WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 17, 602-615. Research Article ISSN 2277–7105

EVALUATION OF *IN-VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *TINOSPORA CORDIFOLIA*

G. Babu[1]*, Ch. Srinivas Reddy[2], R. Sai Sindhu[1] and Dr. M. Chinna Eswaraiah[3]

[1]Pharmaceutical Chemistry, [2]Pharmaceutics, [3]Pharmacognosy, Anurag Pharmacy College, Ananthagiri, Kodad, Suryapet, Telangana.

Article Received on
31 October 2017,
Revised on 21 Nov. 2017,
Accepted on 11 Dec. 2017
DOI: 10.20959/wjpr201717-10290

***Corresponding Author**
G. Babu
Pharmaceutical Chemistry,
Anurag Pharmacy College,
Ananthagiri, Kodad,
Suryapet, Telangana.

ABSTRACT

Tinospora cordifolia is a popular medicinal plant known to both the Ayurveda and Chinese traditional medicines since ancient time. The aim of this research was to study the qualitative phytochemistry and to determine the antioxidant and anti-inflammatory activities of Tinospora cardifolia. Different solvent extracts of the crude drug were tested for their in vitro antioxidant activity by using Griess reagent, to know the Nitric oxide scavenging activity were determined by spectrophotometric method. Methanol extracts of the total plant and stem were evaluated for anti-oxidant and anit-inflammatory activity, antioxidant potential as compared with standard, vitamin C and Diclofenac. Methanol extract also exhibited potent anti-inflammatory activity. anti-oxidant and anit-inflammatory activity is reported for the first time in this paper with Methanol extract.

KEYWORDS: *Tinospora cordifolia*, Soxhlet's apparatus, antioxidant, Nitric Oxide scavenging activity, Griess reagent, Methanol extract, Hexane extract, anti-inflammatory activity.

INTRODUCTION

A herbal is a book containing the names and descriptions of plants, usually with information on their medicinal, tonic, culinary, toxic, hallucinatory, aromatic, or magical powers, and the legends associated with them. A herbal may also classify the plants it describes, may give recipes for herbal extracts, tinctures, or potions, and sometimes include mineral and animal

medicaments in addition to those obtained from plants. Herbals were often illustrated to assist plant identification.

Herbals were among the first literature produced in Ancient Egypt, China, India and Europeas the medical wisdom of the day accumulated by herbalists, apothecaries and physicians. Herbals were also among the first books to be printed in both China and Europe. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation and natural chemicals found in foods and body tissue which are said to have beneficial health effects.

To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or the dietary antioxidants, vitamin A, vitamin C and vitamin E.

Antioxidant dietary supplements do not improve health nor are they effective in preventing diseases as shown by randomized clinical trials including supplements of beta-carotene, vitamin A and vitamin E singly or in different combinations having no effect on mortality rate[1][2] or cancer risk. Supplementation with selenium or vitamin E does not reduce the risk of cardiovascular disease. Oxidative stress can be considered as either a cause or consequence of some diseases, an area of research stimulating drug development for antioxidant compounds for use as potential therapies.

Industrial antioxidants have diverse uses, such as food and cosmetics preservatives and inhibitors of rubber or gasoline deterioration.

Inflammation

The word inflammation comes from the Latin *"inflammo"*, meaning *"I set alight, I ignite"*. Inflammation is part of the body's immune response. Initially, it is beneficial when, for example, your knee sustains a blow and tissues need care and protection. However, sometimes inflammation can cause further inflammation; it can become self-perpetuating. More inflammation is created in response to the existing Inflammation does not mean

infection, even when an infection causes inflammation. Infection is caused by a bacterium, virus or fungus, while inflammation is the body's response to it.

Examples of diseases and conditions with chronic inflammation include

- Asthma
- Chronic peptic ulcer
- Tuberculosis
- Rheumatoid arthritis
- Chronic periodontitis
- Ulcerative colitis and Crohn's disease
- Chronic sinusitis
- Chronic active hepatitis (there are many more).

Mechanism of action

The main action of NSAID'S is inhibition of arachidonate cyclooxygenase enzyme (COX). The COX is a bifunctional enzyme having two distinct activities. The main cyclooxygenase action gives PGG2 and peroxidase, which converts PGG2 to PGH2. Different NSAID'S inhibit the enzyme by different mechanisms but all act at the first of the two sites. COX1 is present in stomach, kidneys and blood vessels whereas COX2 is seen in activated leucocytes and other inflammatory cells.

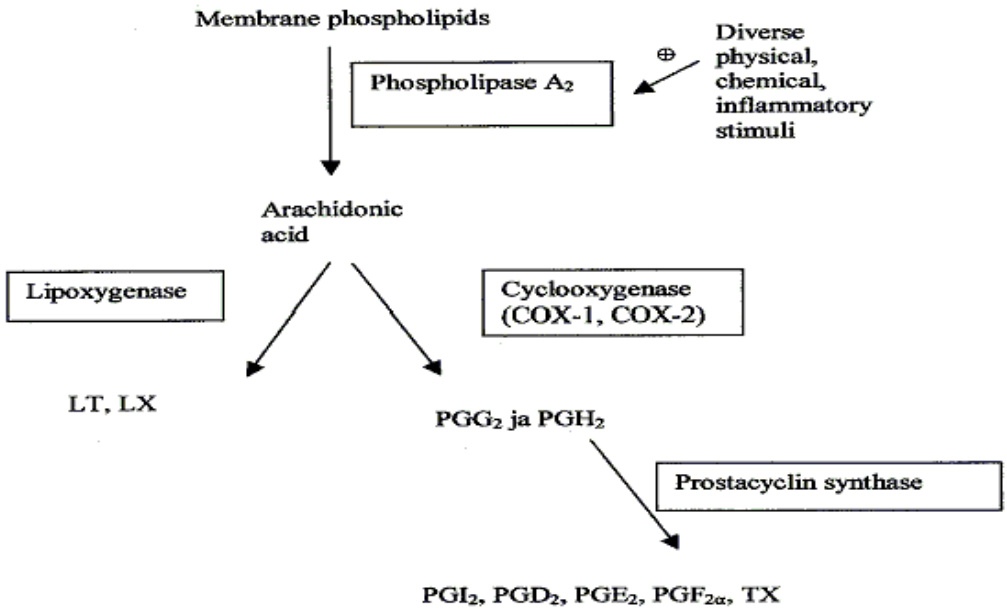

Figure 1: Mechanism of action.

As per our knowledge antioxidant potential and anti-inflammatory activity of *Tinospora cardifolia* whole plant and stem are reported for the first time in this paper.

LITERATURE REVIEW

Plant Profile

***Tinospora cordifolia*: Guduchi.**

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Ranunculales
Family:	Menispermaceae
Genus:	Tinospora
Species:	T. Cordifolia

Fig 2: Leaves and Fruits of *Tinosora cCordifolia.*

PHARMACOLOGICAL ACTIVITIES

Anticonvulsant activity

The anticonvulsant activity was accessed by determining and comparing the test group with that of the standard drug treated group. The petroleum ether extract having the % inhibition of extension phase is 35.3% and the ethanolic extract having % inhibition phase of 61.1%. The ethanolic extract treated animals was found to be significantly good activity compared to standard drug treated cases (Murthy et al., 2012).

Murthy JR, Meera R, Venkataraman S, Satpute BT, Chidambaranathan N, Devi P. Phytochemical investigation and anticonvulsant activity of leaves of *Tinosporacordifolia* Miers. international journal of pharmaceutical sciences, 2010; 2: 522-527.

Anti-oxidant activity

Tinosporacordifolia stem extract has shown to produce immunological activity because of the presence of arabinogalactan. Probably all thesesecondary metabolites from the three plants contribute to provide a synergistic effect and greater inhibition for the microbes under investigation. It was further noticed that those extract combinations with *T. cordifolia* showed better inhibition and susceptibility to various pathogens. This study supports the traditional use of *T. cordifolia* and indicated that it contains some major bioactive compounds inhibiting the growth of microorganisms there by proving very effective source of derived drugs (Debnath et al., 2014).[2]

Debnath M, Khandelwal M, Lal P, Jain R. Evaluation of Heavy Metal Distribution and Antibacterial Activities of Medicinal Plants Tinosporacordifolia, Ocimum sanctum and Piper Nigrum International Journal of Pharmaceutical Sciences and Drug Research, 2014; 6: 229-234.

Immunomodulatory activity

T. cordifolia extract in human immuno-deficiency virus positive patients. For this, they assessed the efficacy of *T. cordifolia* extract (TCE) in HIV positive patients in randomized double blind placebo controlled trial. After clinical examination TLC, DLC, ESR, platelet count, hemoglobin and CD4 count were done and the results showed significant reduction in eosinophil due to that TCE treatment (Mathew et al., 1999).[10]

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Immunostimulatory activity

The immunostimulatory effect of leaf extract of *T. cordifolia*on(a) specificimmunity (antibody response), (b) non-specific immunity (neutrophil activity) and (c) disease resistance against Aeromonashydrophilain O. mossambicususing ethanol and petroleum ether extracts of the leaves. They observed that the fish injected with both the extractat a dose of 8 mg/kg were protected against experimental infection with virulent A. hydrophilaand concluded that

the potentiality of *T. cordifolia*leaf extracts for use as an immunoprophylactic to prevent diseases in finfish aquaculture.

Sudhakaran DS, Srirekha P, Devasree LD, Michael RD. Immunostimulatory effects of *Tinosporacordifolia* Miers leaf extracts in Oreochromismossambicus. Indian Journal of Experimental biology, 2006; 44: 726-732.

Anti-cancer/Anti-tumor Activity

T. cordifolia extracts (TCE) in vitro inhibited cell proliferation and induced celldeath in a dose-dependent (25-75µg/ml) and time dependent (24-120 hours) manner in oral squamous cell carcinoma cell line along with a significantcytostaticeffect. Hence; it may have therapeutic potential in cancer. Differentiation and antitumor functions of tumor-associated macrophages (TAM) derived dendritic cells (DC) obtained romtumor-bearing host administered with alcoholic extract of *T. cordifolia* (ALTC). Their study indicates that the *T. cordifolia* can influence the myeloid differentiation of bone marrow progenitor cells and the recruitment of macrophages in response to tumor growth in situ (Mishra, 2015).

Mishra R, Kaur G.*Tinosporacordifolia*induces differentiation & senescence pathways in neuroblastoma cells. Molecular Neurobiology, 2015; 52: 719-33.

Cognition (Learning and Memory) Activity

T. cordifolia extract effects on learning and memory in normal and cyclosporine induced memory deficit rats. Alcoholic and aqueous extracts of the wholeplant of *T. cordifolia* was administered orally for 15 days in two groups of rats. Both alcoholic and aqueous extracts of TC produced a decrease in learning scores in Hebb William maze and retention memory indicating enhancement of learning and memory.

Anti-inflammatory and Wound Healing Activity

The dried stem of *T. Cordifolia* produced significant anti-inflammatory effect in both acute and sub acute models of inflammation. *T. cordifolia* has been found to be more effective than acetylsalicylic acid in acute inflammation but insub acute inflammation, the drug is inferior to phenylbutazone.

Patgiri B, Umretia BL, Prajapati PK, Shukla VJ. Anti-inflammatory activity of aqueous extract of *Tinospora cordifolia*. Ayurveda, 2014; 35: 108-10.

Anti-bacterial activity

The methanolic extracts of in vitro grown plants and callus showed a broadspectrum of activity against all the bacterial strains at the testedconcentration of 10 –**20µg/disc for** Staphylococcus aureus, S. typhi (8mm) (Madhu et al).

Cardio-protective

Ethanolic extract of *T. cordifolia* at various dose levels showed dose dependentreduction in infarct size and in lipid peroxide levels of serum and hearttissue80. The cardioprotective activity of an herbal formulation "Caps HT2", which contains methanol extract of *T. cordifolia*, has antioxidant, anticoagulant, platelet antiaggregatory, lipoprotein lipase releasing, anti inflammatory and hypolipidaemic activity in rat (Mukeshwar et a., 2012).

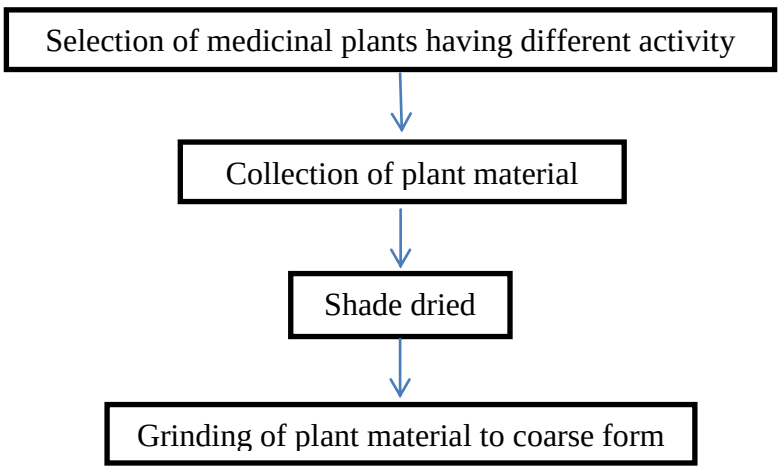
Pandey Mukeshwar, Chikara SK, Vyas MK, Sharma R, Thakur GS, Bisen PS. *Tinosporacordifolia*: a climbing shrub in health care management Int J Pharm Bio Sci, 202; 3: 612-628.

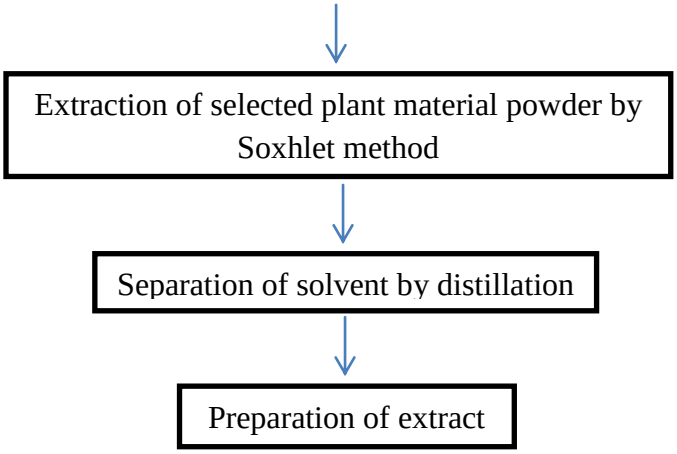
Anti-malaria (HMS) Activity

The effect of aqueous extract of *T. cordifolia* along with chloroquine in thetreatment of three cases of hyper-reactive malarious splenomegaly (HMS) was studied. Aqueous extract of *T.cordifolia* (500mg) added to CQ base (300mg) weekly and CQ prophylaxis including spleen enlargement, Hb, serum IgM and well-being have been observed up to six months. The 10results showed regression of spleen by 37-50% after six weeks and 45-69% after six months (Baskar et al., 2009).

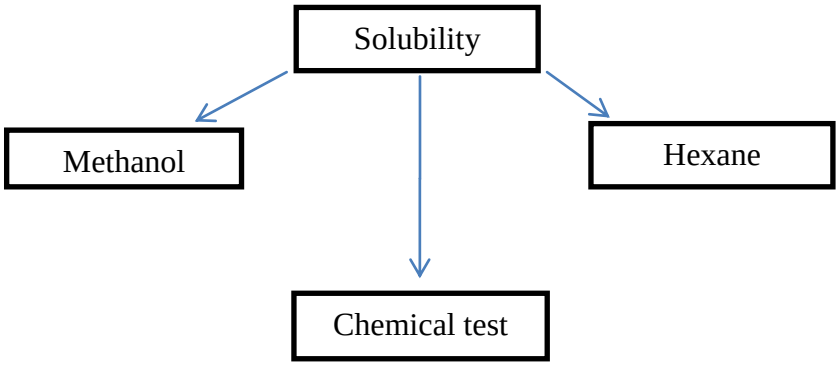
PLAN OF WORK

Phase-I: Preparation of extract.





Phase-II: Phytochemical evaluation



Phase-III: In Vitro Activities.

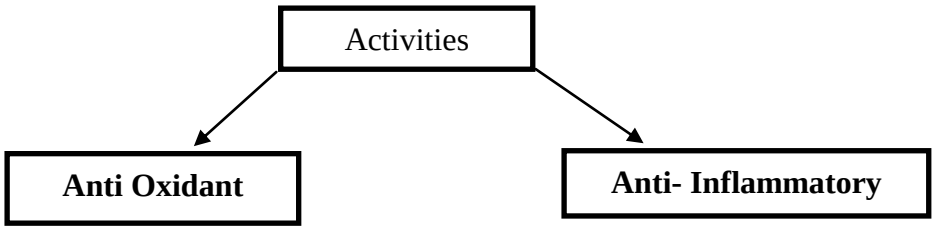


Fig 3: Schematic representation of the research methodology.

MATERIALS AND METHODS

Collection of Plant Materials

The plants *Tinosporacordifolia* of the family Menispemaceae were obtained from kondapally area surroundings, Telangana, India. The Authenticity of the plant species was confirmed by Professor Ch. Chinna Eswaraiah, Ph.D. Director of Anurag Pharmacy College, Telangana. The plant leaves were dried and ground to sawdust form, which was then kept in air-tight brown bottle until use.

Extraction Procedure

Ethanol, Methanol, Bovine serum albumin (5%w/v aqueous solution), Diclofenac sodium, 1N Hydrochloricacid and Phosphate buffer. Preparation of plant extracts. The dried leaves powder of *Tinosporacordifolia* L was extracted with methanol, ethanol and hexane. The extracts were filtered while the residue was further Abstract *Tinosporacordifolia* L. belongs to the family Menispemaceae found in southern region of India, which is used in the treatment of variety of illnesses, such as malaria, asthma, hepatitis, dermatitis and rheumatism. The qualitative phyto-chemical screening showed the presence of steroids, flavonoids and alkaloids. The Ethanol and Hexane fractions of the leaves of *Tinosporacordifolia* L. were subjected to In vitro Anti-inflammatory activity by HRBC membrane stabilization method in various concentrations i.e. 62.5, 125µg/ml. All the extracts showed positive response as compared to standard Diclofenac sodium. The Ethanol extract showed maximum activity. The order of effect of different extracts were represented as follows Methanol >Hexane.

Extracted under the same conditions twice. Both extracts were evaporated under reduced pressure. The percentage yield of ethanol and hexane were 94.2% and 92.1% respectively. Preliminary phytochemical screening A portion of residue from each extract was subjected for phytochemical analysis in order to see the presence of alkaloids, steroids and flavonoids.

Method of Extraction

Continuous hot percolation process by using Soxhlet apparatus.

Materials

1. Soxhlet apparatus
2. Methanol
4. Hexane
5. Shade dried coarse powder of *Tinospora cordifolia*.

Methanolic Extract

The shade dried coarsely powdered of *Tinosporacordifolia* (100gms) was extracted with methanol (60-70°C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Greenish brown coloured residue was obtained. The residue was then stored in desiccator.

Hexane Extract

The shade dried coarsely powdered of *Tinospora cordifolia* (100gms) was extracted with hexane (50-60°C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Greenish brown coloured residue was obtained. The residue was then stored in desiccator.

RESULTS OF PHYTOCHEMICAL TESTS

The results of Phytochemical tests are mentioned below.

Table 1: Qualitative chemical examination of extracts.

Sl.No	Chemical constituents	Tests	Hexane extract	Methanol extract
1	Alkaloida	Mayer's test	+	+
2	Carbohydrates	Molish test	+	+
3	Glycosides	Legal's test	+	+
4	Saponins	Froth test	_	_
5	Phytosterol	Salkowski test	+	+
6	Phenols	Ferric chloride	+	+
7	Fixed oils	Stain test	+	+
8	Tannins	Ferric chloride	+	+
9	Diterpenes	Cupper acetate	+	+
10	Proteins and amino acids	Ninhydrin test	_	+
11	Flavonoid	Lead acetate test	_	_

(-) A sign indicates absence of constituent in the respective screening test; (+) sign indicates the presence of a constituent in the respective screening test.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined according to the method.[28] 2 ml of 20 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations prepared in ethanol and the mixture incubated at 25^{0c} for 30 min. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. The nitric oxide radicals scavenging activity was calculated. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples using Eq.(1). IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear

regression algorithm. Nitric oxide scavenging activity is measured by using following formula,

% inhibition= (Absorbance of control- absorbance of sample) / Absorbance of control X 100.

Table 2: Nitric oxide scavenging activity of Standard Ascorbic acid.

S.No	Con.(µg/ml)	% inhibition
1	Control	-
2	100	99.52
3	200	99.61
4	300	99.71
5	400	99.80
6	500	99.90

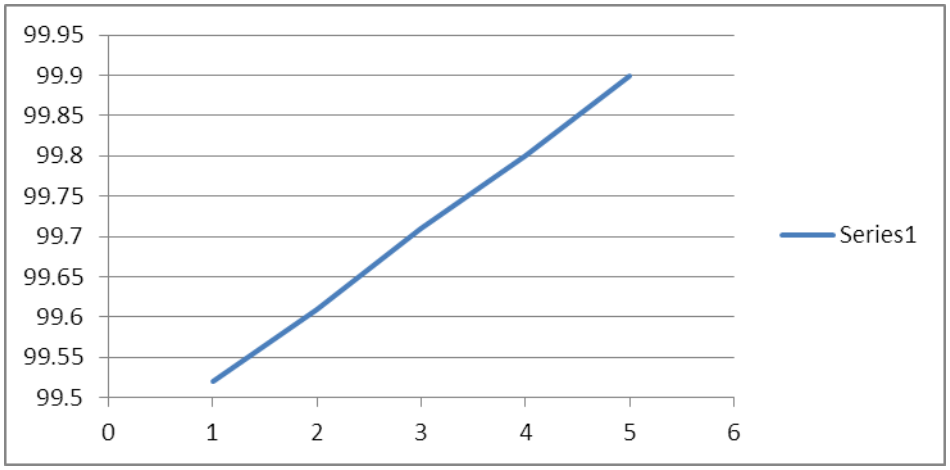


Figure 4: Standard plot of ascorbic acid.

Table 3: Nitric oxide scavenging activity of *Tinosporacordifolia* with different concentration.

S.No	Con.(µg/ml)	%inhibition
1	Control	-
2	100	23.19
3	200	25.142
4	300	26.04
5	400	26.133
6	500	29.14

From theresult of the present study, various fractions of theleaves of *Tinosporacordifolia*, Weresubjected to *Invitro* anti-inflammatory activity in various concentrations i.e. 62.5, 125µg/ml and the percentage inhibition of different extracts of leaves of *Tinospora cordifolia* by Protein denaturation method is depicted in Table.

Table 4: Anti inflammatory activity of *Tinospora cordifolia* extracts.

S.No.	Con.(µg/ml)	% inhibition	
1	Control	Ethanol extract	Hexane extract
		-	
2	62.5	92.4	91.4
3	125	94.2	92.1

Table 5: Anti inflammatory activity of Standard Diclofenac Sodium.

Sl.No.	Conc.(µg/ml)	%inhibition
1	Control	Diclofenac Sodium
		-
2	62.5	93.8
3	125	94.4

ACKNOWLEDGEMENTS

We express *our* heartfelt thanks to Mr. G.BABU, Assistant Professor, Department of Pharmaceutical Chemistry, for invaluable guidance and constant encouragement during the course of this study.

We express *our* sincere thanks to Dr. M. Chinna Eswaraiah, Principal, Anurag Pharmacy College, Kodad for providing all the facilities on carrying out the study.

We express *our* sincere thanks to Dr. B. Raja, Professor and Head, Department of Pharmaceutical Analysis and Ch. Srinivas Reddy, Associate professor for providing all the facilities on carrying out the study.

CONCLUSION

Herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and mainstream system. India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant based formulations, used in folk medicine and known to rural communities in India.

Medicinal plants play a central role not only as traditional medicines, but also as trade commodities. In the present work Pharmacological and Phytochemical investigation of *Tinospora cordifolia*was performed. The plant species were standardized and compared as per WHO guidelines.

Successive solvent extraction was done using soxhlet. The deterioration time of plant material depends upon the amount of water present in the plant material. If the water content

is high, the plant can be easily deteriorated due to fungus. Preliminary phytochemical screening of *Tinospora cordifolia*showed the presence of carbohydrates, glycosides, flavonoids, phenols, tannins and amino acids in the crude drug.

Tinospora cordifolia stem extracts exhibited marked dose dependent antimicrobial activity in vitroagainst both gram positive and gram negative bacteria and can be used as a good therapeutic approach for infectious disease management and therapy. Methanolicextract was found to be more potent against both the group of bacteria. The *Tinosporacordifolia*stem has shown different types of phytochemicals. Methanolic extract of *Tinospora cordifolia* stem exhibited better antioxidant potential also.

The In vitro studies on leaves *Tinospora cordifolia* showed the presence of significant anti-inflammatory activity. The methanolic extract showed more anti inflammatory activity. TheActivity may be due to the presence of steroids, flavonoids and alkaloid. Our future aim is to isolate the chemical constituents responsible for the anti-inflammatory.

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