (Shimadzu UV-1700 PC, Japan, Yokohama) and the individual peaks were observed

2.3 Determination of Total Phenolics

With a few adjustments, the standard protocols[16,17] were followed to determine the total phenolic content in the extract fractions. One of the modifications was dissolving the extracts

in DMSO rather than water. 3 mL of water were added to 0.2 mL of sample (2 mg / mL in DMSO). After adding a tiny amount (0.5 mL) of two-fold diluted FCR, the mixture was stirred. Following, 3 minute duration, 2 mL of a 20% sodium carbonate solution was added, and the tubes were then cooled after one minute in a boiling water bath. With a spectrophotometer, the absorbance was measured at 650 nm in relation to a blank for the reagent. The measure used to quantify the amount of phenols in the test samples was mg catechol equivalent per gram, or mg CE/g.

2.4 Microorganisms and Growth Conditions.

The current work pathogenic microorganisms came from the Clinical Microbiology Laboratory, Chandigarh, India. Among them was Pseudomonas aeruginosa and Escherichia coli, two Gram-negative bacteria. The bacterial culture on nutritional agar slants was kept at 4 ^{o}C.

2.5 Antibacterial study

In this study, the gram-negative *E. coli* and *Pseudomonas auregonas* was employed to examine the antibacterial activity of the ethanolic leaf extract of the *Tinospora cordifolia.* The choice of bacteria as the gram-negative bacteria for our antimicrobial study was based on its ready ease to use throughout work. The selected these bacteria were cultured in a Luria Bertani broth[18] (containing 1.5 % agar) at 37◦C with initial concentration of 1.5-5×10^{5} CFU/mL. Subsequent to overnight growth, 2 mL of *E. coli* was aliquoted into a separate container then incubated with the dispersion of 50µ/mL, 100 µ/mL concentration of leaf extract at 37◦C for 24 h. For comparison, a similarly prepared *E. coli* sample without any nanomaterial was used as the control.

2.6 Anti-oxidant Activity by DPPH procedure

Ethanolic leaf extract preparation of Tinospora cordifolia: The shade dried and then powdered leaf of Tinospora cordifolia was extracted with the organic solvent ethanol (35-40^{o}) with the help of the Soxhlet Apparatus set up on the heating mantle. Later, the solvent was evaporated with Rotavapor.

2.7 DPPH Assay:

DPPH free radical scavenging activity of the leaf extract of T**c** was estimated by using the stable free radical DPPH. An aliquot 1.00 mL of standard or extract solution at various concentrations was added to 1.00 mL of 0.10 mM DPPH solution in the solvent methanol and the spectrophotometer absorbance of mixture observed at 518 nm after 20 min of incubation. Ascorbic acid was used as positive control.

3. Results and Discussions

Powdered plant leaf material was subjected for extraction by Soxhlet extraction procedure, after the careful extraction process, pharmacognostical assessment of ethanolic extract was subjected to different phytochemical screenings for preliminary observation of a variety of phytoconstituents.[19] The leaf extract revealed that, Alkaloids, Carbohydrates, Phenols, Steroids, Starch, Tannins, and Glycocides (Table.1). The free radical scavenging potential of standard and extract analyzed by DPPH protocols[20] are depicted in with a characteristic absorption at 517nm. Leaf extract exhibited gradual increasing (Fig.2) percentage inhibition with rising concentration at 517 nm in spectrophotometer as an antioxidant by DPPH assay.[20] FTIR Analytical technique was used to determine available functional groups identification of organic molecules in the extract. It has been showed some significant peaks[21], among them, one sharp peak showing (C-H) aromatic ring with a peak value of 2930 cm^{-1}, Aromatic C=C at 1450 cm^{-1}, 1560 cm^{-1} followed by amines and amides (NH) with a peak value of 2100 cm^{-1} aldehyde and ketones (C = O) with a peak value at 1705-1713 and Amide functional group at 1632 cm^{-1}. The peak observed for the presence alkyl halides (C-H) with a peak value of 1120 and 1080-1100 for the phenolic compounds (Fig 3).

Table-1. Results of qualitative test for the observed functional groups of Tinospora cordifolia

S.No	**Functional groups**	**Ethanolic leaf extract of Tinospora cordifolia**
1	Alkaloids	+ve
2	Phenols	+ve
3	Flavonoids	-ve
4	Carbohydrates	+ve
5	Saponin	-ve
6	Glycosides	+ve
7	Starch	+ve

8	Tannin	+ve
9	Proteins	-ve
10	Sterol/Steroid	+ve
Note: +ve =present, -ve = absent		

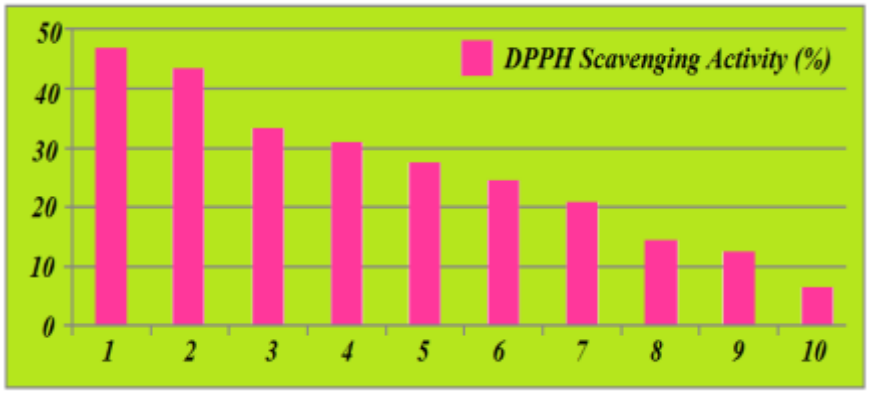

Figure-2. Antioxidant study of Tinospora cordifolia by DPPH assay

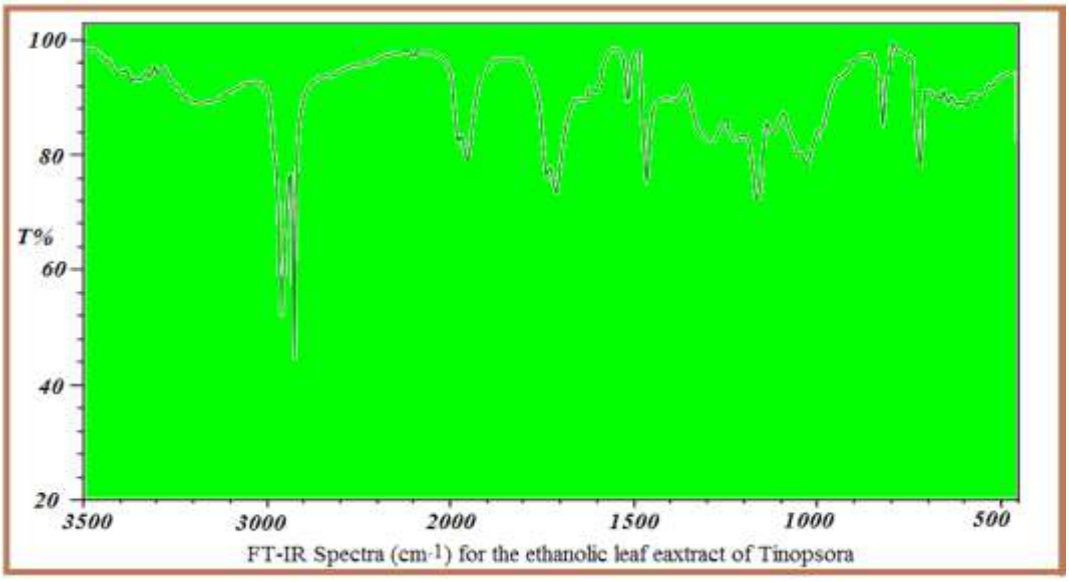

Figure 3. FT-IR Spectral Peaks for the ethanolic extract of Tinospora cordifolia

In the present study, effects of ethanolic extract of Tinospora cordifolia had been evaluated against gram positive (P.aeruginas) and gram-negative (E. coli) bacteria[1-10] where as antifungal activity[22] was obtained against Aspergilus Niger and Candida Albicans. Existed glycosides, phenols, tannins, alkaloids, and sterols, which may be further responsible for their antibacterial (Table.2, and Fig 4) and antifungal properties (Table.3), according to the phytochemical analysis for different functional groups in the sample.

Table-2. Antibacterial activity of Tinospora cordifolia

S.No	Name of the Bacteria	Tinospora cordifolia	
		50 µg / mL Inhibition rate	100 µg/mL Inhibition rate
1	*E.Coli*	60.65%	99.90%
2	*P.aeruginas*	61.00%	99.80%

$$\text{Inhibition rate } = \frac{(Control\ Concentration - Concentration\ of\ Bacteria)}{Control\ Concentration} x\ 100$$

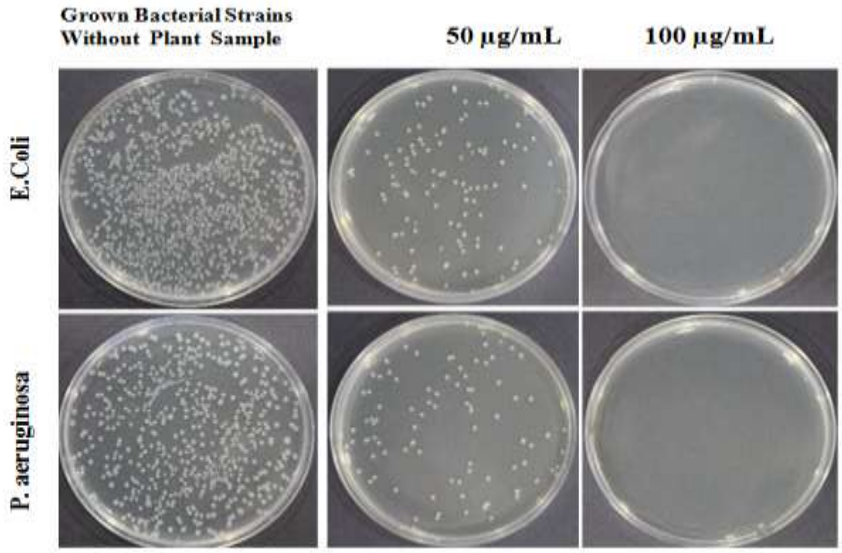

Figure. 4. Antimicrobial activity of the ethanolic leaf extract of Tc (Top row represents E.Coli and bottom row represents Pseudomonas aeruginosa**).**

The majority of reports[7-12] that are now available indicate that higher plants' antibacterial properties are caused by secondary metabolites like tannins, alkaloids, flavonoids, and other phenolic chemicals.[3-9, 22] Spices have been shown to possess antibacterial properties against respiratory tract infections due to the presence of monoterpenes, sesquiterpenes, alcohols, and

aldehyde. There have been reports of membrane integrity loss and proton motive force dissipation due to cyclic terpene chemicals.[23] Thus, the presence of certain of these phytochemicals in addition to phenolic compounds may partially account for the antibacterial activity seen in this investigation. T. cordifolia shown that its ethanolic leaf extract ability to suppress pathogenic test microorganisms (Table 1). Merely, one of the most frequent barriers to the healing process is wound infection. It is crucial that wound dressings have strong antibacterial qualities for this reason. Herein, the antibacterial activity of sponges was investigated in vitro against two bacterial strains, Gram-negative E. coli and P. aeruginosa, which causes most infectious diseases. As shown in Fig 4. the Col group displayed a 60.65 % survival ratio for P. aeruginosa and 61.00 % for E. coli, respectively for the ethanolic plant leaf extract samples of T.Cordifolia (Tc) respectively at the 50 µg/ml. Almost all P. aeruginosa (99.90 %) and E. coli (99.90 %) were completely inhibited or killed by the (TC) ethanolic plant extracts. These results suggested that the plant extracts had a robust antibacterial activity.

Table-3. Antifungal activity of Tinospora cordifolia

Sample	**Inhibition rate**	
Ethanolic Leaf extract of Tc (Concentration)	***A. Niger***	***C. Albicans***
Chloramphenicol (30 mg)	93.30 %	92.01 %
Control 10 µg/ml	-	-
20 µg/ml	30.01 %	27.01 %
30 µg/ml	45.35 %	43.27 %
50 µg/ml	78.15 %	76.30 %
100 µg/ml	91.80 %	89.98 %

The identification of unknown components in GC-MS is crucial for determining the provenance of plants. A (GC-MS) analysis was performed on a crude ethanol (2 µl) extract that contained various Tinospora cordifolia components (Table.4). Instruments and chromatographic circumstances GC-MS examination was carried out on a GC-500 Perkin Elmer, Japan system containing a AXOC-30i auto analyst and gas chromatograph interfaced to a mass spectrometer (GCMS) instrument retaining the subsequent conditions; column

Elite-1 attached silica capillary column (40 ×0.25 mm × ID x 1µm of capillary column, composed of (100% DP siloxane), operational in electron impact mode at 70 eV; helium (99.99%) was used as transporter gas at a persistent flow of 1ml / minute, temperature 200-250°C; ion-source temperature 280°C. The oven temperature fixed at 120°C (isothermal for 3 min), with an increase of 10°C/minutes, to 250 °C/minutes, then 5°C/minutes to 280°C/min, finish with a 8 minutes isothermal at 280°C. Mass spectra were occupied at 70 eV; a scan intermission of 0.5 seconds and fragments from 45 to 450 Da. The name and molecular weight of the unknown constituent are determined by comparing its spectrum to those of the recognized constituents that are kept in the NIST library.

Table 4. Bioactive compounds identified in the Ethanolic crude extract T.Cordifolia Leaves in GC–MS [24-25]

Sr No	*R. time*	*Area/ Height(A / H)*	*Compound Name*	*Mol. formula*	*Mol. weight (g/mol)*	*Biological functions*
1	11.221	3.03	3-(Dimethylsiloxy)-3,3-dimethyl-1-propene	$C_5H_{13}OSi$	144	Anti-fouling
2	26.787	2.96	Phytol	$C_{20}H_{40}O$	296	Anti-inflammatory, Anticancer Antimicrobial, Diuretic
3	24.235	2.45	Flexricin P-4	$C_{21}H_{38}O_4$	354	Anti-cancer, Anti-fungal
4	8.255	5.95	Cyclohexene-d1	C_6H_9D	83	Anti-tumor
5	15.831	3.28	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone	$C_{13}H_{18}O_2$	206	Antihistamine
6	25.175	2.65	E-15-Heptadecenal	$C_{17}H_{32}O$	252	Anti-phytopathogenic, Anti-inflammatory
7	17.329	2.98	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)	C13H18O	190	Anti-oxidant

8			2-Vinylbicyclo [2.1.1] hexan-2-ol	$C_8H_{12}O$	124	No activity recorded
9	14.889	4.53	Alpha [5-Methyl-2,3,4,5-tetrahydro-2-furyl) glucine	$C_7H_{13}NO_3$	159	Urinary incontinence, helps to cure renalheart disorder, anti-inflammatory
10	15.982	4.68	1,3-Oxathiane, 5-isopropyl-2-methyl-	$C_8H_{16}OS$	160	Anti-proliferative, Food improvement agent
11	16.767	2.95	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	Antimicrobial
12	5.968	6.16	7-Methyl-2-oxepanone	$C_7H_{12}O_2$	128	Antimicrobial
13	8.710	3.02	N-Carbobenzoxy-DL-leucine	$C_{14}H_{19}NO_4$	265	Anticancer
14	11.967	6.21	5-Methoxypyrrolidin-2-one	$C_5H_9NO_2$	115	Cholesterol acyltransferase
15	8.800	5.11	2-Methoxy-5-methylthiophene	C_6H_8OS	128	Anti-bacterial lung infection
16	3.219	1.66	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118	Antidermatophytic, Antidiabetic
17	26.193	5.88	Octanoic anhydride	$C_{16}H_{30}O_3$	270	Anti-bacterial and Anti-fungal
18	5.382	5.09	Protoanemonin	$C_5H_4O_2$	96	Anti-fungal, Anti-cancerous, Flavor and fragrance agents
19	4.484	3.50	Furan, 2,5-dimethyl-	C_6H_8O	96	Biofuel, Scavenger for singlet oxygen

20	3.520	3.89	Ketocyclopentane	C_5H_8O	84	Antidiabetic
21	15.045	4.89	Syringol	$C_8H_{10}O_3$	154	Antimicrobial, Bacteriostat
22	24.782	2.76	Butyl 2-ethylhexyl phthalate	$C_{20}H_{30}O_4$	334	Antifungal, Medical tubing, Used in blood storage bags
23	7.917	4.30	4-Hydroxybenzenesulfonic acid	$C_6H_6O_4S$	174	Dental antiseptics, Fungal degradation
24	14.613	10.61	hydroquinone	$C_6H_6O_2$	110	Dermatologic agent, Antibacterial, Anti-cancer, Anti- influenza
25	20.959	29.85	Kinic acid	$C_7H_{12}O_6$	192	Neuropharmacological agent
26	9.712	7.32	Guaiacol	$C_7H_8O_2$	124	Bone repair, Anti-bacterial, Antifungal activity
27	14.380	3.86	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	Anti-proliferative

4. Conclusions

In summary, the current study explored that the presence of numerous functional groups of phytochemicals in *T. cordifolia ethanolic leaf* extract were accountable to exhibiting considerable antioxidant, antimicrobial activities. At this moment, it is tricky to say which component(s) of the Tc ethanolic leaf extract are most effective for the above determined activities. Nevertheless, further phytochemical studies are essential to isolate the active compound (s) accountable for these pharmacological activities. Additional investigations are needed for the evaluation of the absolute reasons behind the exhibited potential activities such as Antioxidant and Antibacterial properties.

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5. References

1. Bellini, M. F., Angeli, J. P. F., Matuo, R., Terezan, A. P., Ribeiro, L. R and Mantovani, M. S. (2006). Antigenotoxicity of Agaricus blazei mushroom organic and aqueous extracts in chromosomal aberration and cyto kinesis block micronucleus assays in CHOk1 and HTCcells. Toxicol in Vitro. 20(3), 355–360.
2. Rohit, S., Prajapati, P.K. (2016). Screening of Antimicrobial activity of Guduchi Ghana (dried aqueous extract of Tinospora cordifolia (Willd.) Miers). Journal of Ayurvedic and Herbal Medicine. 2(6), 209-212.
3. Pandey, A.K. (2007). Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed Pariheniumhisterophorus: an in vitro study," National Academy Science Letters. 30 (11-12), 383–386.
4. Kumar, S., Sharma, U.K., Sharma, A.K. and Pandey, A.K. (2012). Protective efficacy of *Solanum xanthocarpum* root extracts against free radical damage: phytochemical analysis and antioxidant effect. *Cellular and Molecular Biology*. 58 (1), 171–178.
5. Umretia, B., Vaishnav, P., Patgiri, B., Shukla, V. (2013). Immunomodulatory activity of Guduchi Ghana (Aqueous Extract of Tinospora cordifolia Miers). *NJIRM.* 4(3), 90-6.
6. Sowjanya, K., Vaishnavi, V., Sandhya V., Uppuluri, S. (2019). The multi-activity herbaceous vine - Tinospora cordifolia. Asian J. Pharmaceut and Clinical Res. 12 (3). 23-26.
7. Jagetia, G. C., Rao, S.K. (2006). Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifolia*) in Ehrlich ascites carcinoma bearing mice," *Biological and Pharmaceutical Bulletin*. 29 (3) 460–466.
8. Deepika, S., Prabir, K.Ch.(2016). Chemistry and Pharmacology of Tinospora cordifolia. Natural Product Communication. 12 (2), 299 – 308.
9. Adhvaryu, M. R., Reddy, N., Parabia, M.H. Effects of four Indian medicinal herbs on Isoniazid-, Rifampicin- and Pyrazinamide-induced hepatic injury and immunesuppression in guinea pigs. (2007). *World Journal of Gastroenterology*. 13 (23), 3199–3205.
10. S. S. Singh., Pandey, S.C, Srivastava, S., Gupta, V.S., Patro, B. And Ghosh, 2003 A.C. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi)," *Indian Journal of Pharmacology*. (35) 2, 83–91.
11. Trease, E. A., Evans, B.(1983).Medicine and Plants, Pitman, London, UK, 3rd edition.
12. Mishra, A.K., Mishra, A., Kehri, H.K., Sharma, B. Sharma and Pandey, A.K.(2009). Inhibitory activity of Indian spice plant Cinnamomum zeylanicum extracts against

Alternaria solani and Curvularia lunata, the pathogenic dematiaceousmoulds. Annals of Clinical Microbiology and Antimicrobials, 8 (9).

13. Anu, Ch., Rina, Das., Kiran M., Dinesh Kumar, M.(2024). Indian herb Tinospora cordifolia and Tinospora species: Phytochemical and therapeutic application. Heliyon. 10., e31229.
14. Papitha, R., Lokesh, R., Kaviyarasi, R., Selvaraj, C. (2016). Phytochemical Screening, FT-IR and Gas Chromatography Mass Spectrometry Analysis of Tinospora cordifolia (Thunb.) Miers. International Journal of Pharmacognosy and Phytochemical Research. 8(12), 2020-2024.
15. Sowmya, M., Ramesh, S., Ganne V.S.R., Ramesh, S., Jalantha, P., Sujatha, P. L., Indumathi, R. (2024). Phytochemical Analysis of Tinospora Cordifolia by Gc-Ms and Evaluation of its Antiurolithiatic Potential by In Siilico. Indian Vet. J. 101 (4) , 39 – 48.
16. F. Khan., Arvind, S.J., Poonam, B. (2022). Investigation of Antimicrobial and Antioxidant Potential of Tinospora cordifolia by In-vitro Methods. Journal of Drug Delivery & Therapeutics.12(6-S), 36-41.
17. Said, R., Jadhav, S. L., Kamble, S.C. (2023). Tinospora cordifolia a Medicinal plant with many roles: A Review. Res. J. Pharmacognosy and Phytochem. 15(1), 87-90.
18. Manuri, B., Dong-Chiao, Y, Yu-Chun F., Si-Yu, L, Shing-Yi, S. (2023). A novel graphene oxide-1,3,4-oxadiazole-2,5-diamine nanomaterial with enhanced bactericide activity. Journal of the Taiwan Institute of Chemical Engineers. 153, 105208.
19. Preety, D., Shiv Kumar, J., and Nalini, S. (2020). Preliminary phytochemical screening, FTIR and GC-MS analyses of aqueous, ethanolic and methanolic extracts of stem of Tinospora cordifolia (Willd.) Miers for search of antidiabetic compounds. Annals of Phytomedicine 9(2): 183-197.
20. Ilaiyaraja, N., F, Khanum. (2011).Antioxidant Potential of Tinospora cordifolia Extracts and their Protective Effect on Oxidation of Biomolecules. Pharmacognosy Journal. 3 (20), 56-62.
21. Papitha, R., Lokesh, R., Kaviyarasi, R., Selvaraj, C. (2016) Phytochemical Screening, FT-IR and Gas Chromatography Mass Spectrometry Analysis of Tinospora cordifolia (Thunb.) Miers. International Journal of Pharmacognosy and Phytochemical Research. 8(12), 2020-2024.

22. Patil, R.C., Kulkarni, C.P., and Ashu, P. (2017). Antifungal and phytochemical properties of Tinospora cordifolia, Azadirachta indica and Ocimum sanctum leaves extract. Journal of Medicinal Plants Studies. 5(5),23-26.
23. Mishra, A., Kumar, S., Bhargava, A., Sharma, B., Pandey, A.K.(2011). Studies on in vitro antioxidant and antistaphylococcal activities of some important medicinal plants, Cellular and Molecular Biology, 57 (1), 16–25.
24. Anuradha, S. G., Anamika, S., Kapila, K. (2020). Analysis of therapeutic value of Tinospora cordifolia. Asia Pacific Journal of Multidisciplinary Research, 8 (1), 27-41.
25. Gaurav et al. (2022). GC–MS Metabolomics and Network Pharmacology-Based Investigation of Molecular Mechanism of Identified Metabolites from Tinospora cordifolia (Willd.) Miers for the Treatment of Kidney Diseases. Phcog Mag. 18, 548-58.