STUDIES ON ANTIBACTERIAL ACTIVITY AND IMMUNOMODULATORY POTENTIAL OF *Tinospora cordifolia* IN POULTRY

THESIS

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

BY

PATIL MUKESH SUBHASH

Enrolment No. V/02/971

Post Graduate Institute of Veterinary and Animal Sciences, Akola

MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY, NAGPUR- 440 006.

(INDIA)

2009

DECLARATION OF STUDENT

I hereby declare that the experimental Research work and interpretation of the thesis entitled **"Studies on antibacterial activity and immunomodulatory potential of *Tinospora cordifolia* in poultry"** or part there of has not been submitted for any other degree or diploma of any University nor the data have been derived from any thesis / publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

Date: 13/07/2009

Signature
Mr. Patil M . S.
Enroll. No. V / 02 / 971

Counter signed by
Chairman Advisory Committee with date.

DECLARATION OF ADVISORY COMMITTEE

Mr. Patil Mukesh Subhash has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled **"Studies on antibacterial activity and immunomodulatory potential of *Tinospora cordifolia* in poultry"** submitted by him is the result of research work is sufficient to warrant its presentation to the examination in the subject of **Veterinary Pharmacology & Toxicology** for the award of **Master of Veterinary Science** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part there of has not been previously submitted by him for a degree of any other university.

Place : Akola
Date : 13.07.2009

Signature
(Dr. G. D. Ranvir)
Associate Professor of Pharmacology,
[Advisor / Guide]

ADVISORY COMMITTEE

	Name & Designation		**Signature**
1.	**Dr. G. D. Ranvir** (Assoc. Prof. of Vet. Pharmacology)	(Chairman)	
2.	**Dr. S. W. Hajare** (Asst. Prof. of Vet. Pharmacology	(Member)	
2	**Dr. A. Y. Kolte** (Professor & Head of Vet. Medicine)	(Member)	
3.	**Dr. V. P. Pathak** (Prof. (CAS) of Vet. Pathology)	(Member)	
4.	**Dr. P. M . Kapale** (Asstt. Prof. of Vet. Medicine)	(Member)	

CERTIFICATE

This is to certify that the thesis entitled **"Studies on antibacterial activity and immunomodulatory potential of *Tinospora cordifolia* in poultry"** submitted by **Mr. Patil Mukesh Subhash** to the Maharashtra Animal & Fishery Sciences University in partial fulfillment of the requirement for the degree of **Master of Veterinary Science** in **Veterinary Pharmacology & Toxicology** has been approved by the students advisory Committee after examination in collaboration with the External Examiner.

Name & Signature of External Examiner	Signature with seal Head of Department	Name & Signature Advisor / Guide
PROF. A.GOPALA REDDY	Deptt of Pharmacology Toxicology P. G. College of Vet. Sc. & AH Sciences (M A F S U, Akola	Designation (Dr. G.D. Ranvir)

ADVISORY COMMITEE

Name & Designation		Signature
1. **Dr. G.D.Ranvir** (Associate Professor of Pharmacology)	(Chairman)	
2. **Dr. S.W.Hajare** (Asst.Prof. of Vet. Pharmacology)	(Member)	
3. **Dr. A.Y.Kolte** (Professor & Head of Vety. Medicine)	(Member)	
4. **Dr. V.P. Pathak** (Professor (CAS) of Vety. Pathology)	(Member)	
5. **Dr. P.M. Kapale** (Assistant Professor of Vety. Biochemistry)	(Member)	

(Dr. V.H. Kalbande)
Signature with Seal
Dean / Associate Dean

ACKNOWLEDGMENT

"The culmination of research work is a corner stone in the life of any student with the research guide being the driving force behind."

Success is not be possible lonely without involvement of many minds and hands to beautify it. Acknowledgement is not just an formality to express thank but the words lacks to express my feeling to all who helped me to mould this research work, without their help I cannot have put up my thesis. My acknowledgments are many more than what I am expressing.

I fell immense pleasure in taking the opportunity of expressing my whole hearted sense of gratitude and sincere indebtedness to my erudite guide ***Dr G. D. Ranvir,*** *M.V.Sc., Associate Professor, Department of Veterinary Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences, Akola, for suggesting me this topic of field applicability concern, his constant encouragement and untiring efforts during the whole span of investigation.*

I have great pleasure in expressing my sincere and whole hearted thanks to ***Dr. S. W. Hajare,*** *M.V.Sc., Ph.D., Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Post Graduate Institute of Veterinary and Animal Sciences, Akola for providing the valuable support and time to time guide line for the completion of my work, without his cooperative hands this research work will never competed.*

I must greatfully obliged to ***Dr.A.Y.Kolte*** *M.V.Sc., Ph.D., Professor and head, Department of Veterinary Medicine,* ***Dr V.P. Pathak*** *M.V.Sc., Ph.D.,Associate professor, Department of veterinary pathology,* ***Dr.P.M.Kapale,*** *M.V.Sc.,., Assistant Professor, Department of Veterinary Biochemistry, Post Graduate Institute of Veterinary and Animal Sciences, Akola, for their suggestions during this research work.*

I wish to express my sincere feeling of gratitude to ***Dr. S.Z. Ali., M.V.Sc., Ph.D.,*** *Professor and University head, Department of Animal Genetics and Breeding,* ***Dr. S.V. Kuralkar,*** *M.V.Sc., Ph.D., Associate Professor Department of Animal Genetics and Breeding , Post Graduate Institute of Veterinary and Animal Sciences, Akola for their timely help and suggestion.*

*My inexplicable gratitude goes to **Dr.,V.H.Kalbande** Associate Dean, Post Graduate Institute of Veterinary and Animal Sciences, Akola for providing necessary facilities to pursue this study & their generous help in all academic matters.*

*I think, the words with me are insufficient to express the feeling of my heart to acknowledge by beloved Mother **Sau.Shobha** and **Father Subhash Chintaman Patil** whose constant encouragement, inspiration and blessing always help me make my dreams come true and I thank them from the depth of my heart for building up in me a positive human being. I also express my deep sense of gratitude where emotions are involved to my Sisters **Miss. Archana Subhash Patil** and **Miss Gayatri Sarjerao Salunkhe** for their ever most love, encouragement and affection. I also express my thanks to Bacchaparty of my family, **Sonu, Nanu, Abhishek, and Diksha** as their cheerful and innocent faces always make me fresh and happy.*

*I feel unique pleasure in extending deep-hearted gratitude and sincere thanks to my Colleague **Awesh Rangnath, Prashant, Vijay, Ashok, Karadkar, swapnil, Rakesh, Sopan, Bhushan, santosh, Ajit, Giri Suhil Pandao, , Sonali, Smita, Madhvi** for his commendable help, altruistic attitude, pains taking efforts and excellent company being at back and call to accomplish the work. Genuine appreciation and thanks to my Juniors **Sanny, Rahul ,Deepak, Sandeep, Snehal, shubha, Anjali** for his everwilling and untiring assistance.*

*All my friends deserve my special thanks for keeping me socially alive and for always being there for me. I am fortunate to have friends like, **Dr borekar sir , Chadure Sir,** from whom I received immense affection & moral support always when I needed it.*

*In this perspective, I wish to exert my eulogy and wholehearted endearment to my UG batchmates, **Dr. Mahesh, , Dr.Pendharkar, ,** for their altruistic guidance.*

*I am very much thankful to **P.L.Thakur**, Junior Veterinary Officer, Poultry Research Centre, PGIVAS, Akola for his moral support and valuable help. I am also thankful to **Shakuntalabai** for their generous cooperation during my research work.*

***"Coming together is a beginning, keeping together is a progress; working together is a success".** During my stay at this institute I was fortunate enough to receive the kind cooperation from almost everyone in one way or the*

other. It is extremely difficult to thank all of them individually by name. This shortcoming may please be pardoned.

Above all The Almighty, ***"God"*** *I want to thank for bringing new hope & ray of light in my life through achievements as well as failures. Whatever good come out of this work credit is his.*

Patil

(PATIL MUKESH SUBHASH)

Place: Akola

Univ .Enrolment No. V/02/971

Date: 13.07.2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS / ABBREVIATIONS

Symbols / Abbreviations		Long forms
BC	:	Before Christ
cm	:	centimeter
CMIR	:	Cell Mediated Immune Response
CNS	:	Central Nervous System
CRD	:	Completely Randomised Desigh
Bact. Ctrl	:	Bacterial control
^0C	:	Degree centigrade
DNCB	:	Dinitro Chloro Benzene
DNFB	:	Dinito fluro Benzene
Ext.Ctrl	:	Extract Control
F.D.A.	:	Food and Drug Administration
Hb	:	Hemoglobin
gm	:	gram
hrs.	:	Hours
HPLC-UV	:	High Performance Liquid Chromatography-Ultra Violet
IgG	:	Immunoglobulin G
IgM	:	Immunoglobulin M
IL	:	Interleukin
INF	:	Interleukin Neutralizing Factor
LC-MS	:	Liquid Chromatography Mass Spectrophotometry
MIC	:	Minimal Inhibitory Concentration
mg	:	milligram
mg/kg	:	milligram per kilogram
mm	:	millimeter
ml	:	milliliter
mg/ml	:	milligram per milliliter
MTCC	:	Microbial Type culture collection
NMR	:	Nuclear Magnetic Resonance
O.D	:	Optical density
PCV	:	Packed Cell Valume
PHA	:	Phyto Agglutinin
PO	:	Per os

RBC	:	Red blood cells
SE	:	Standard error
Spp.	:	Species
TNF	:	Total Neutralizing Factor
UV	:	Ultraviolet
USA	:	United State of America
µg	:	microgram
µl	:	micro liter

Introduction

CHAPTER I

INTRODUCTION

Several new generations of synthetic antibiotics are developed due to ineffectiveness and emergence of resistant pathogenic bacteria (Parida *et al.* 2006). These drugs are not cost prohibitive, free from toxicity, adverse reactions and side effects that may cause more harm than the benefits (Atal and Kapoor, 1982). With the advancement in the scientific knowledge on plant drugs their active bio-molecules are being isolated, identified and characterized into more effective therapeutic remedy.

Different medicinal properties of *Tinospora cordifolia* including antibacterial, immunomodulatory are well recognized in the Ayurvedia and also documented in Materia Medica, Siddha and Unani medicine. Rigveda and Atharavaveda (500 B.C.) described health benefits of ancient knowledge in man and animals. Atharavaveda (12000 B.C.), Charak samhita and Sushrut samhita (1000-500 B.C.) mentioned 700 important herbs of therapeutic significance (Dash and Sharma, 2001). The therapeutic potential of herbs in the discovery of new drug has not been fully exploited (Dubey *et.al* 1997). It has been estimated that out of 250000 – 500000 plants species, a very few plant species are investigated so far in relation to their phytochemical, biological and pharmacological activities.

Modern research has also clarified beneficial effects of plant drugs. World Health Organization has advocated greater use of herbal medicine for safe healthcare reported that more than 80% world's population solely relies on medicinal plants for their primary health care (Fansworth *et al.* 1986). The herbal remedy is being effective, safer, cost prohibitive and eco-friendly accepted world wide as an alternate source for synthetic drugs (Branter and Grein, 1994, Cowan, 1999, Stockwell, 1998).

Extract fractions derived from 119 plants have found their prototypes in traditional medicines. About 25% of therapeutically useful drugs such as aspirin,

quinine, vincristine, codeine, digitalis and ephedrine have their prototype directly or indirectly in the plant. Higher plant derived 130 drugs are being used as a single drug entity under traditional systems of medicine and some of them are modified and are used for economic purpose (Newman *et al*, 2000). FDA (USA) also approved greater use of herbal medicines has suggested guidelines for standardization of plant extracts. The medicinal plants are continuously used in the treatment and prophylaxis of bacterial, viral and fungal infections in many parts of world (Umadevi *et al* 2007). Thus, the plants are considered as valuable segment of modern medicine (Fransworth and Bengel, 1977) which is also investigating for economic reasons.

Tinospora cordifolia is well recognized and documented as a single drug entity in the Ayurveda and Unani systems of medicine. It is used in many cosmetic and herbal preparations for health benefits (Ali, 1990, Kohli and Jain, 2006). Different medicinal properties of this herb are documented in the ancient manuscripts and have been reported by several workers. Different parts of this plant are valuable in the treatment of vata, burning sensation, dyspepsia, hyperdyspepsia, fever, gout, general debility, jaundice, spleenopathy, anemia, skin diseases, flatulence, jaundice, rheumatism, urinary diseases (Khan *et al.*, 1989) and as nutrient (Patel and Patel, 1956).

In vitro antibacterial activity of ether extract (Gupta, *et al* 1956), stem decoction (Tatthe, et al 1992), aqueous, ethanolic and chloroform extracts (Jeyachandran, et al 2003), aqueous root extracts (Agarwal, et al 2008), aqueous and methanol extracts (Girish and Satish, 2008), methanolic leaf extract (Mahesh and Satish, 2008) and aqueous extract (Mohana, et al 2008) of *Tinospora cordifolia* have been reported.

However, repots pertaining to antibacterial evaluation of *T. cordifolia* stem extracts at different concentrations and immunomodulatory potential of *Tinospora cordifolia* stem powder during extreme hot summer in broiler birds has not found in the reviewed literature. Therefore the present investigation was carried out to

evaluate antibacterial activity and immunomodulatory potential of *Tinospora cordifolia* in poultry with the following objectives.

1. To prepare aqueous and ethanolic hot extracts of stem of *Tinospora cordifolia* and to determine its percentage of extractability.

2. To study the antibacterial activity of these extracts alone and potent extract in combination with reference drug ciprofloxacin against common pathogenic bacteria by disc diffusion and tube dilution method *in vitro*.

3. To determine minimal inhibitory concentration (MIC) of most potent antibacterial extract by tube dilution technique *in vitro*.

4. Phytochemical studies of these extracts (Qualitative analysis) for presence of active principles and

5. To evaluate immunomodulatory potential of *Tinospora cordifolia* stem powder employing DNCB skin sensitivity test in poultry.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Emergence of bacterial resistance to the available antibiotic and the spread of infectious diseases evolved in man and animals. Most of new generation antibiotics, antimicrobials are not found safe, their side effects and toxicities have curtailed their use in therapy. On the contrarly herbal drugs are gaining wide acceptability due to affordable, safer, eco-friendly attributes world wide. *Tinospora cordifolia* possesses different medicinal properties and is a valuable remedy in the treatment of fever and diabetes and being used as a general tonic in debilitating diseases in man. Its anticancer, antioxidant and antidiabetic, immunomodulatory properties are documented in the ancient manuscripts (Kirtikar and Basu, 1935) which is scientifically recognized and are supported through various investigations. Available literature on *T. cordifolia* special reference to its *in vitro* antibacterial activity and immunomodulatory potential in poultry are reviewed in this chapter under following headings botanical description, distribution, phytochemistry, antibacterial, immunomodulatory, pharmacological activities, medicinal properties and uses.

2.1 Botanical description

The herb *Tinospora cordifolia* (Family: Minospermicaea) is a large, glabrous, deciduous, climbing shrub, woody, slender (Plate. I) and perineal. Its stem is thick, soft, warted. Shoot is succulent. Root is adventitious, slender and long tuberous. Leaf is broadly ovate, alternate, 2-4 inches long, petiolate, roundish, ovulate, entire, cordate at base, acutely apexed, smooth, thin and glaucous beneath. Flowers are small yellow or greenish yellow or green, unisexual. Male flowers are clustered fascieled. Female flowers are solitary, drupes. Fruits are ovoid, succulent, lustrous and red when ripe. Seeds are bean shaped, curved, white and warty (Kirtikar and Basu, 1969, Sharma *et al.*, 2005, Prajapati *et al.*, 2003, Dhiman, 2006).

Plate. I: The herb *Tinospora cordifolia* Miers.

2.2 Geographical distribution

The herb *T. cordifolia* is widely distributed throughout the tropical region in India, Ceylon, Burma and Assam (Robert and Henry, 2006). Its various vernacular names are listed below (Kirtikar and Basu, 1935, Sharma *et al.* 2005., Dhiman, 2006)

Arabic	:	Gilo
Assam	:	Siddhilata, Amarlata
Bengali	:	Golancha, Giloe
English	:	Gulancha tinospora, Tinospora
Gujarati	:	Gulvel, Galo
Hindi	:	Gulancha, Giloy, Amrita, Gulbel
Kannad	:	Amruta balli, Yuganiballi, Madhuparni
Malayalam	:	Chittamritu, Amritu
Marathi	:	Gulvel
Panjabi	:	Gilo, Garham, Palo
Sanskrit	:	Guduchi, Amtita
Tamil	:	Amurutavalli, Cintilikkoti
Telugu	:	Thippateega, Amruta.
Urdu	:	Gilo

2.3 Phytochemical studies

Many modern drugs have their prototype in plants. Due to advancement in molecular research in biological field and organic chemistry the active chemical constitutes from plant are being isolated, identified and characterized using modern analytical tools. Available literature on phytochemical investigations of medicinal plants are herewith reviewed under this subhead.

Kidwai *et al.* (1949) have obtained giloin (glycoside), gilenin (non glycoside) and gilosterol the bitter principles from stem extract of *Tinospora cordifolia*.

Chatterjee and Ghosh (1960) reported tinosporine, the furanoid bitter principle from stem extracts of *Tinospora cordifolia*.

Hanuman *et al.* (1986) isolated and identified diterpenoid furanolactone from chloroform extract of stem of *Tinospora cordifolia* by atomic and column NMR chromatography. Whose structure was similar to clerodane diterpenoid.

Bhatt *et al.* (1988) have isolated and identified clerodane diterpenoid compounds from stem extract of *Tinospora cordifolia* by FT-NMR chromatography.

Hanuman *et al.* (1988) isolated clerodane furanoditerpene-2 from stem of *Tinospora cordifolia* which was structurally similar to furanoditerpene.

Bhatt and Sabata (1989) isolated and identified furanoid diterpene glucoside from chloroform stem extract of *Tinospora cordifolia* by NMR chromatography.

Khan *et al.* (1989) isolated and identified 18- nor-clerodane diterpana-o-glucoside (Tinosporaside) from ethanolic stem extract of *Tinospora cordifolia*.

Adhila *et al.* (1991) isolated and identified cis-clerodane furanoditerpene from fractionated chloroform, petroleum ether and methanol extracts of shoot part of *Tinospora cordifolia* on the basis of spectral data.

Gangan *et al.* (1994) isolated nor-diterepene furanoglycoside as tetra acetate viz. cordifoliside D and cordifoliside E from butanol extract of stem part of *Tinospora cordifolia* by 1D and 2D NMR spectroscopy.

Maurya *el al.* (1995) isolated and identified tinosponone and tinocordioside from stem extract of *Tinospora cordifolia* and their structure was illustrated by spectroscopic and chemical co-relation method.

Maurya *et al.* (1997) isolated and identified sesquiterpene glucoside, tinocordifolioside from stem extract of *Tinospora cordifolia* by spectroscopic method.

Chintalwar *et al.* (1999) isolated and identified arabinogalactan from stem of *Tinospora cordifolia* by methylation, partial hydrolysis and carboxyl reduction analysis. They noted polyclonal mitogenic activity of polysaccharides against B-cell without involvement of macrophages in the cell proliferation.

Jahfer (2003) have purified trimethylsilated polysaccharide from *Tinospora cordifolia* by GC-MS. They also obtained glucose, arabinose, rhamnose, xylose, mannose and galactose as other constituents.

Prajapati *et al.* (2003) reviewed phytochemical constituents was obtained from *T. cordifolia* such as tinosporin, columbin, chasmanthin, palmarin, berberine, tinosporon, tinosporic acid, tinosporol, giloin, giloinisin, pyrolidine, diterpeniod furanolactone, 18 norclerodane,-o-glucoside, adultetrahydrofuranolignane, octacosanol, noracosan 15-one and beta-sistosterol.

Jahfer and Azadi (2004) isolated, purified methylated alditol acetate derivatives with the terminal linkage of glucose, 4-xylose, 4-glucose, 4, 6, glucose and 2, 3, 4, 6-glucose, by methylation, hydroxylation, reduction and acetylating polysaccharide of *Tinospora cordifolia*.

Maurya *et al.* (2004) isolated and identified clerodane furanoditerpene glucoside acetates as amritosides A, B, C, and D from stem of *Tinospora cordifolia* by spectroscopic method.

Kapoor (2005) reported principles such as giloin (glycoside), gilenin (nonglycoside), gilosterol, tinosporine, furanoid from stem extract of *Tinospora cordifolia* as bitter principles.

Sharma *et al.* (2005) have documented different chemical constituents from various parts of *Tinospora cordifolia* namely tinosporine, tinosporon, tinosporic acid, tinospord, tinosporide, tinosporidine, columbin, chasmanthin, palmarin, berberine, giloin, gilonisin, 1,2, pyrolidine, diterpenoid, furanolactone, 18 nor-cleradonediterpine-o-glucoside, aryltetrahydrofuranolignon, octacosanol, nonacoson-15-one and beta sistosterol, cordifolon, manoflorine, tambetarine, cardiofoliosides A and B, phenolic legnan, 3,4, tetrahydrofuraon, arabinogalacton.

Ahmed *et al.* (2006) reported active principles such as 20-betahydroxyecdysona, tinosporaside, cardioside and columbin from 70% ethanolic extract of three different species of *Tinospora* viz. *Tinospora cordifolia, Tinospora melabrica* and *Tinospora crispa*) by reverse- phase high performance liquid chromatography-UV-diode array detection method and from the extract of *Tinospora cordifolia* by column chromatography and H^1 and ^{18}C NMR.

The mass spectral data obtained by reverse phase column in water acetonitrile gradient was detected by HPLC-UV-DAD method. They suggested that these methods were successful in separating biomarker compounds from these three species of plants obtained from different regions of India. Yield of these biomarker compounds was comparatively higher in *Tinospora cordifolia* species grown in the Jammu province than other regions.

2.4 Pharmacological studies

Ikram *et al.* (1987) reported the antipyretic activity of hexane and chloroform extracts of eight Pakistani medicinal plants including *Tinospora cordifolia* against yeast induced pyrexia in rabbit. They observed ineffectiveness of hexane extract at 1.6 gm/kg body weight following oral administration but was found safe in mice.

Dahanukar *et al.* (1988) has reported protective effect of stem decoction of *Tinospora cordifolia* against caecal ligation induced abdominal sepsis in wistar rats at 100 mg/kg body weight p.o. dose.

Rege and Dahanukar (1993) observed dose dependent phagocytic and killing effect of macrophages on monocyte macrophage cell line (Candida spp.) and the peritoneal macrophages in rats and human by the extract of *Tinospora cordifolia*.

Nagarkatti *et al.* (1994) has reported hepatoprotective effect of *T. cordifolia* through modulation of kupffer cell activity during hepatic injury. Significant increase in half life of carbon collide revealed deteriorating activity on kuffer cells which was reduced by *T. cordifolia* through activation at 100 mg/kg dose against liver fibrosis in rat.

Maurya *et al.* (1995) has reviewed pharmacological properties of *Tinospora cordifolia* such as hypoglycemic, antihyperglycemic, CNS depressant, antibacterial, antidiarrheic, antimicrobial, antipyretic, antifungal, antiarthritic, antiallergic, hepatoprotective, analgesic, immunomodulatory, antineoplastic, antistress, antidiabetic, antitumor, adoptogenic, hypotasine, antioxidant, antiendotoxic, and diuretic.

Dhuley (1997) has studied antitoxic effect of four Indian herbs including *Tinospora cordifolia* on macrophages function of mouse treated with ochratoxin-A. They observed that the extracts of *Asparagus recimosus*, *Tinospora cordifolia*, *Withania somenifera*, *Picorrhiza kurroa* were effective on ochratoxin induced suppression of chemotactic activity and production of IL-1 and TNF- alpha from macrophages *in vitro*.

Spelman (2001) while reviewing traditional use and indications of *Tinospora cordifolia* reported significant anti-infective activity of *Tinospora cordifolia*.

2.5 Antibacterial studies

Gupta *et al.* (1956) reported antibacterial activity of ether extract of *Tinospora cordifolia* against *Mycobacterium tuberculosis* where growth of this bacterium was significantly inhibited by *T. cordifolia* extract at 1:50,000 concentrations *in vitro*.

Dhar *et al.* (1968) reported *in- vitro* antiviral activity of 50 % ethanol stem extract of *Tinospora cordifolia* after 48-72 hours at 37 °C against Ranikhet disease

Dhar *et al.* (1968) screened 285 medicinal plants including *Tinospora cordifolia* for biological activities against bacteria, fungi, protozoan, helminth parasite, where ethanol extract of *Tinospora cordifolia* was found ineffective against bacteria, fungi and protozoan at 50% concentration. The ether extract of this plant was also ineffective against experimentally induced parasitic infection in albino rats on 9th day.

Thatte *et al.* (1992) reported antibacterial activity of *Tinospora cordifolia* stem decoction against experimentally induced *E .coli* infection in mice. There was significant improvement in the mortality rate, bacterial clearance and phagocytic intracellular bactericidal activity in Swiss mice *in-vitro* but the extract was ineffective *in-vivo*.

Jeyachandran *et al.* (2003) reported antibacterial activity of aqueous, ethanolic and chloroform stem extracts of *Tinospora cordifolia* against *E. coli, Proteus vulgaris, Enterobacter faecalis, Salmonella typhi, Staphylococcus aureus, Serratia marcescens* by disc diffusion method. The ethanolic extract showed significant antibacterial activity against all test organisms but the chloroform extract was moderately effective except on *Staphylococcus aureus, Serratia marcescens* and the aqueous extract was marginally effective against *E. coli*.

Samy (2005) has reported antimicrobial activity of the methanolic extract of *Zinziber officinalae, Asteracantha longidfolia, Citrus acida, Salacia microsperma and Tinospora cordifolia.*

Agarwal *et al.* (2008) reported antimicrobial activity of aqueous root extract of *Tinospora cordifolia* against four fungal and two plant pathogenic bacteria obtained from cluster bean seed. The antifungal and antibacterial activity of this extract was concentration dependant. The absolute (100%) concentration of the extract was more effective against these pathogenic microbes as compared to Bavistine and Streptomycin.

Girish and Satish (2008) has screened antibacterial activity of aqueous and methanol extracts of five different plants belonging to different family viz. *Boerhavia diffusa, Cassia auriculata ,Cassia lantana, Eclipta alba* and *Tinospora cardifolia* against *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis* and *Yersinia enterocolitica* by agar diffusion method. They observed significant antimicrobial activity of methanol leaf extract of *Tinospora cardiofolia, Boerhaavia diffusa* and *Eclipta alba in vitro.*

Mahesh and Satish (2008) reported antimicrobial activity of methanolic leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer,* and *Zizyphus mauritiana.* They observed significant antibacterial activity of ethanolic extract of these plants against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescence, Sthaphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* and antifungal activity against *Aspergillus flavus, Dreschlera turcica and Fusarium verticillioides.*

Mohana *et al* (2008) screened aqueous extracts of eight higher medicinal plants namely *Argemona mexicana, Caesalpinia coriaria, Decalepis hamiltonii, Euphorbia tirucalli, Leucas aspera, Phyllanthus amarus, Phyllanthus niruri, Tinospora cordifolia* and *Tribulis terrestris* against eleven human pathogenic

bacteria by cup diffusion method and isolated active constituents from these plants. They concluded that the *Caesalpinia coriaria* Wild has significant antibacterial activity against all test bacteria where other plants were marginally effective.

2.6 Immunomodulatory studies

Morgan *et al.,* (1976) reported effect of high environmental temperature on immediate hypersensitivity reaction in chickens in which anaphylactic reaction following challenge with BSA in previously sensitized bird was significantly reduced within 2 hour prior to challenge.

Regnier and Kelly (1981) reported effect of heat stress on cell mediated immune response and antibody production in chickens *in vivo* and *in vitro.* They observed significant decrease of wattle swelling sensitized by DNFB and PHA (phyto haemagglutinin) exposed to 36^0 C temperature for 5 days that might be due to involvement of corticosterone and other serum factors (thymic hormones lymphokines and other substances) in decrease CMI response.

Valsala *et al.* (1981) evaluated CMI response following sensitization and challenge with DNCB after fourteen days. They observed increase in 2 to 2.5 mm thickness of the skin at 24 hours was normal response in duck which was taken into account as baseline data and was implied in evaluating CMI response. There was progressive reduction in thickness of skin which was almost normal after 48 to 72 hours. Histology of skin showed slight necrosis of epidermis, pronounced diffuse edema of skin, increase in heterophils, lymphocytes, macrophages, engorged blood vessels, lymphatic dilatation and scattered erythrocytes in the tissue after 24 hours where congestion and edma was less evident and there was infiltration of macrophages and lymphocytes around the blood vessels after 48 hours. The macrophage and lymphocytes were scattered and edema subsided subsequently after 72 hours as compared to control ducks which was given acetone

Henken *et al.*, (1983) reported effect of environmental temperature on physiological and immunological response in pullets immunized with sheep RBC. They observed significant differences in each heat treatment, type of immunization nutrition and length of heat treatment period and increased feed conversion, feed intake in birds exposed to low temperature without alteration in the growth rate and weight gain but during high temperature (30-40 ^{O}C) caused significant decrease in feed intake, growth rate without change in weight gain and feed conversion.

Atal *et al.* (1986) reported immunomodulatory potential of some indigenous plants including *Tinospora cordifolia* against chronic infections and immunological disorders in mice. The phagocyte function was improved without affecting humoral or cell mediated immunity by ethanolic extract of *Tinospora cordifolia* and *Ocimum gratissimum*.

Atal *et al.* (1986) reported that the 95 % ethanolic stem extract of *Tinospora cordifolia* had enhanced phagocytic activity of reticulo-endothelial system without affecting humoral and cell mediated immune response that was indicative of immunomodulatory activity of this plant.

Rege *et al.* (1989) observed enhanced intracellular killing capacity of macrophages and polymorphonuclear cells and reduced mortality following treatment with *T. cordifolia* extract at 100mg/ kg dose twice a day for 7 days against *E. coli* infection showed immune competence in rats.

Devegowda *et al.* (1990) studied influence of Livol (containing *Tinospora cordifolia*) supplemented in the diet of broiler at 0.25 and 0.5 % level. They observed that the body weight gain and feed efficiency of Livol supplemented groups were significantly increased than that of control at all ages. 0.5% Livol supplemented group was superior as compared to 0.25% level.

Patwardhan *et al.* (1990) discussed screening methods for immuno modulatory agents and plants. They observed that the plant *Tinospora cordifolia* was effective immunomodulatory agent.

Dhande *et al.* (1991) studied the effect of Livol supplementation on performance of broilers added in diet at 02% and 0.5% levels. The Livol supplemented groups gained 18.81% and 22.8 % more body weight than control. They observed the cumulative feed consumption in control and treatment groups 3098, 3246.5 and 3427.2 gm with the cumulative feed efficiency ratio 3.00, 2.60 and 3.34 respectively. The Livol supplementation at these dose levels not showed any significant difference in body weight than control. However, these two groups showed significantly higher weight gain than control. The feed efficiency in these treatment groups were 2.51, 2.23 and 2.21 respectively showed better efficiency of Livol supplementation. The growth rate of Livol treated groups was higher by 4% than the control group and concluded that the use of Livol in broiler diet at 0.5% level was economical.

Ensminger (1992) mentioned daily water requirement in poultry during 3rd to 6th week ranged from 90 to 260 liters/1000 birds. Further they mentioned that 10% body water loss cause physiological disorder and 20% loss results in death which also affected the growth and production of birds. They also recorded that third week onwards the body weight of male and female broiler birds increased from 560gm to 1690gm and 515 gm to 1430 gm respectively.

Bhattacharya *et al* (1994) studied the anti stress activity of herbal formulation 'Zeetress' on albino rats and mice. They observed that the Zeetress increased tolerance of mice to anoxic stress and attenuated stress induced increase levels of tribulin, an endocoid marker of stress in rat brain and significant anti stress activity was indicative of putative adaptogenic property and protective effect against adverse environmental factors in organism. These findings provided the evidence for decrease in hypothalamo-puitiutory-adrenocortical activity induced by stress.

Sharma *et al* (1995) studied the effect of alcoholic extract of *Tinospora cordifolia* in modulation of norepinephrine, dopamine, 5-HT, and 5 hydroxy indolic acetic acid levels in experimental rats.

Agarwal and Singh (1999) reviewed immunomodulators from plant extracts and synthetic pesticides during *in vivo* and *in vitro* studies mostly in animals and humans in Indian laboratories. They mentioned some of promising plants having immunomodulatory potential such as *Asparagus rarcemosus, Curcuma longa, Tinospora cordifolia, Azadiracta indica, Ocimum sauctum, Panax ginseng, Picordiza kurroa, Withania somnifera* and so on. Further they identified some plants as rasayana and reviewed pharmacological properties including *T. cordifolia* (guduchi) such as antibacterial, antiageing, antiallergic, antirheumatic and immuno stimulant. These plants showed *in vitro* and *in vivo* immunomodulatory property.

Bhat and Gupta (2008) evaluated effect of antistress agent such as Vit C, E, sodium bicarbonate and ammonium chloride incorporated in feed with ration of 210 WHL pullets during summer. They observed improvement in the egg production of different treatment groups of birds was given antistress agents and significant improvement was observed with Vit C treated group followed by ammonium chloride, sodium bicarbonate and ammonium chloride respectively. They concluded that the Vit C, Vit. E, sodium bicarbonate and ammonium chloride may be helpful in ameliorating extremely high temperature during summer season.

Samy *et al.* (2008) reviewed some important bioactive compounds and role of medicinal plants including *T. cordifolia* in ayurvedic system of medicine. They mentioned that the *T. cordifolia* Miers is a rich source of Vit C which inhibited the growth of bacteria and strengthen immune system through enhanced killing ability of macrophage. Further they mentioned the use of *T. cordifolia* in ayurvedic formulation for the treatment of various ailments in human being such as cancer, chronic fever, diabetes, fistula, gastritis, lack of appetite, migraine, prostatic enlargement where in 15 per cent of *T. cordifolia* as a single drug entity was used and given at 4 gm powdered mixture twice daily before meal with water. The 10 per

cent formulation of *T. cordifolia* is used in cure of cysts, hair problems, leucoderma, as liver tonic, and urinary tract diseases and 20 per cent formulation for skin diseases and intestinal problems.

Thatte *et al.* (1994) observed that there was significant increase in number of colony forming unit and macrophage activation by *Tinospora cordifolia* at 100mg/kg dose for 10 days treatment.

Bapat *et al.* (1995) reported immunotherapeutic potential of *Tinospora cordifolia* with and without percutaneous transhepatic surgical billiary drainage in human patients. They observed improved killing capacity of polymorphonuclear cells by *Tinospora cordifolia* thereby host defense without post surgical mortality.

Sohni *et al.* (1996) reported therapeutic efficacy of herbal formulation consisting of five different medicinal plants including *Tinospora cordifolia* against hepatic amoebiasis through enhanced immunomodulatory activity in golden hamster. There was enhanced HA titer, leucocytic migration without affecting T cell count that results in cure of hepatic amoebiasis.

Dhuley (1997) observed immunomodulatory effect of *Tinospora cordifolia* against immunosupression induced by carcinogen and ochratoxin. They observed significant decrease in chemotactic activity of murine macrophages and enhanced production of interlukin-1 and TNF in ochratoxin induced immunosupression and restoration of peptides, interlukin-1 and TNF following treatment of ethanolic extract of *Tinospora cordifolia* against carcinogenesis in mice.

Kapil and Sharma (1997) demonstrated IgG antibodies following treatment with *Tinospora cordifolia* in serum of mice. The humoral and cell mediated immune response was dose dependant. Further they isolated clerodane furano diterpene glycosides, syringin, cordion, cordioside and cordifoliosides A & B from *Tinospora cordifolia* which was responsible for anti-complement and immunostimulatory effect.

Chintalwar *et al.* (1999) reported that the aqueous extract of *Tinospora cordifolia* enhanced phagocytic and bactericidal activity of neutrophil and macrophages. They isolated arabinogalactan (polysaccharide) from stem extract of *Tinospora cordifolia* which was responsible for polyclonal mitogenic activity against B-cell and thus its immunomodulating potential.

Muffarrej Al *et al.,* (1999) reviewed effect of heat stress on the humoral antibody response, cell mediated immunomodulation related to breed and sex, metabolic, physiological and blood biochemical alterations due to heat stress. They mentioned reduction in delayed type of hypersensitivity and impairment of lymphocytes function observed due to heat stress in mice. The humoral and cell mediated immunity was due to rise in blood corticosterone and complex mechanism involved in nervous, endocrine and immunological interactions.

Bishayi *et al.* (2002) reported hepatoprotective and immunomodulatory effect of *Tinospora cordifolia* against carbon tetrachloride induced hepatotoxicity in rats. They observed significant reduction in serum glutamine oxaloacetate transaminase, glutamate pyruvate aminotransferase and alkaline phosphatase and billirubin by *T. cordifolia* extract at 100 mg/kg body weight dose for 15 days. The functional capacity of peritoneal macrophages was increased in rat. On the basis of observations they concluded that the *Tinospora cordifolia* extract may be the critical remedy against carbon tetrachloride induced liver toxicity and immunomodulation.

Diwanay *et al.* (2004) reported immunoprotective effect of extracts of *Withania sominifera* (Linn.), *Tinospora cordifolia* (Miers) and *Asparagus racemosus* (Wild) and its formulations in cyclophosphamide treated mouse ascetic sarcoma. There was increased white cell count, hameagglutination and haemolytic antibody titre with *Withania sominifera* and *Tinospora cordifolia* and alkaloid free polar fraction of *Withania sominiafera.*

Nair *et al.* (2004) isolated and characterized the alpha-D-glucan (polysaccharide) from *Tinospora cordifolia* and elucidated immunomodulatory

properties on lymphocyte and tumor cell line. They observed non cytotoxic and non proliferative effect of glucan on normal lymphocyte and tumor cell line but activation of macrophages, T-cells and B-cells. The killing of tumor cell line and activation of normal lymphocytes through synthesis of IL-1 beta, IL-6, IL-12, IL-12p40, IL-18, INF-gamma, TNF-alpha and monocyte chemoattractant protein without production of IL-2, IL-4, IL10, TNF-alpha and TNF-beta was dose dependent. Further there was no oxidative stress due to increased nitric oxide production by nitric oxide synthtase in lympohocyte. The oxidative stress, water solubility, high molecule activation of lymphocyte and cytokine profile were confirmed immunoprotective potential of alpha-D-glucan. There was significant action on macrophages which was dose dependent.

Khobragade *et al* (2005) studied the heamo-immuno-biochemical profile of broilers fed with herbal supplemented feed containing 0.5 % and 1% levels of the plant powder of *Tinospora cordifolia* and *Leptadenia reticulata.* In their investigation they observed that there was significant increase in PCV, blood plasma glucose, phosphorus and total lipid levels. They also studied immunomodulatory potential of this plant and reported that the combination of 1% levels of the both plants showed higher HI titer and significantly higher skin thickness with the 1% level of *Leptadenia reticulata.* They concluded that supplementation of *Tinospora cordifolia* and *Leptadenia reticulata* improves the immune status in commercial broilers.

Singh (2005) reported antimalarial activity of aqueous extract of *Tinospora cordifolia* in combination with chloroquine against hyperactive malarious spleenomegaly patients. The aqueous extract and chloroquine base for first 6 weeks followed by later 6 weeks were showed regression of spleen, decreased IgM and increased hemoglobin in the recovered patients.

Nair *et al.* (2006) investigated immunomodulatory potential of (1, 4) - alpha-d-glucan obtained from *Tinospora cordifolia* against RAW 264.7 macrophages. The phygocytosis, binding and internal ligation of opsonized zymosan A was inhibited by glucan, however macrophages treated with anti CD11b mAb fallowed by glucan did

not show inhibitory effect on TNF-alpha synthesis. They found that the glucan induced TNF-alpha synthesis, translocation, cytokine production was responsible for immune system activation in dose dependent manner which was inhibited by NF K-beta inhibitors such as caffeic acid phenethyl ester or curcumin.

Spleman *et al.* (2006) revived immunomodulatory activity of herbal medicine including *Tinospora cordifolia in vitro* and *in vivo.* They observed modulation of cytokines viz. IL-1, IL-6, TNF and INF by *Tinospora cordifolia.* The broad spectrum effect of cytokines in autoimmune disorders and chronic degenerative changes suggested further investigation.

Desai *et al.* (2007) investigated protective effect of arabinogalactan polysaccharide "G1-4A", obtained from stem of *Tinospora cordifolia* against endotoxin induced sepsis in mice. They observed 100% protection against lipopolysaccharide induced mortality in pretreated mice with G1-4A due to increase in serum TNF-alpha and IL-1-beta which was subsequently reduced with challenged lipopolysaccharide followed by G1-4A treatment. The endotoxic shock was modulated by G1-4A through enhanced soluble TNF- alpha and release of nitric oxide from murine macrophage.

Siddiqui *et al* (2007) studied the effect of supplementation of *Withania somnifera* and *Tinospora cordifolia* on haematological parameters in experimentally induced immunosuppression by cyclophosphamide in broiler birds. They observed that the combined treatment with *Withania somnifera* and *Tinospora cordifolia* were significantly increased TLC, lymphocytes, Hb, and PCV as compared to immunosuppressed birds of control group. Finally they concluded that if these plants fed in combination regularly than single treatment have additive effect leading to prevention of immunosuppression in broiler birds.

Ghamdi (2008) reported effect of heat stress on immunomodulatory response in broiler chickens exposed to high temperature during summer in closed system. They observed significant decrease in plasma antibodies (IgM, and IgG)

level in all experimental groups during heat stress but lymphocytes, neutrophil and neutrophil lymphocyte ratio were significantly differed in all experimental groups which was indicative of attenuated immunoresponse due to heat stress in chickens.

2.7 Medicinal properties and uses

Chopra *et al.* (1956) have documented medicinal properties and therapeutic uses of *Tinospora cordifolia* stem such as bitter, stomachic, antipyretic, alternative and aphrodisiac. Stem juice in fever, while mixed in honey in the treatment of jaundice, stem decoction mixed in fruits of *Piper longum* and honey in fever associated with cough, while mixed in sweet oil in encephalitis (Chatterjee and Pakarashi, 1991). Starch obtained from stem aqueous extract as tonic (Ambasta, 1986), anti-diarrhoeal, antidysentric and antacid, leaf aqueous extract in fever (Chatterjee and Pakrashi, 1991) leprosy. Roots are valuable as emetic in the visceral obstructions. Fruits are valuable in jaundice and rheumatism (Ambsta, 1986). Other parts in general debility, dyspepsia, fever and urinary diseases (Negi and Pant, 1994).

Ali (1990) has mentioned that more than two hundred indigenous drugs including *Tinospora cordifolia* (gilo) documented in the Unani Materia Medica.

Katewa and Arora (1997) mentioned folklore uses of bark paste of *Tinospora cordifolia* in the treatment of piles, swollen rectum. Raw stem pieces in rheumatism are used in Udaipur District of Rajasthan.

Kumar and Jain (1998) documented traditional uses of leaf juice and stem extract of *Tinospora cordifolia* in fever, jaundice and as antidote in snake bite is being practiced by tribal peoples of Prakasam District in Andra Pradesh (India).

Sharma *et al.* (2005) mentioned medicinal properties of *Tinospora cordifolia* such stem as bitter, astringent, sweet, thrombogenic, anodyne, antihelmentic,

alternate, antiperiodic antifungal, antipyretic, antiemetic, digestive, carminative, stomach appetizer, and so on and is valuable in burning sensation, jaundice, hyperdyspepsis, vomiting, fracture, fever, inflammation, gout, debility, skin diseases, leprosy, anemia, cough, asthma, infertility, urinary disorders, rheumatoid arthritis and eye diseases, leaf in cure of jaundice.

2.8 Drug Interaction Studies

Harle and Gaikwad, (2004) showed increased absorptive permeability of dexamethasone on isolated rat ileum ex vivo fallowing acute (400mg/kg PO) and chronic (150 mg/kg/day for 5 days) treatment with light petroleum ether extract of *Tinospora cordifolia* in male wistar rats. They suggested that the inhibitory effect of *Tinospora cordifolia* on P-glycoprotein deserves herb drug interaction and increased absorptive permeability of dexamethasone.

Material & Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Plant material

The *Tinospora cordifolia* Miers (Family: Minospermicaea) stem part was employed in this investigation.

3.1.1 Collection and authentication of plant materials

Tinospora cordifolia stems were collected from University campus of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and confirmed from the Botanist cum Director of the Nagarjun Medicinal Plant Garden of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra.

3.2 Processing of Plant Materials

The fresh, matured stems *of Tinospora cordifolia* were collected, weighed and chopped into small pieces (Plate. II) with the help of cutter was shade dried in the department laboratory. The moisture content was found to be 57.14% (Dry yield on wet-weight basis 42.68%). Dried plant material was powdered through pulverizing mill of local folk vender. Powder thus obtained was sieved (67.8% kg w/w) to get fine powder (Plate. III) was employed in this investigation.

3.2.1 Extraction

Fine powder of *T. cordifolia* was subjected to soxhelet's extraction using aqueous and ethanolic solvents. For the sake of uniformity in the extraction and to determine percentage extractability of each extract 50 gm of plant material was used in each trial.

Plate. II: Chapped stem of *Tinospora cordifolia* Miers.

Plate. III: Stem powder of *Tinospora cordifolia* Miers.

3.2.1.1 Preparation of Extraction Thimble

Extraction thimble was prepared using ordinary filter paper (size 12x3 cm) into which the plant material was placed. Then the thimble was sealed at both the ends using sterilized adsorable cotton. The plant material containing thimble was tightened with the help of sterilized cotton thread.

3.2.1.2 Preparation of Aqueous Extract

Fifty grams of stem powder of *Tinospora cordifolia* subjected to soxhlet's extraction using distilled water. The plant material was placed in extraction thimble and was inserted into soxhelt's reservoir. Distilled water filled 2/3rd capacity of the reservoir up to demarcated mark was flushed in to the heating flask.

The content was boiled and recycled till the colorless solvent reappear in the reservoir. This process was repeated several times to get aqueous extract. The solvent extract was cooled and was filtered through what man filter paper No.1. It was transferred to previously weighed petri plates and evaporated at room temperature under laminar air flow. The residue (extract) was stored under refrigeration was used as an when required in this investigation.

3.2.1.3 Preparation of Ethanolic Extract

Fifty grams of stem powder of *Tinospora cordifolia* was subjected to soxhlet's extraction using ethanol. Similarly procedure as described above was followed for preparation of ethanolic extract.

3.2.1.4 Extractability Percentage

The percentage extractability of both the aqueous and ethanolic extracts was determined taking difference in the weight of extract before and after weighing the petri plates with and without extract. For the preparation of different concentrations such as 100 %(1gm/ml), 50% (0.5gm/ml) and 10% (0.1gm/ml) for respective extracts both the crude extracts were weighed and then reconstitute in their

respective solvents. For 100%, 50% and 10% the stock solution as 5 gm/5ml, 2.5 gm/5ml and 0.5 gm/5ml was prepared These extracts were evaluated for physicochemical properties such as color, consistency, odor and test.

3.3 Preparation of Herbal Discs

The sterilized blank filter paper discs (Himedia) were collectively weighed taking 20 discs at each time with the help of electronic weighing balance. Similar procedure was repeated thrice. Average weight of each blank disc so obtained was 12 mg. As there was no difference in average weight of blank discs, blank discs were separately impregnated with the aqueous and ethanolic extracts at at 100 %(1gm/ml), 50% (0.5gm/ml) and 10% (0.1gm/ml) concentrations taking twenty discs at each time. When each extract was completely dissolved in their respective solvents, it was taken in tuberculin syringe and was impregnated drop by drop in each disc until the disc until disc was fully saturated. These discs were air dried at room temperature under laminar airflow and again collectively weighed thrice taking 20 discs at each time.

Accordingly collective weight of extract disc of different concentrations was obtained and the extract actually adsorbed in single disc was determined. This procedure was repeated thrice. These discs were stored in airtight screw capped vials was used as and when required in this investigation.

3.4 Antibacterial Studies

Both the aqueous and ethanolic extracts of stem of *Tinospora cordifolia* herbal discs at 100 %(1gm/ml), 50% (0.5gm/ml) and 10% (0.1gm/ml) concentrations were evaluated for antibacterial activity against four different pathogenic bacteria as per the disc diffusion and tube dilution methods described by Bauer *et al.* (1966) and Cruickshank *et al.* (1975) *In vitro*. Where one of the bacteria namely *Streptococcus*

pyogenes that was proposed to screen was not available from the commercial source, hence the study was carried out using four different bacteria detailed as below.

3.4.1 Bacterial Culture

Lyophilized pure pathogenic bacterial culture namely *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2453) and *Salmonella typhimurium* (MTCC 1251) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh were employed in this study. After thawing ampoule sealed lyophilized bacterial culture at room temperature was sub-cultured in nutrient broth (Himedia) was maintained in the Department of Veterinary Pharmacology and Toxicology, Akola were employed in this study.

3.4.2 Disc diffusion test

Each of the four bacterial cultures viz. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonellla typhimurium* were grown in nutrient broth at 37^0C for overnight incubation. The bacterial culture was diluted using sterile nutrient broth (10^{-3} to 10^{-9}). Each dilution of bacterial culture was poured on nutrient agar plates incubated at 37^0C then the excess broth in the plates was discarded after three minutes of incubation. Then the surface of nutrient agar was swabbed with sterile cotton and herbal discs were placed along with the reference drug disc with the help of sterile forceps aseptically. it was incubated at 37^0C. After 24 hours of incubation petri-plates were examined for zone of inhibition was measured in mm scale and also compared with the reference standard drug ciprofloxacin (5-ug/disc).

For herbal-drug interaction studies similar procedure was followed with the most potent extract and its combination with reference drug ciprofloxacin and the zone of inhibition was measured in mm scale.

3.4.3 Tube dilution test

On the basis of results of disc diffusion test potent extract concentrations were selected for screening antibacterial activity by tube dilution test. A loopful of 24-hours-old broth culture of each bacterium under test was revived in 5 ml of nutrient broth taken in test tube and was incubated at 37^0C for 4 hours. Bacterial dilution 10^{-3} to 10^{-9} was made. In each dilution two extract-impregnated herbal discs was added and were incubated overnight at 37^0C for overnight incubation. 50 µl of each bacterium was plated in Petri plate was covered with nutrient agar was further incubated at 37^0C for 24 hrs. The colony count of respective bacterium was assessed in each petri plates during pilot study. Based on observations 10^{-9} bacterial dilution was selected for the final test.

The bacterial dilution 10^{-9} was used in the final test and the colony count of respective bacteria was assessed. Similar procedure was followed as described above for final test.

3.5 Minimal Inhibitory Concentration

The lowest concentration of the extract that inhibited the growth of bacteria *in vitro* referred as MIC. Therefore MIC of ethanolic stem extract of *Tinospora cordifolia* determined spectrophotometrially observing optical density per the method described by Pelczar *et al.*, (1986) involving tube dilution technique. A set of test tubes containing bacterial dilution (10^{-9}) was added with increasing amount of extract.

In each set one test tube were kept as bacterial control (no extract) and other test tubes as extract control (no bacteria). The bacterial control and respective bacterial culture added with graded amount of the extract with the extract control was incubated at 37^0C for 24 hours.

The concentration of ethanolic stem extract of *Tinospora cordifolia* inhibiting growth of bacteria was assessed spectrophotometrically. The optical density of each treatment was determined at wavelength 490-nm. The absence of growth or change in turbidity was assessed by measuring optical density (O.D.). Thus, the MIC of ethanolic extract of stem of *Tinospora cordifolia* that prevent the growth of bacteria *In-vitro* was determined.

3.6 Phytochemical studies

Both the stem extracts of *Tinospora cordifolia* was analyzed for presence of active phytochemical constituents employing various chemical tests (qualitative analysis) as the methods described by Trease and Evans (1985), Rosenthaler, (1930).

3.6.1 Test for Alkaloids

A small amount of the extract was added to 1.5 % v/v hydrochloric acid and was filtered through whatman No.1 filter paper. Filtrate thus obtained was used for testing presence of alkaloids in the extract.

3.6.1.1 Dragendroff's reagent

17 grams of bismuth sub-nitrate and 200gm tartaric acid dissolved in 800 ml of distilled water (Solution-A). 160 gm Potassium iodide mixed in 400 ml distilled water (Solution-B). For preparation of final reagent equal proportion v/v (1:1) of both A and B solutions were added.

Working standard was prepared using 50 ml of prepared reagent solution added with 100 gm tartaric acid and adjusted volume of working standard up to 500 ml in distilled water. This reagent spread on Whatman's filter paper was dried. Test

extract was applied on pretreated reagent filter paper with the help of capillary tube. Development of orange to red color indicates presence of the alkaloids.

3.6.1.2 Mayer's reagent (Potassium mercuric iodide reagent)

1.36 gm of mercuric chloride was dissolved in 60 ml of distilled water and 5 gm of potassium iodide dissolved in 10 ml of distilled water. It was mixed and diluted up to 100 ml volume using distilled water for preparation of Mayer's reagent. Little amount of test filtrate added with few drops of the above reagent was taken in a watch glass. Development of cream colure precipitate indicating presence of alkaloids.

3.6.1.3 Wagner's reagent

The reagent was prepared by dissolving 1.27 gm of iodine and 2 gm of potassium iodide in 5ml of distilled water and was diluted up to 100 ml. The reagent added in test filtrate. The appearance of flocculent brown perception was suggestive for the presence of alkaloids.

3.6.1.4 Marme's reagent (Potassium cadmium iodide)

20 gm potassium iodide is dissolved in 20 ml of water and solution is added to 10 gm of cadmium iodide dissolved in 50 ml of water. This reagent added with test filtrate develops yellow precipitate which is amorphous at first and then turns crystalline. The matrix is soluble in excess of reagent and ethanol indicating the presence of alkaloids.

3.6.2 Test for Reducing Sugars

3.6.2.1 Molisch's test

Few mg of test extract dissolved in 0.5 ml of water. It was mixed with 2 drops of 10% solution of alpha-naphthol in alcohol and was added 1 ml of concentrated sulphuric acid from the side of inclined test tube, so that the acid formed a layer beneath the aqueous solution. Appearance of red ring at common surface of immiscible liquids was indicative the presence of sugars.

3.6.2.2 Fehling's solution test

Fehling's A (Copper sulfate solution) and B (Sodium potassium tartarate in alkali) was mixed in equal proportion and was added in test extract. It was heated. Development of red precipitation of cuprous oxide indicates the presence of reducing sugar.

3.6.2.3 Barfoed's test

This reagent is prepared by dissolving 13.3 gm of crystalline neutral copper acetate in 200 ml of 1% acetic acid solution. The test extract dissolved in water was heated with a little amount reagent. Appearance of red colour to the test due to formation of cuprrious oxide within two minutes indicates presence of monosaccharides.

3.6.2.4 Benedict's reagent

Fehling's reagent (0.5 ml) added with few mg of extract was heated in water bath for 10 minutes. Development of red precipitation indicates presence of reducing sugars.

3.6.3 Test for Glycosides

3.6.3.1 Benedict's reagent

Contents of the test used in reducing sugar was filtered and added with dilute hydrochloric acid. pH of is adjusted to alkaline. Equal volume of filtrate and benedict's reagents was mixed and heated. Development of brownish color indicates presence of glycosides.

3.6.3.2 Fehling's reagent

Contents of the test employed in reducing sugar were cleared by adding few drops of hydrochloric acid and was boiled for 5 minutes. Fehling's reagent added further to record any change in reduction. Appearance of brownish red color to the test indicates presence of glycosides.

3.6.4 Test for Tannins

Ethanol extract is taken separately in water was warmed and filtered. The filtrate was used for estimation of tannins.

3.6.4.1 Ferric chloride test

Few drops of ferric chloride solution were added to a little quantity of filtrate. Appearance of green color to the test indicates presence of tannins.

3.6.4.2 Potassium dichromate test

Test filtrate was added with potassium dichromate appearance of dark yellow color indicates presence of tannins.

3.6.4.3 Lead acetate test

Few drops of lead acetate solution added to the test filtrate. Development of precipitation indicates presence of tannins.

3.6.5 Test for Sterol

3.6.5.1 Salkowski's reaction

Little amount of test extract mixed in chloroform in which 2 ml of sulfuric acid was added by sides of test tube. The contents was shaken for few minutes imparting red color in chloroform layer and a greenish yellow fluorescence to the lower layer was indicative presence of sterols.

3.6.6 Test for Anthraquinones

3.6.6.1 Borntrager's test

A little amount of test extract was added to 5 ml of 10% sulfuric acid was boiled for few minutes in test tube. The hot content was filtered through Whatman No.1 filter paper and then cooled. The contents were shaken with benzene and half of its volume with 10 % ammonia solution. Development of pink ring to the ammonical layer indicates presence of anthraquinones.

3.6.7 Test for Flavonoids

A small quantity of test residue is dissolved in 5 ml ethanol (95%) and treated with few drops of concentrated hydrochloric acid and 0.5 mg of magnesium turnings. Development of pink or magenta color within 3 minutes indicates presence of flavonids.

3.6.8 Test for Proteins

3.6.8.1 Biuret test

Few mg of residue was mixed in water and added to 1ml of sodium hydroxide (4% w/v) solution followed by 2 ml copper sulfate. Appearance of violet or pink color to the test showed presence of proteins.

3.6.8.2 Xanthoprotein test

Little amount of test extract was dissolved in water and added with 0.5 ml of concentrated nitric acid. Development of white or yellow color indicates presence proteins.

3.6.9 Test for Amino Acids

3.6.9.1 Ninhydrine test

A small amount of test extract was added in alcoholic solution of Ninhydrine 0.1% w/v. Development of violet or purple color indicates presence of amino acids.

3.6.10 Test for Saponins

3.6.10.1 Foam test

A few mg of test extract added in small amount of water and sodium bicarbonate solution. The contents were vigorously then shaken. Formation of froth to the test was considered for presence of saponins.

3.7 Immunological Studies

3.7.1 Procurement and maintenance of experimental birds:

Thirty boiler birds weighing 350 to 360 gm with average age of 3 week immunized on farm were procured from M/s, Tranbakeao gavande commercial poultry farm, akola. They are randomly divided into three groups. Each group was comprised of 10 birds. The experimental protocol was as under.

Group I — This group of birds were kept as untreated control under normal feeding and watering conditions.

Group II — This group of birds was given *T. cordifolia* stem powder @ 4 g/kg feed fed for three week (21 days)

Group III — This group of birds was given *T. cordifolia* stem powder @ 8 g/kg fed for three week (21 days)

All the experimental birds were sensitized at the end of 6^{th} week after 21 post treatment days and were challenged at the end of 8^{th} week were naturally exposed to hot environmental temperature during summer (46^0C to 48 ^{0}C ± 2 ^{0}C). These groups of birds were maintained on deep litter system at Poultry Research Center, PG Institute of Veterinary Sciences, Akola was fed with commercial diet procured from M/s, Tranbakeao gavande commercial poultry farm, akola.. Free access of drinking water (RO water) was given during experimental period.

3.7.2 Physical parameters

3.7.2.1 Feed consumption

The average feed consumption of different experimental groups of birds was recorded from total feed offered to respective group of birds. On the basis of

average weekly feed consumption per bird during entire experimental period was recorded.

3.7.2.2 Body weight/ gain

The average weight of different groups of birds before the treatment and after treatment was recorded. From respective weekly weight, the initial weight (3rd week) of each bird was subtracted. On this basis average weekly weight gain per bird in respective treatment group was determined.

3.7.2.3 Feed conversion ratio

On the basis of results of average weekly body weight gain and average weekly feed consumption per bird was determined in respective groups.

3.7.2.4 Water intake

The water intakes in different group of experimental birds were calculated differencing the amount of drinking water offered and left over per day /per group of birds. On this basis average weekly water intake per bird was determined group wise. The average water intake during the experiment in each group was recorded.

3.8 DNCB skin hypersensitivity reaction

Cell mediated immune (CMI) response was assessed following sensitization and challenge with 1% 2-4 Dinitroclorobenzene (DNCB) in acetone. All three experimental groups of birds were sensitized at the end of 6th week and challenged after 14days at the end of eight week to assess CMI as per the method described by Valsala *et al* (1981) and Sreeramulu (1989).

3.8.1 Preparation of site

The thoracolumbar site lateral to the vertebral column on both left and right were selected and prepared for testing. The feathers were removed. The sensitizing dose was applied at left side while challenging dose was applied at the right side of thoracolumbar region.

3.8.2 Sensitizing with DNCB

While sensitization, 0.2 ml of 1% DNCB solution was poured drop by drop on thoracolumbar region using 1ML tuberculin syringe. The test area was demarcated by a metallic ring of 2 cm diameter having raised border .Thus, diameter of area exposed during challenge and sensitization remained the same. The running down of DNCB in surrounding area was prevented by allowing the solution to get evaporated with mouth blowing.

3.8.3 Challenging with DNCB:

The challenge dose of DNCB (0.2 ml) was applied to the area apposite to the site of sensitization on 14^{th} day post sensitization.

3.8.4 Measurement of challenging area:

The thickness of skin and diameter of spreading lesion at the site of challenge, indicating reaction zone, was measured at 24, 48, and 72 hours after challenging with DNCB by using vernier calipers.

3.9 Statistical analysis

Data of this study was statistically analyzed by CRD, factorial CRD (Snedcor and Cochran, 1967). The values were considered as significant at 1% ($P<0.001$) and 5% ($P<0.05$) level of significance.

Results & Discussion

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation was carried out to evaluate antibacterial activity of stem extracts against pathogenic bacteria by disc diffusion and tube dilution techniques *in vitro* and immunomodulatory potential of stem powder of *T. cordifolia* in poultry. The results of various investigations are described in this chapter.

4.1 Extractability

Table 1 and Fig 1 depicted percent extractability and some physical properties of aqueous and ethanolic stem extract of *Tinospora cordifolia.* The average yield of extract from 50 gm stem powder of *T. cordifolia* was found to be 2.42 gm and 2.15 gm in aqueous and ethanolic solvents respectively. Percentage of extractability of these extracts was 4.83 and 4.29. There was significant difference in amount of extract obtained however there was no significant difference observed in percent extractability of both the extract.

It might be due to difference in solubility of extract in different solvent. Both the extracts were radish brown to yellowish brown in color under normal light and green under UV light. These extracts were solid and sticky in consistency, bitter in taste and pleasant to mint in odour.

4.2 Herbal discs

Table 2 and Fig 2. depicted amount of aqueous extract of *T. cordifolia* adsorbed at 10, 50 and 100 percent concentrations on to the discs and the actual amount of extract in each disc. There was no significant difference in the weight

Table 1. Percent extractability and some physical properties of *Tinospora cordifolia* Miers stem.

Type of extract	Solvent used	Powder used* (gm)	Extract obtained* (gm)	Percent Extractability	Color		Consistency	Taste	Odor
					Normal	UV			
Aqueous (hot)	Distilled water	50.00 ± 0.00	02.42 [a] ± 0.08	4.83	Reddish brown	Green	Solid sticky	Bitter	Pleasant
Ethanolic (hot)	Ethanol	50.00 ± 0.00	02.15 [b] ± 0.32	4.29	Yellowish brown	Green	Solid sticky crystalline	Bitter	Mint

* Mean of three observations ± SE, Mean value of superscript "a" significantly differs from "b" ($P < 0.05$)

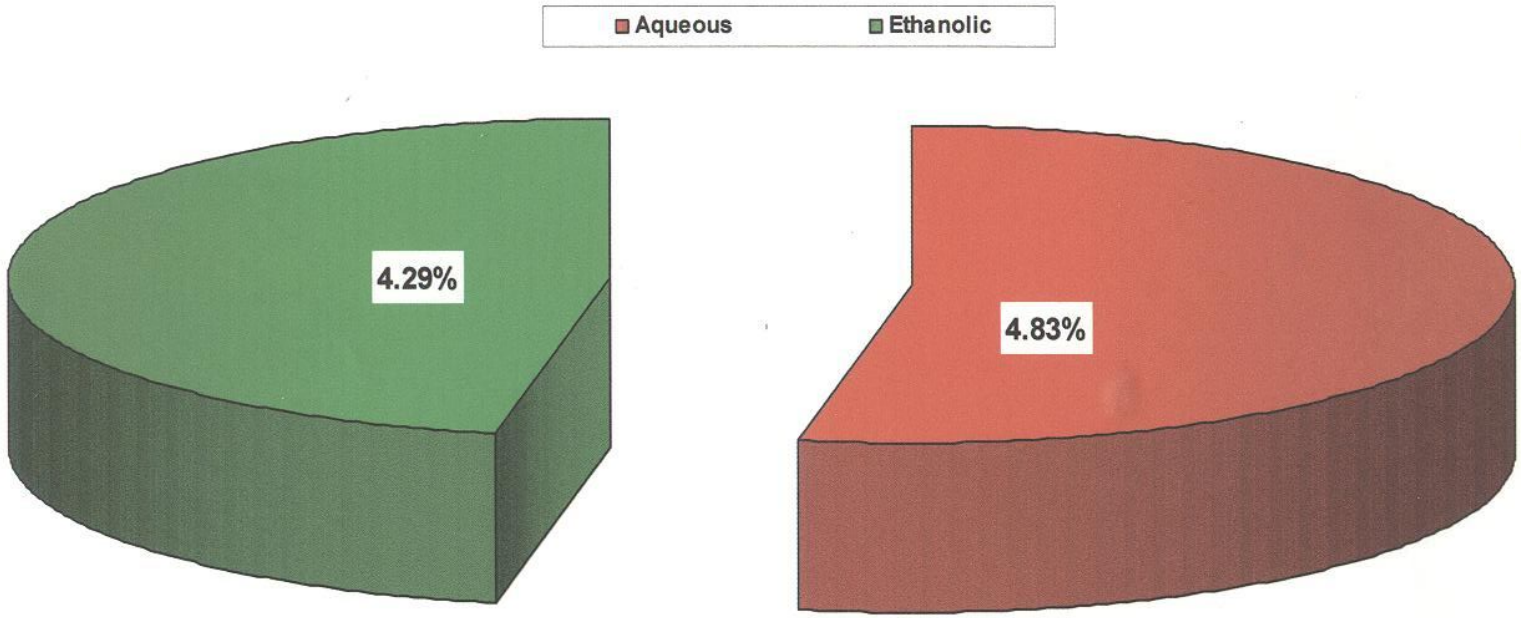

Fig.1 Showing Percent Extractability of aqueous and ethanolic hot extracts of *Tinospora cordifolia*

Table 2. Amount of aqueous extract of *Tinospora cordifolia* stem impregnated on the blank disc/ discs

Type of Extract	Concentrations (g/ml)	Weight (mg)		
		Blank Disc§	Extract Impregnated Disc§	Extract in each disc
Aqueous (hot)	1.0 gm/ml (100%)	250 250 250	700 702 705	22.5 22.6 22.75
	Mean ±SE	**250±0.00**	**702.33*[a] ±1.45**	**22.67**[a] ± 0.07**
	0.5g/ml (50%)	250 250 250	603 600 600	17.65 17.50 17.00
	Mean ±SE	**250±0.00**	**601.00[b] ±1.00**	**17.17[b] ± 0.17**
	0.1g/ml (10%)	250 250 250	530 529 529	15.00 13.95 13.95
	Mean ±SE	**250±0.00**	**529.33[c] ±0.33**	**13.97[c] ± 0.01**

§= Weight of twenty discs at each time

**significantly higher (P<0.01) than other extracts

* significant (P<0.01) than other extracts

Means with similar superscript are not significantly differs

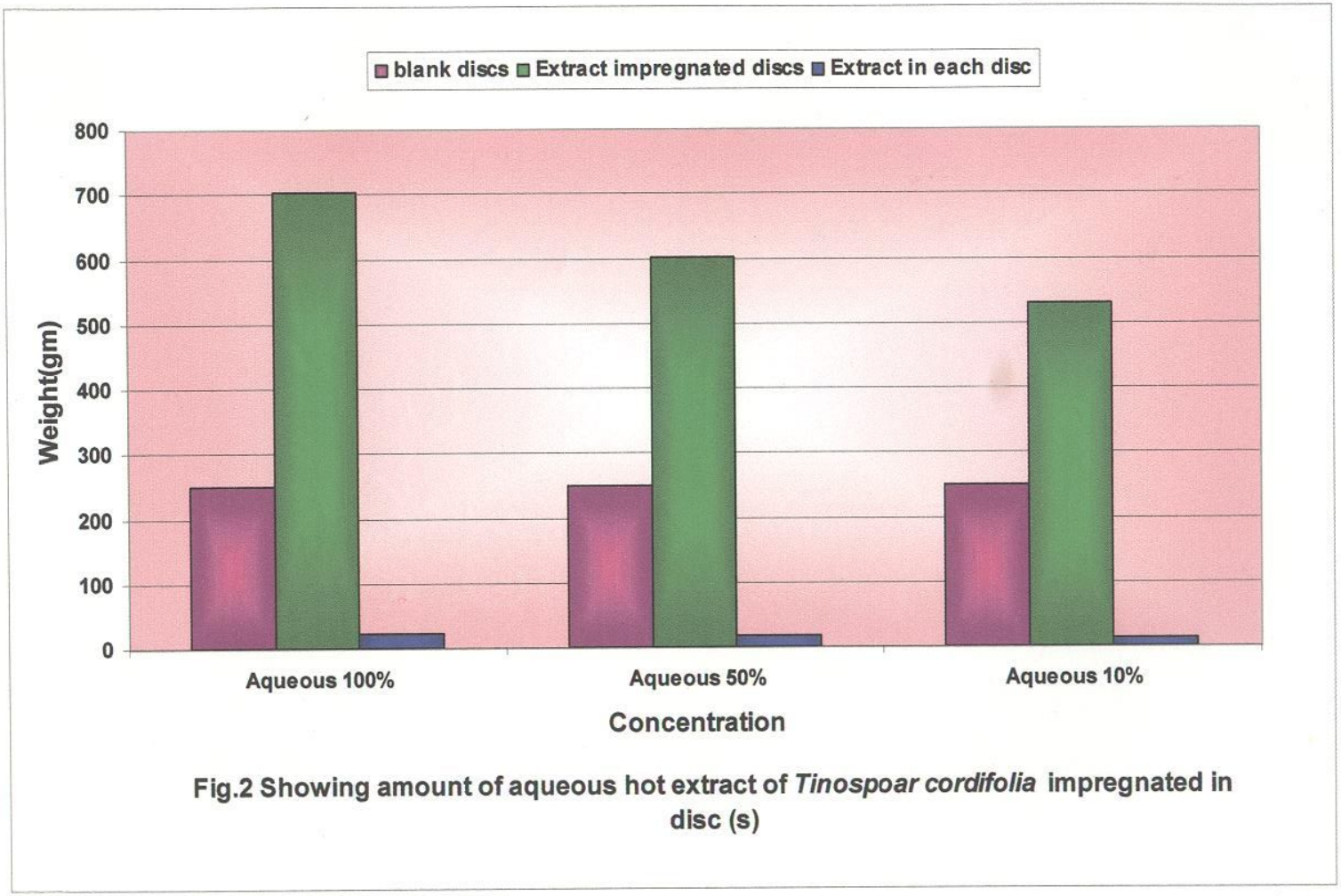

Fig.2 Showing amount of aqueous hot extract of *Tinospoar cordifolia* impregnated in disc (s)

blank discs (250.00±0.00mg) after several repeated trials. The amount of aqueous extract at 100% concentration adsorbed on the discs (702.33±1.45 mg) was significantly higher than 50% (601.00±1.00mg) and 10 % 529.33±0.33 mg) concentrations and the amount extract in each disc (22.67± 0.07mg) was also significant than other extract concentrations.

An amount of ethanol stem extract of *T. cordifolia* adsorbed on to the disc (s) is presented in Table 3 and Fig 3. The weight of twenty blank discs was identical after several repeated trials. The weight of extract impregnated discs at 10 % concentration (613.67±0.33mg) was significantly higher($P<0.01$) than at 50% (564.67 ± 0.88 mg) concentration and 100% (540.67±0.67mg) concentration. The amount of extract impregnated in each disc was also found significant ($P<0.01$) at 10% concentration (18.30 ± 0.10mg) than 50% (15.33±0.04 mg) and 100% (14.53±0.03mg) concentration.

It is clear from the above results that the adsorption of aqueous and ethanolic hot extract on blank disc was not uniform which varied from solvent to solvent. This might be due to varied active constituents and its solubility in different solvents with different molecular weights and density. The heat treatment might have influence on solubility of the constituents. Significant difference in weight of extracts pointed out in this study might be due to difference in physico-chemical properties of the extract and its impregnation on the disc (s). Weight of aqueous extract varies from 100% > 50% > 10% and ethanolic extract 10%> 50%> 100%. No literature was available to support these findings.

4.3 Antibacterial studies

The antibacterial activity of stem extracts of *T. cordifolia* at different concentrations was evaluated against four different pathogenic bacteria by disc diffusion and tube dilution methods. The results of this investigation are described parameter wise under this heading.

Table 3. Amount of ethanolic extract of *Tinospora cordifolia* stem impregnated on the blank disc/ discs

Type of Extract	Concentrations (g/ml)	Weight (mg)		
		Blank Disc§	Extract Impregnated Disc§	Extract in each disc
Ethanolic (hot)	1.0 gm / ml (100%)	250	542	14.6
		250	540	14.5
		250	540	14.5
	Mean ±SE	**250±0.00**	**540.67 ^{c} ±0.67**	**14.53 ^{c} ±0.03**
	0.5g/ml (50%)	250	566	15.8
		250	563	15.65
		250	565	15.75
	Mean ±SE	**250±0.00**	**564.67 ^{b} ±0.88**	**15.33 ^{b} ±0.04**
	0.1g/ml (10%)	250	614	18.2
		250	614	18.2
		250	613	18.5
	Mean ±SE	**250±0.00**	**613.67** ** ^{a} ±0.33**	**18.30** ** ^{a} ±0.10**

§= Weight of twenty discs at each time Means with similar superscript are not significantly differs
**Significantly higher (P<0.01) than other extracts

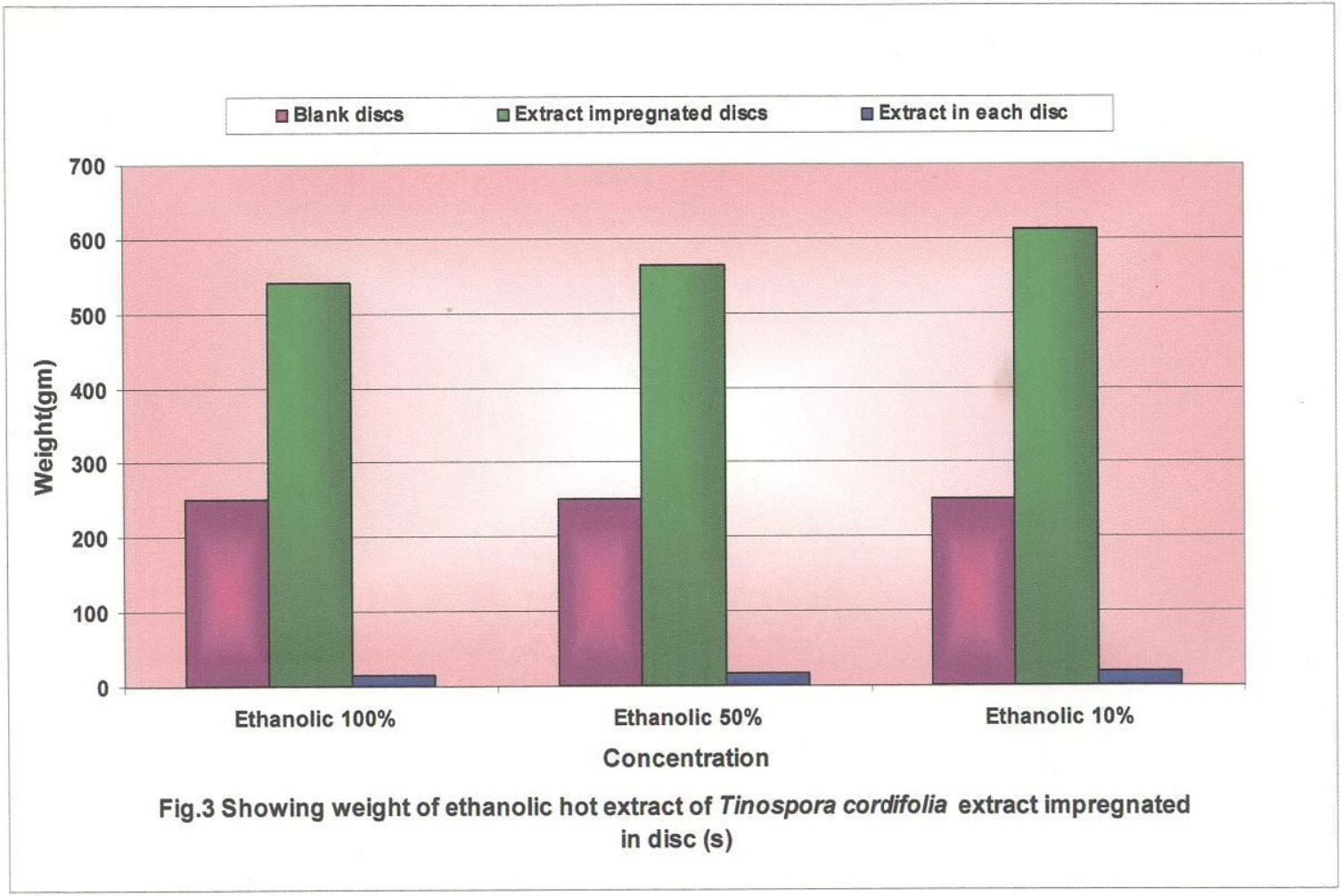

Fig.3 Showing weight of ethanolic hot extract of *Tinospora cordifolia* extract impregnated in disc (s)

4.3.1 Disc diffusion method

Zone of inhibition of *T. cordifolia* extracts and reference drug ciprofloxacin against *Escherichia coli* is given in Table 4 and Plate IV. The mean zone of inhibition of aqueous extract at 100% (9.33±0.67mm) concentration was significant than 50% (7.67±0.67mm) concentration but 10 % concentration was found ineffective. Where average zone of inhibition of ethanol extract 50% (9.67±0.33mm) and 10% (9.00±0.33mm) concentration was statistically similar and was ineffective at 100% concentration. Zone of inhibition produced by both the extracts was significantly ($P<0.01$)lesser than ciprofloxacin (28.00±1.00 mm).

Table 5 and Plate V. Shows zone of inhibition of different extracts of *T. cordifolia* and reference drug ciprofloxacin against *Pseudomonas aeruginosa*. Both the aqueous and ethanolic extracts at different concentrations were showed ineffectiveness of the extracts against *Pseudomonas aeruginosa*.

Mean zone of inhibition of *T. cordifolia* stem extracts against *Salmonella typhimurium* are presented in Table 6 and Plate VI . The aqueous extract was ineffective against *Salmonella typhimurium* at different concentrations. The ethanol extract 50% and 10% concentrations were showed statistically similar zone of inhibition (11.33±0.67mm and 10.00±0.57mm) which was comparatively significantly lesser ($P<0.01$) than ciprofloxacin (25.33±1.20mm), a reference standard drug.

Average zone of inhibition of *T. cordifolia* extracts at different concentrations against *Staphylococcus aureus* are given in Table 7 and Plate VII. Zone of inhibition of aqueous extract at 100% and 10% concentrations were 7.67±0.67mm and 10.22±1.92 mm respectively which was comparatively lesser ($P<0.01$) than reference drug ciprofloxacin (37.33±0.88mm). However 50 % concentration was found ineffective. The ethanol extract 100%, 50% and 10% concentrations were showed zone of inhibition 11.33±2.18 mm, 7.67±0.67mm and 7.00±0.00 mm respectively which was comparatively much lesser ($P<0.01$) than ciprofloxacin (37.33±0.88 mm), a reference standard drug.

Table 4. **Zone of inhibition of different extracts of *Tinospora cordifolia* and reference drug (ciprofloxacin) against *Escherichia coli***

Extracts	Zone of inhibition (mm) ± SE§			Ciprofloxacin (5 µg/disc)
	1.0 gm / ml (100%)	0.5g/ml (50%)	0.1g/ml (10%)	
Aqueous (hot)	9.33 [a] ± 0.67	7.67± 0.67 [b]	No Zone	28.00 ±1.00 **
Ethanolic (hot)	No Zone	9.67 [a] ± 0.33	9.00 [a] ± 0.33	

§= mean of three observations, Means with similar superscript are not significantly different,
**Significantly higher (P<0.01) than extracts

Table 5. **Zone of inhibition of different extracts of *Tinospora cordifolia* and reference drug (ciprofloxacin) against *Pseudomonas aeruginosa***

Extracts	Zone of inhibition (mm) ± SE§			Ciprofloxacin (5 µg/disc)
	1.0 gm / ml (100%)	0.5g/ml (50%)	0.1g/ml (10%)	
Aqueous (hot)	No Zone	No Zone	No Zone	37.66±3.71
Ethanolic (hot)	No Zone	No Zone	No Zone	

§= mean of three observations,

Plate. IV **Zone of inhibition by different stem extracts *of T. cordifolia* and ciprofloxacin against *Escherichia coli***

A : Aqueous extract 100% (1gm/1ml)

B : Aqueous extract 50% (0.5gm/1ml)

C : Aqueous extract 10% (0.1 gm/1ml)

D : Ethanolic extract 100% (1gm/1ml)

E : Ethanolic extract 50% (0.5gm/1ml)

F : Ethanolic extract 10% (0.1gm/1ml)

G : Reference standard drug Ciprofloxacin (5µg/disc)

Plate. V **Zone of inhibition by different stem extracts *of T. cordifolia* and ciprofloxacin against *Pseudomonas areuginosa***

A : Aqueous extract 100% (1gm/1ml)

B : Aqueous extract 50% (0.5gm/1ml)

C : Aqueous extract 10% (0.1 gm/1ml)

D : Ethanolic extract 100% (1gm/1ml)

E : Ethanolic extract 50% (0.5gm/1ml)

F : Ethanolic extract 10% (0.1gm/1ml)

G : Reference standard drug Ciprofloxacin (5µg/disc)

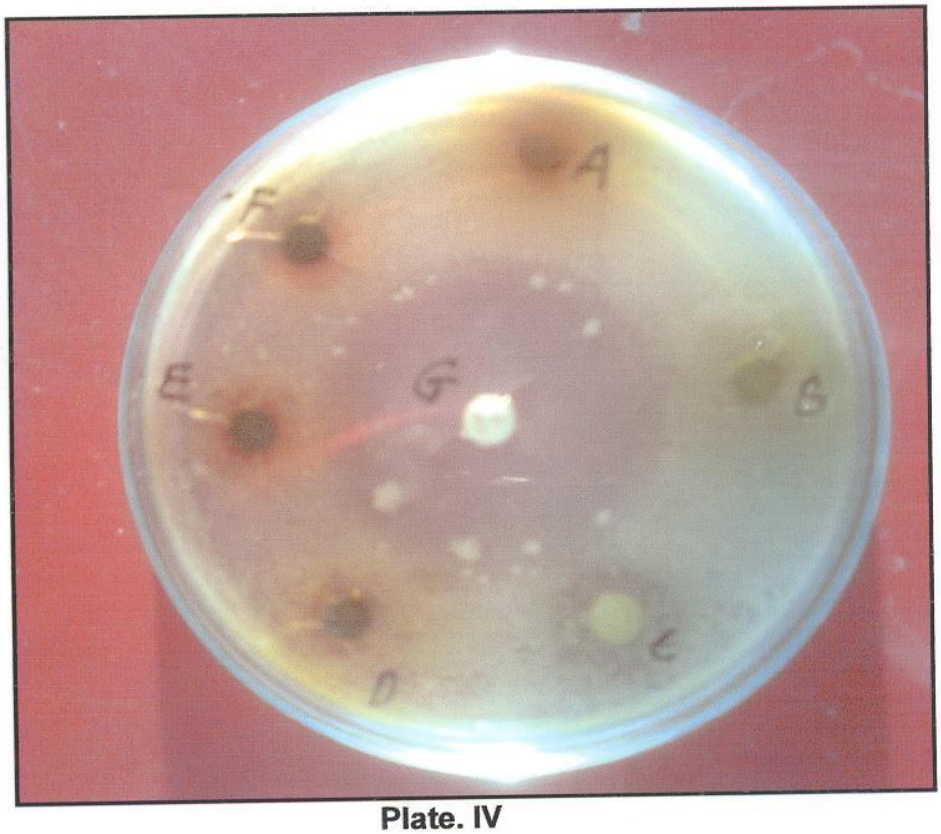

Plate. IV

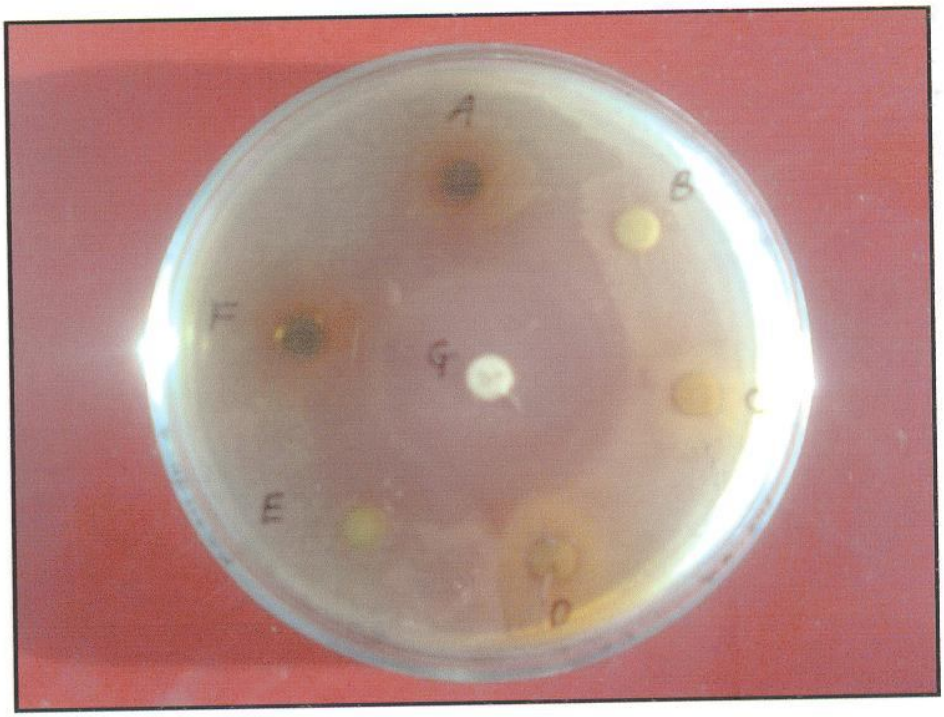

Plate. V

Table 6. **Zone of inhibition of different extracts of *Tinospora cordifolia* and reference drug (ciprofloxacin) against *Salmonella typhimurium***

Extracts	Zone of inhibition (mm) ± SE§			Ciprofloxacin (5 µg/disc)
	1.0 gm / ml (100%)	0.5g/ml (50%)	0.1g/ml (10%)	
Aqueous (hot)	No Zone	No Zone	No Zone	25.33** ±1.20
Ethanolic (hot)	No Zone	11.33±0.67 [b]	10.00±0.57 [b]	

§= mean of three observations,
**Significantly higher (P<0.01) than other extracts

Means with similar superscript are not significantly different

Table 7. Zone of inhibition of different extracts of *Tinospora cordifolia* and reference drug (ciprofloxacin) against *Staphylococcus aureus*

Extracts	Zone of inhibition (mm) ± SE§			Ciprofloxacin (5 µg/disc)
	1.0 gm / ml (100%)	0.5g/ml (50%)	0.1g/ml (10%)	
Aqueous (hot)	7.67 [b] ± 0.67	No zone	10.22 [b] ± 1.92	37.33**±0.88
Ethanolic (hot)	11.33** [a] ± 2.18	7.67 [b] ± 0.67	7.00 [b] ± 00	

§= mean of three observations Means with similar superscript are not significantly different

**Significantly higher (P<0.01) than aqueous and ethanolic extract extracts ** [a] significantly higher (P<0.01)

Plate. VI **Zone of inhibition by different stem extracts *of T. cordifolia* and ciprofloxacin against *Salmonella typhimurium***

A : Aqueous extract 100% (1gm/1ml)

B : Aqueous extract 50% (0.5gm/1ml)

C : Aqueous extract 10% (0.1 gm/1ml)

D : Ethanolic extract 100% (1gm/1ml)

E : Ethanolic extract 50% (0.5gm/1ml)

F : Ethanolic extract 10% (0.1gm/1ml)

G : Reference standard drug Ciprofloxacin (5µg/disc)

Plate. VII **Zone of inhibition by different stem extracts *of T. cordifolia* and ciprofloxacin against *Staphylococcus aureus***

A : Aqueous extract 100% (1gm/1ml)

B : Aqueous extract 50% (0.5gm/1ml)

C : Aqueous extract 10% (0.1 gm/1ml)

D : Ethanolic extract 100% (1gm/1ml)

E : Ethanolic extract 50% (0.5gm/1ml)

F : Ethanolic extract 10% (0.1gm/1ml)

G : Reference standard drug Ciprofloxacin (5µg/disc)

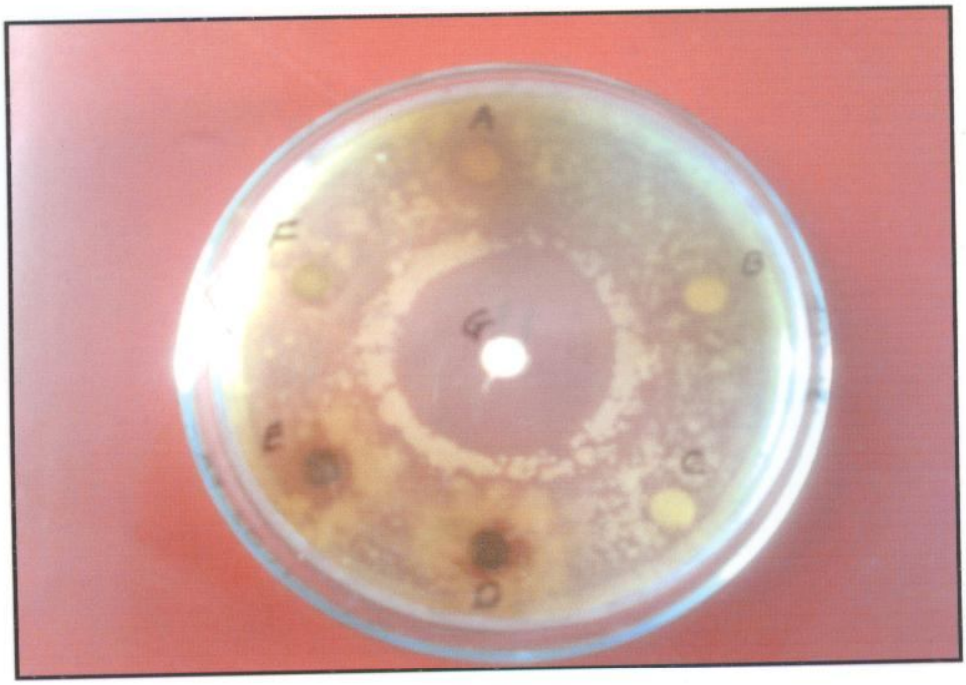

Plate VI.

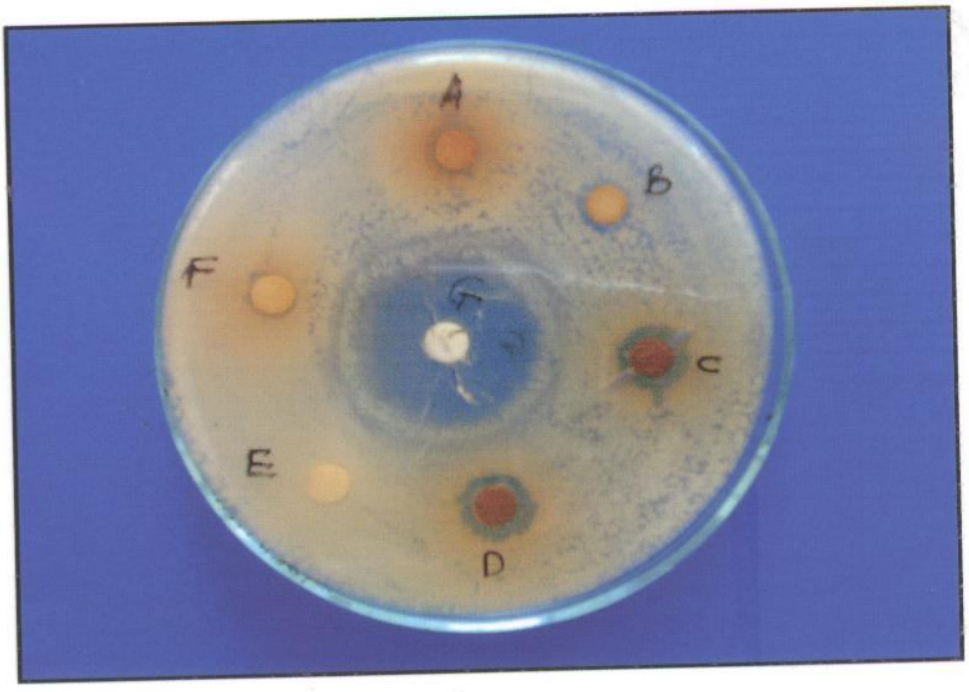

Plate VII.

Table 8. depicted average zone of inhibition of aqueous extract of *T. cordifolia* against different pathogenic bacteria. The aqueous extract 100% and 50% concentrations was only effective against *Escherichia coli* and *Staphylococcus aureus* whose antibacterial activity was much lesser ($P<0.01$) than ciprofloxacin and was found ineffective against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The 10% concentration was also found ineffective against all test bacteria.

Average zone of inhibition of ethanol extract of *T. cordifolia* against different pathogenic bacteria is depicted in Table 9. The ethanol extract at all concentrations were found effective against *Staphylococcus aureus* however 10% and 50% concentration was effective against *Escherichia coli* and *Salmonella typhimurium*. The ethanolic extract at all concentrations were ineffective against the bacterium *Pseudomonas aeruginosa*. As compared to zone of inhibition of ciprofloxacin (25.33±1.20mm to 37.66±3.71mm) the ethanol extracts at all concentrations were showed significantly($P<0.01$)lesser zone of inhibition (7.00±00 mm to 11.33±2.18 mm) against susceptible bacteria.

The comparative zone of inhibition of aqueous and ethanolic extracts of *T. cordifolia* against different bacteria is presented in Table 10 and Fig 4.

Zone of inhibition of both the aqueous and ethanol extracts at different concentrations was much lesser than reference drug ciprofloxacin against susceptible bacteria. But these extracts at tested concentrations were ineffective against *Pseudomonas aeruginosa*.

Table 11. depicted sensitivity pattern of different bacteria against extracts of *T. cordifolia* and ciprofloxacin. All test bacteria were less sensitive to sensitive to both the aqueous and ethanol extracts exposed at different concentrations. The ciprofloxacin sensitivity of bacteria to these extracts concentration was comparatively much lesser than highly sensitive a reference drug where as *Pseudomonas aeruginosa* was found resistant.

Table 8. Zone of inhibition of aqueous hot extract of *Tinospora cordifolia* stem and reference drug (ciprofloxacin) against different bacteria

Extract	Concentration Used (gm/ml)	Zone of inhibition (mm) ± SE§			
		E.coli	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Staphylococcus aureus*
Aqueous hot extract	1.0 (100%)	09.33 [a] ±0.67	No Zone	No Zone	07.67 [a] ± 0.67
	0.5 (50%)	07.67 [b] ± 0.67	No Zone	No Zone	No zone
	0.1 (10%)	No Zone	No Zone	No Zone	10.22 [a] ± 1.92
Reference drug (Ciprofloxacin)	5µg/disc	28.00**±1	37.66**±3.71	25.33**±1.20	37.33**±0.88

§= mean of three observations Means with similar superscript are not significantly different

**Significantly higher (P<0.01) than aqueous extracts

Table 9. Zone of inhibition of ethanolic hot extract of *Tinospora cordifolia* stem and reference drug (ciprofloxacin) against different bacteria

Extract	Concentration Used (gm/ml)	Zone of inhibition (mm) ± SE§			
		E.coli	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Staphylococcus aurius*
Ethanolic hot extract	1.0 (100%)	No Zone	No Zone	No Zone	11.33** [a] ± 2.18
	0.5 (50%)	9.67 [a] ± 0.33	No Zone	11.33 [a] ± 0.67	7.67 [b] ± 0.67
	0.1 (10%)	9.00 [a] ± 0.57	No Zone	10.00 [a] ± 0.57	7.00 [b] ± 00
Reference drug (Ciprofloxacin)	5µg/disc	28.00 **±1.00	37.66**± 3.71	25.33**± 1.20	37.33**± 0.88

§= mean of three observations Means with similar superscript are not significantly different

**Significantly higher (P<0.01) than aqueous and ethanolic extract extracts

** [a] Significantly higher (P<0.01) than 10% and 50% ethanol extract

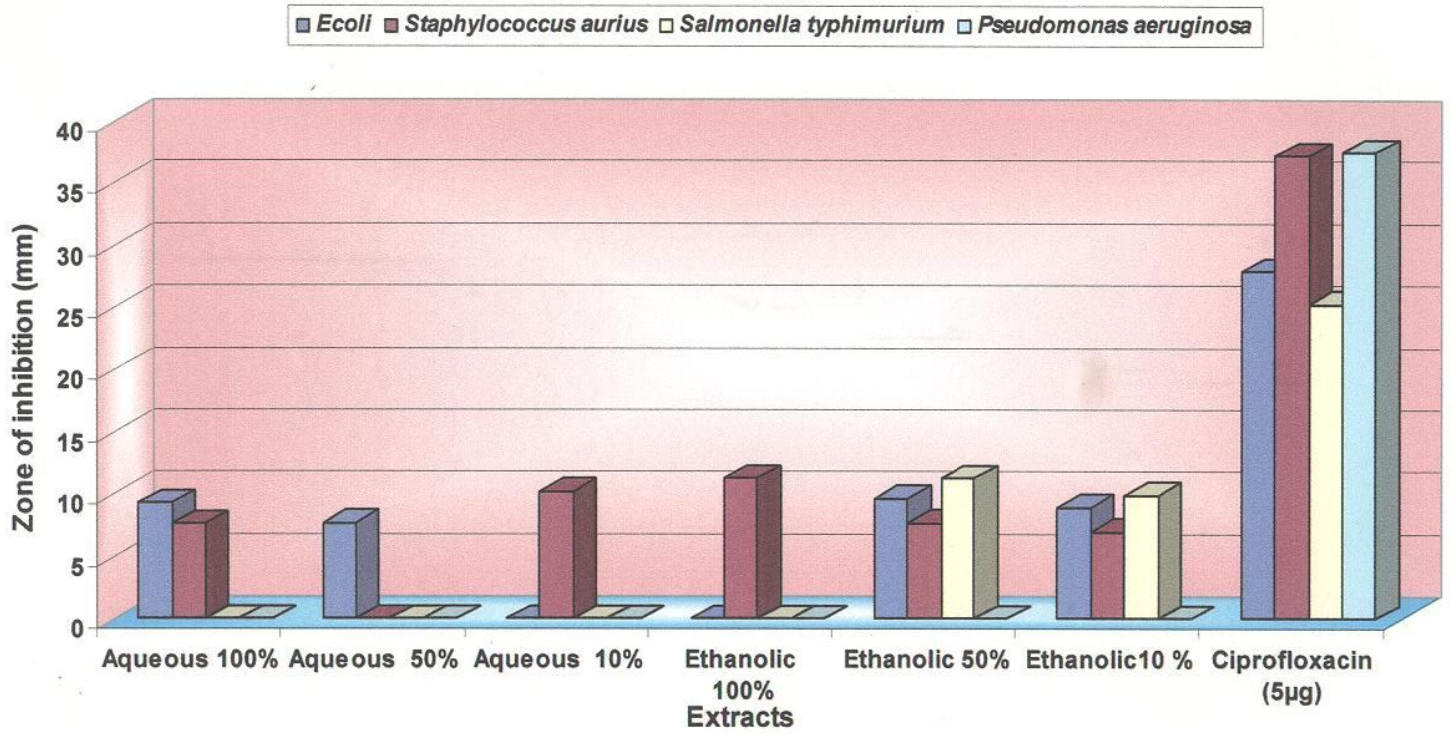

Fig .4 Zone of inhibition by aqueous and ethanolic extracts of *Tinospora cordifolia* and reference drug(ciprofloxacin) against different bacteria

Table 10. Comparative zone of inhibition of different extracts of *Tinospora cordifolia* stem and reference drug (ciprofloxacin) against different bacteria

Type of Extracts	Concentration (gm/ml)	Zone of inhibition (mm) ± SE§			
		Escherichia coli	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Staphylococcus aureus*
Aqueous hot	1.0 (100%)	9.33 [a] ± 0.67	No Zone	No Zone	7.67 [b] ± 0.67
	0.5 (50%)	7.67 [b] ± 0.67	No Zone	No Zone	10.22 [a] ± 1.92
	0.1 (10%)	No Zone	No Zone	No Zone	No Zone
Ethanolic hot	1.0 (100%)	No Zone	No Zone	No Zone	11.33 [a] ± 2.18
	0.5 (50%)	9.67 [a] ± 0.33	No Zone	11.33 [a] ± 0.67	7.67 [b] ± 0.67
	0.1 (10%)	9.00 [a] ± 0.57	No Zone	10.00 [a] ± 0.57	7.00 [b] ± 00
Ciprofloxacin	5µg/disc	28.00**±1.0	37.66**± 3.71	25.33**±1.20	37.33**± 0.88

§= mean of three observations

Means with similar superscript are not significantly different

**significantly higher (P<0.01) than both the extracts

Table 11. **Sensitivity pattern of bacteria against different hot extracts of *Tinospora cordifolia* stem and reference drug (ciprofloxacin)**

Extract Discs	Concentration (gm/ml)	Sensitivity Pattern			
		E.coli	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Staphylococcus aurius*
Aqueous	1.0 (100%)	+	R	R	+ +
	0.5 (50%)	+	R	R	R
	0.1 (10%)	R	R	R	+ +
Ethanolic	1.0 (100%)	R	R	R	+ +
	0.5 (50%)	+	R	+ +	+
	0.1 (10%)	+	R	+ +	+
Ciprofloxacin	5µg/disc	+ + + +	+ + + +	+ + + +	+ + + +

R : Resistant
\+ : Less Sensitive (Zone< 10 mm)
+ + : Sensitive (Zone between 10-15 mm)
+ + + : Moderately sensitive (Zone between 15-20 mm)
+ + + + : Highly sensitive (Zone > 20)

It is evident from the above results that both the aqueous and ethanolic extract of *Tinospora cordifolia* was effective against susceptible pathogenic bacteria. The ethanolic extract was more effective than aqueous extract. This implied that the gram positive bacteria were more susceptible to extract than gram negative bacteria might due to presence of outer cell membrane. Since the zone of inhibition of ethanolic extract was significantly higher against *Salmonella typhimurium* was indicative better permeation of active principles of this plant extracts where the *Pseudomonos aeruginosa* showed intrinsic resistance due to restrictive outer membrane barrier and trans envelop multi drug resistance pump.

The result of present investigation also highlight the fact that the organic solvent extracts showed higher antibacterial activity due to either polar or non-polar active antibacterial principles which were extracted through organic medium (Girish and satish,2008 Significant antibacterial activity of ethanolic stem extract of *Tinospora cordifolia* against *E.coli, Salmonella typhi, Staphylococcus aureus* including other bacteria and marginal effectiveness of aqueous extract against *E. coli* by disc diffusion method was reported by Jeyachandran, *et al* (2003)

The concentration dependant anti fungal and antibacterial activity of aqueous root extract was reported by Agarwal, *et al* (2008) where 100% concentration was most effective against plant pathogens. Significant antibacterial activity of five different plants including *Tinospora cordifolia* against *E.coli, Pseudomonos aeruginosa, Salmonella typhi, Staphylococcus aureus* and other bacteria have been reported by Girish and Satish, (2008). Mahesh and Satish 2008 who observed significant antibacterial activity of methanolic leaf extracts of different plants including *Tinospora cordifolia* against *E.coli, Staphylococcus aureus* and other pathogenic microorganisms. Mohana, *et al* 2008 observed antibacterial activity of aqueous extract of *Tinospora cordifolia* and other eight medicinal plants against eleven human pathogenic bacteria. These reports are in agreement with our findings.

4.3.2 Tube dilution method

The colony count of different bacteria following treatment with potent ethanolic stem extract of *T. cordifolia* is given in Table 12. The colony count of *Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus* was significantly ($P<0.01$)reduced (100.00±1.67 to 226.00±5.03 cfu/ml) following treatment with treated with 50 % ethanol extract concentration than 10 % concentration (141.67±7.26 to 263.00±4.04 cfu/ml) as compared to bacterial control (197.00±4.36 to 266.67±10.14 cfu/ml). Significant reduction in colony count by 50% concentration was observed against *Salmonella typhimurium* than *Escherichia coli* and *Staphylococcus aureus* than to 10% ethanolic extract concentration as compared to bacterial control.

4.4 Minimal inhibitory concentration (MIC)

The mean optical density (OD) values of four different bacterial cultures treated with ethanol extract, extract control and bacterial control is given in Table 13. Fig 5 Plate VIII, IX and X. MIC of ethanolic stem extract of *T. cordifolia* against *Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus* was found to be 2.0, 1.0 and 1.5 respectively. The susceptibility of respective bacteria to the extract concentration were *Salmonella typhimurium* > *Staphylococcus aureus* > Escherichia *coli.*

Dhar *et al*, (1968) Observed antibacterial ineffectiveness of 50% ethanolic stem extract with MIC value ≤ 25 µg/ml after 24 hours incubation at 37°C is contradicted with our findings might due to difference in concentration used.

Table 12. Colony counts of different bacteria following treatment with *Tinospora cordifolia* stem extract

Treatment	Bacterial colony count (cfu) ± SE§		
	Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*
Bacterial Control	205 [a] ± 2.87	197.00 [a] ± 4.36	266.67 [a] ± 10.14
50% Ethanolic hot extract	**135.33 [c] ± 2.90**	**100.00 [b] ± 1.67**	**226.00 [b] ± 5.03**
10% Ethanolic hot extract	163.33 [b] ± 6.00	141.67 [c] ± 7.26	263.00 [a] ± 4.04

§= mean of three observations

Means with similar superscript are not significantly different

Table 13. MIC of ethanol stem extract (hot) of *Tinospora cordifolia* against different bacteria

Test tube	Mean Optical Density (OD) ± SE		
	Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*
Bacterial control	0.863 ± 0.001	0.553 ± 0.001	0.878 ± 0.002
Extract Control	0.258±0.001	0.258±0.001	0.258±0.001
Extract treatment (mg/ml)			
0.5	0.474 ± 0.001	0.643± 0.001	0. 861 ± 0.001
1.0	0.519 ± 0.001	**0.463* ± 0.001**	0.745 ± 0.001
1.5	0.631±0.001	0.684 ± 0.001	**0.480* ± 0.002**
2.0	**0.470* ± 0.001**	0.500 ± 0.001	0.713 ± 0.001
2.5	0.785 ± 0.001	0.642 ± 0.001	0.640 ± 0.002
3.0	0.853± 0.001	0.909 ± 0.001	0.640 ± 0.001

§= mean of three observations

* Minimal inhibitory concentration

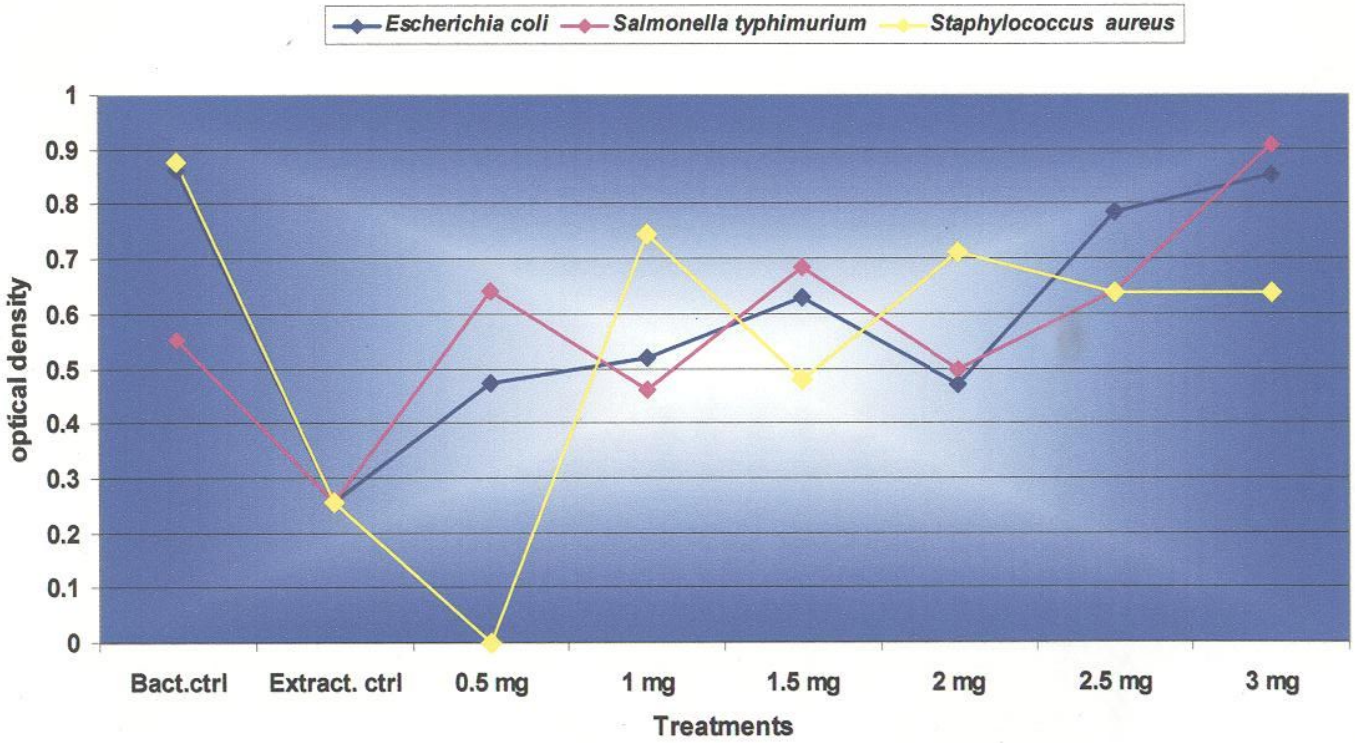

Fig.5 MIC of ethanolic hot extract of *Tinospora cordifolia* against different bacteria

Plate. VIII **MIC of ethanol extract against *Escherichia coli***

A : Bacterial control

B : 0.5 mg/ml

C : 1 mg/ml

D : 1.5 mg/ml

E : 2.0 mg/ml

F : 2.5 mg/ml

G : 3 mg/ml

Plate. IX **MIC of ethanol extract against against *Salmonella typhimurium***

A : Bacterial control

B : 0.5 mg/ml

C : 1 mg/ml

D : 1.5 mg/ml

E : 2.0 mg/ml

F : 2.5 mg/ml

G : 3 mg/ml

Plate. X **MIC of ethanol extract against against *Staphylococcus aureus***

A : Bacterial control

B : 0.5 mg/ml

C : 1 mg/ml

D : 1.5 mg/ml

E : 2.0 mg/ml

F : 2.5 mg/ml

G : 3 mg/ml

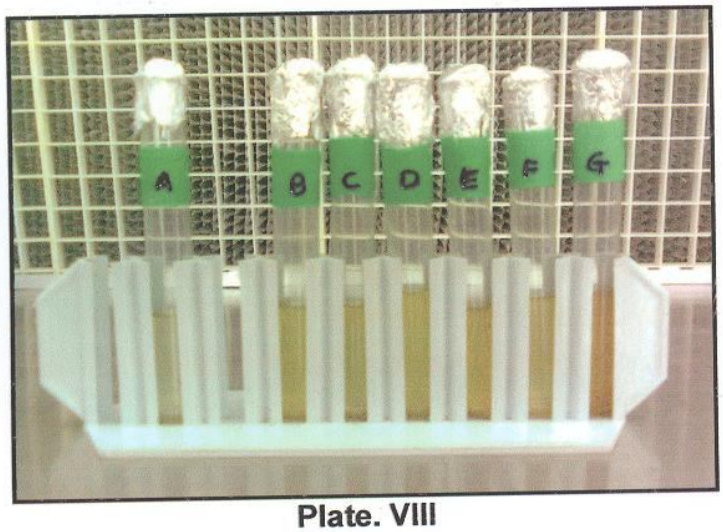

Plate. VIII

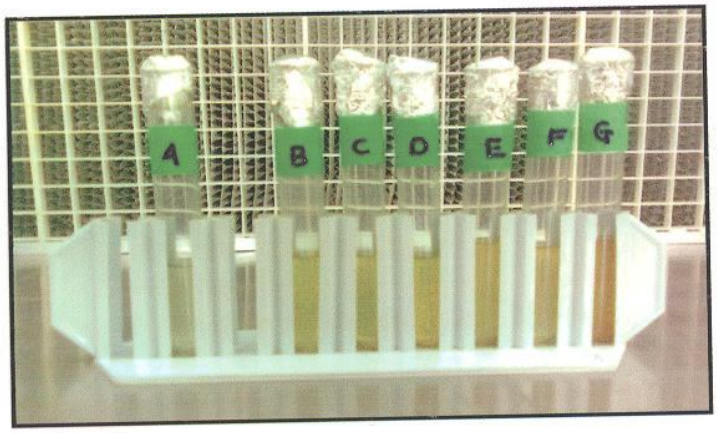

Plate. IX

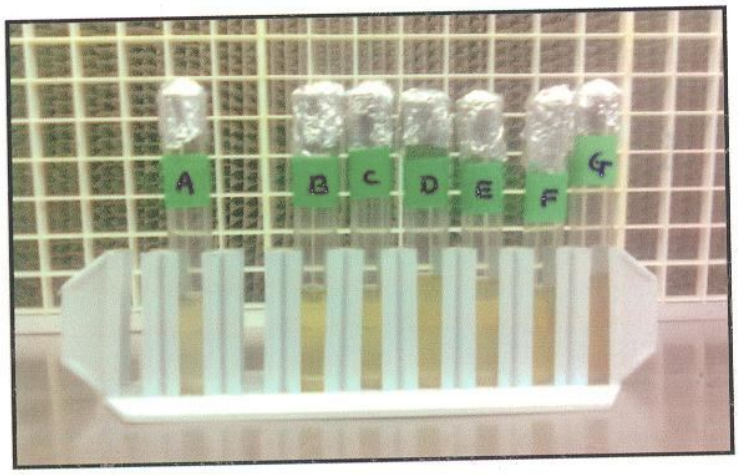

Plate. X

4.5 Drug interaction studies

The ethanol extract of *T. cordifolia* combined with ciprofloxacin a reference drug was evaluated to assess zone of inhibition against different susceptible bacteria was compared with zone of inhibition of ciprofloxacin and extract disc alone.

The results of this investigation are depicted in Table 14 and Plate XI, XII, XIII. The zone of inhibition of ethanol extract (7.33± mm to 8.67± mm), ciprofloxacin (15.67± mm to 27.67± mm) and its combination (13.67± mm to 26.67± mm) was significantly differs ($P< 0.01$). Where the zone of inhibition of combination disc was significantly($P<0.01$)reduced compared to individual extract and ciprofloxacin disc against *Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus* was indicative of drug extract antagonistic effect. Harle and Gaikwad, (2004) who observed interaction of light petroleum ether extract with dexamethasone by increased absorptive permeability on isolated rat ileum .This report is in agreement with our findings. No more information on drug-extract interaction was found in reviewed literature on *Tinospora cordifolia*

4.6 Phytochemical studies

The results of this investigations are depicted in Table 15 and 16. The glycoside, sugar, sterols, flavonoids and saponin were found in the aqueous and ethanolic extracts of *T. cordifolia.*

The presence of glycosides (giloin, gilenin, gilesterol) in the stem extract of *Tinospora cordifolia* was reported by (Kidwai, et al 1949, Gangan, et al 1994, Maurya, et al 1997, Chintalwar, et al 1999) are in agreement with our findings. Patel and Patel (1957) who observed greater amount of protein in the leaf of *Tinospora cordifolia* was contradicted with our findings might be due to the part of plant used.

Plate. VIII **Zone of inhibition by ethanolic extract (50%) *of T. cordifolia,* ciprofloxacin and its combination against *Escherichia coli***

A : Ethanol extract disc (50%)

B : Ethanol extract disc (50%) + Ciprofloxacin

C : Ciprofloxacin

Plate. IX **Zone of inhibition by ethanolic extract (50%) *of T. cordifolia,* , ciprofloxacin and its combination against *Salmonella typhimurium***

A : Ethanol extract disc (50%)

B : Ethanol extract disc (50%) + Ciprofloxacin

C : Ciprofloxacin

Plate. X **Zone of inhibition by ethanolic extract (50%) *of T. cordifolia,* , ciprofloxacin and its combination against *Staphylococcus aureus***

A : Ethanol extract disc (50%)

B : Ethanol extract disc (50%) + Ciprofloxacin

C : Ciprofloxacin

Table 14. Zone of inhibition of ethanolic stem extract of *T. cordifolia*, ciprofloxacin and its combination against different bacteria

Extract / drug	Concentration (gm/ml)	Zone of inhibition (mm) ± SE[a]		
		E.coli	*Salmonella typhimurium*	*Staphylococcus aurius*
Ethanol extract	0.5gm	08.67 [b] ± 0.33	08.00 [c] ± 00	07.33 [c] ± 0.33
Ethanol extract + Ciprofloxacin	0.5 g + 5µg	26.67 [a] ± 0.33	22.67 [a] ± 0.33	13.67 [b] ± 0.33
Ciprofloxacin	5µg/disc	27.67 [a] ± 0.33	22.00 [b] ± 00	15.67 [a] ± 0.33

§= mean of three observations **significantly (P<0.01)
Means with similar superscript are not significantly different

Plate. XI **Zone of inhibition by ethanolic extract (50%) *of T. cordifolia*, ciprofloxacin and its combination against *Escherichia coli***

A : Ethanol extract disc (50%)

B : Ethanol extract disc (50%) + Ciprofloxacin

C : Ciprofloxacin

Plate. XII **Zone of inhibition by ethanolic extract (50%) *of T. cordifolia*, ciprofloxacin and its combination against *Salmonella typhimurium***

A : Ethanol extract disc (50%)

B : Ethanol extract disc (50%) + Ciprofloxacin

C : Ciprofloxacin

Plate. XIII **Zone of inhibition by ethanolic extract (50%) *of T. cordifolia*, ciprofloxacin and its combination against *Staphylococcus aureus***

A : Ethanol extract disc (50%)

B : Ethanol extract disc (50%) + Ciprofloxacin

C : Ciprofloxacin

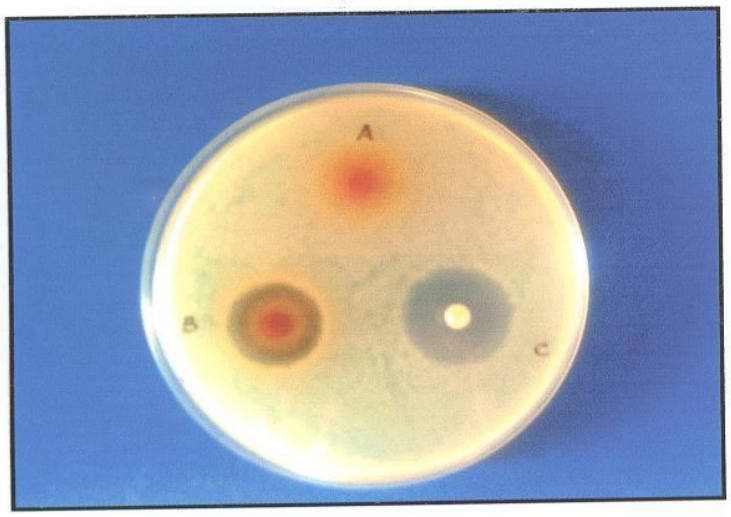

Plate. XI

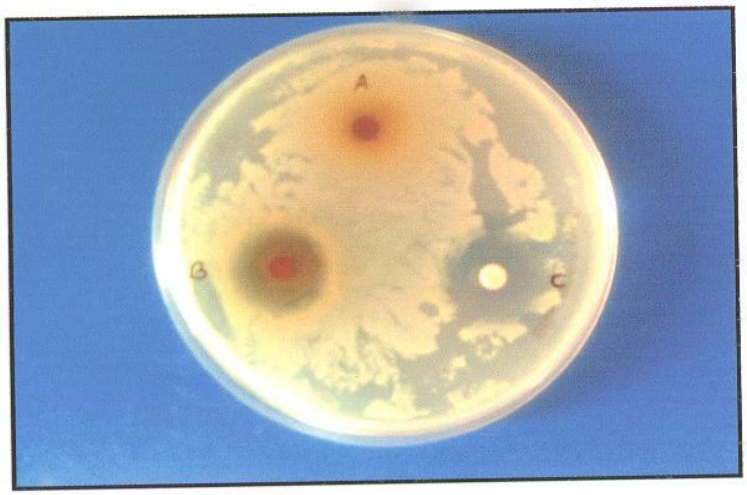

Plate. XII

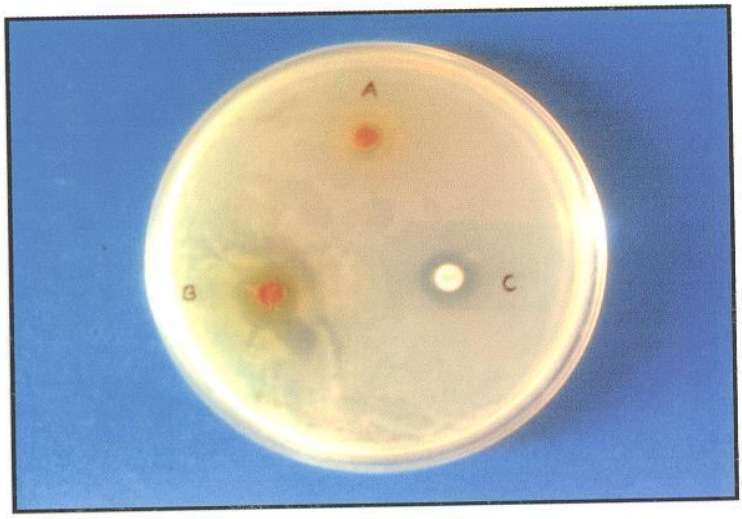

Plate. XIII

Table 15. Phytochemical analysis of ethanolic stem extract of *Tinospora cordifolia* Miers.

Active principles	Test applied	Observations	Inference
Alkaloids	a) Dragendorff's reagent b) Mayer reagent c) Wagners reagent d) Marme's	Orange red color does not develop No Appearance of Cream color ppt Brown flocculent ppt does not developed yellow ppt does not formed	Absent Absent Absent Absent
Glycoside	a) Fehling's reagent b) Benedicts reagent	Brownish red ppt formed Brownish ppt formed	Present Present
Sugar	a) Fehling's reagent b) Benedicts reagent c) Molisch's test d) Barfoed's test	Red ppt develop Red ppt develop Appearance of red ring Red color develop	Present Present Present Present
Tannins	a) Ferric chloride test b) Pottasium dichromate test c) Lead acetate test	Green color does not develop Develop dark yellow color PPT formed	Absent Absent Absent
Sterols	a) Salkowski's reaction	Development of red ring at the junction	Present
Antraquinones	a) Borntrager's test	Ammonical layer does not impart pink color	Absent
Flavonoids	a) Flavonoids test	Pink color sdevelop	Present
Proteins	a) Biurete test b) Xanthoproteic test	Violet pink color does not develop Yellow colour does not develop	Absent Absent
Amino acids	a) Ninhydrine test	Purple colour does not develop	Absent
Saponin	a) Foam test	Froth formed	Present

Table 16. Phytochemical analysis of aqueous stem extract of *Tinospora cordifolia* Miers.

Active principles	Test applied	Observations	Inference
Alkaloids	e) Dragendorff's reagent f) Mayer reagent g) Wagners reagent h) Marme's	Orange red color does not develop No Appearance of Cream color ppt Brown flocculent ppt does not developed yellow ppt does not formed	Absent Absent Absent Absent
Glycoside	c) Fehling's reagent d) Benedicts reagent	Brownish red ppt formed Brownish ppt formed	Present Present
Sugar	e) Fehling's reagent f) Benedicts reagent g) Molisch's test h) Barfoed's test	Red ppt develop Red ppt develop Appearance of red ring Red color develop	Present Present Present Present
Tannins	d) Ferric chloride test e) Pottasium dichromate test f) Lead acetate test	Green color does not develop Does not develop yellow color PPT does not formed	Absent Absent Absent
Sterols	a) Salkowski's reaction	Development of red ring at the junction	Present
Antraquinones	a) Borntrager's test	Ammonical layer does not impart pink color	Absent
Flavonoids	a) Flavonoids test	Pink color not develop	Present
Proteins	c) Biurete test d) Xanthoproteic test	Violet pink color does not develop Yellow colour does not develop	Absent Absent
Amino acids	a) Ninhydrine test	Purple colour does not develop	Absent
Saponin	b) Foam test	Froth formed	Present

The presence of sugar are in agreement with report of Jahfer, (2003) who isolated glucose and polysaccharides such as arabinose, rhamnose, xylose, mannose, and galactose from *Tinospora cordifolia* . Similar observations were also reported by Jahfer and Azadi (2004).

The active constituents such as flavanoides, sterols and saponin were additionally found in the above extracts.

4.7 Immunological studies

In this investigation DNCB dermal hypersensitivity test was employed to assess cell mediated immune response in different treatment groups of broiler birds supplemented with stem powder of *T. cordifolia* in feed and some physical parameters such as body weight, weight gain /loss, feed consumption, feed conversion ratio (FCR) and water intake was also studied. The results of this investigation are as below.

4.7.1 Average weekly body weight: gain/loss

Results of the average body weight and gain in weight following supplementation of *Tinospora cordifolia* in the feed of broiler bird presented in Table 17 and 18. Fig. 6 and 7

In control group (T0) the average weekly body wt. at 3rd, 4th, 5th, and 6th week were 930.90 gm, 970.50gm, 1407.5gm and 1720 gm with gain in weight 276.60gm, 500gm, 312.50 gm respectively. There was significant ($P< 0.01$) increase in body weight and weight gain from 4th week on wards and continued up to sixth week.

In group T1 average weekly body weight during 3rd, 4th, 5th, and 6th week were 636.30 gm 922.50, 1480.50 and 1765.50 gm and gain in weight 286.20gm,

Table 17. Average body weight (gm) per bird/week / birds different treatment groups of broiler fed with stem powder of *Tinospora cordifolia* at different intervals

weeks / Treatment	Body weight (gm)				Treatment mean
	Initial weight at 3rd week	4th	5th	6th	
T0 (Ctrl:)	630.90 ± 7.07	907.50 ± 1.68	1407.5 ± 7.5	1720 ± 7.95	1166.5 ^{c} ± 6.05
T1 (@ 4gm/kg feed)	636.30 ± 11.77	922.50 ± 1.69	1480.5± 5.70	1765.50± 10.25	1201.1 ^{b} ± 7.35
T2 (@ 8gm/kg feed)	650.80 ± 7.28	927.70 ± 2.95	1417.5± 3.89	1915 ± 2.98	1252.8 ^{a} ± 4.27
Week mean	639.33 ^{d} ± 8.70	919.23 ^{c} ± 1.3	1468.5 ^{b} ± 5.70	1800 ^{a} ± 7.06	1206.8**

CD for treatment = 9.25**
CD for week = 10.69**
CD for treatment * week interaction = 18.51**
** significant at 0.01 percent level

The means connected with same superscript do not differ significantly.

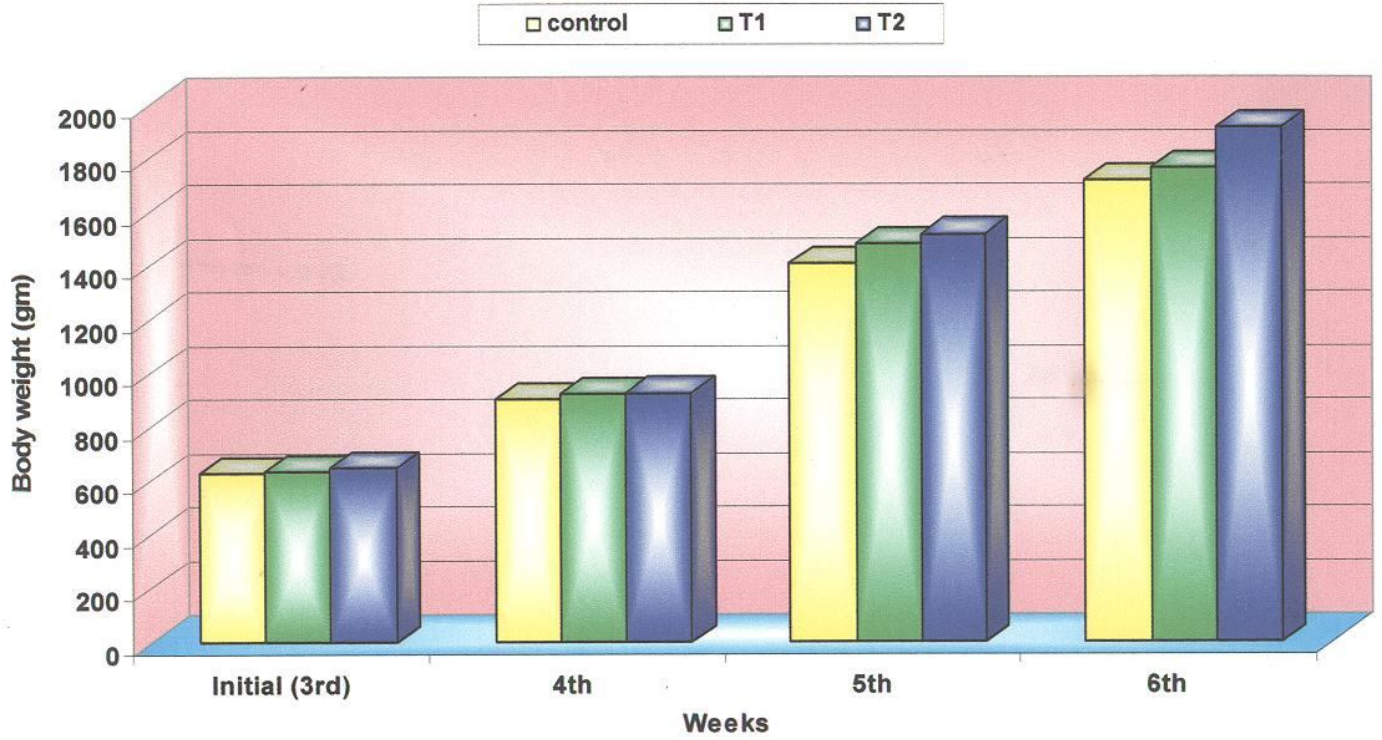

Fig.6 Average weekly body weight (gm) per bird in different treatment group of of broiler fed with stem powder of *Tinospora cordifolia*

Table 18. Average weight gain per bird/week of different treatment groups fed with stem powder of *Tinospora cordifolia*

weeks / Treatment	Weight gain (gm)			Treatment mean
	4th	5th	6th	
T0 (Ctrl.)	276.60 ± 7.28	500 ± 7.10	312.50 ± 13.13	363.03 ^{b} ± 9.17
T1 (@ 4gm/kg feed)	286.20 ± 12.32	558 .00± 4.96	283.50 ± 11.93	375.90 ^{b} ± 9.74
T2 (@ 8gm/kg feed)	276.90 ± 7.91	579.80 ± 8.74	397.50 ± 5.69	418.07 ^{a} ± 7.45
Week mean	279.90 ^{c} ± 9.17	545.93 ^{a} ± 6.93	331.17 ^{b} ± 10.25	

CD for treatment = 14.73**
CD for week = 14.73**
CD for treatment * week interaction =25.53**
** significant at 0.01 percent level

The means connected with same superscript do not differ significantly.

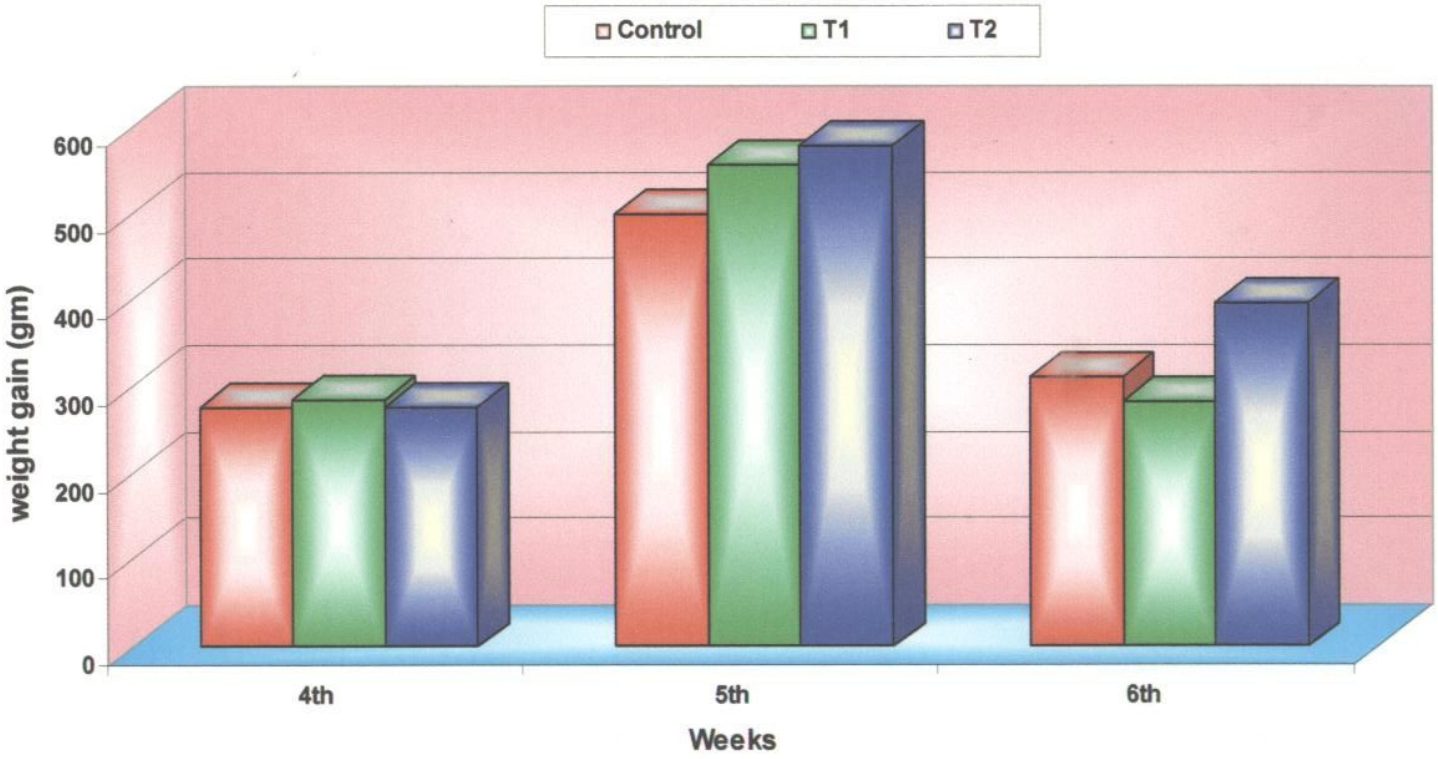

Fig.7 Average weekly weight gain (gm) per bird in different treatment groups of broilers fed with stem powder of *Tinospora cordifolia*

558gm, 283.50 gm respectively. There was significant difference observed in weekly body weight and weight gain however body weight was significantly ($P< 0.01$) increased during 6th week and significantly higher($P< 0.01$) gain in weight during 5th week following *Tinospora cordifolia* feed supplementation @ 4 gm/kg feed for 21 days .

In group T2 average weekly body weight during 3rd, 4th, 5th, and 6th week were 650.80 gm, 927.70gm, 1417.5gm and 1915 gm respectively. That was significantly differ within weekly intervals and was found highly significant ($P< 0.01$) during 6th week. Average weight gain during 4th, 5th, and 6th week were 776.90gm, 579.80gm and 397.50gm respectively which was highly significant($P<0.01$) during 5th week than 6th week compared to 4th week

Comparing between treatment groups T0, T1 and T2 overall body weight in group T2 (1252.8 gm) was significantly increased than T1 group (1201.1 gm) compared to control (1166.5 gm). These values were significantly differs. Overall average gain in body weight in groups T0, T1 and T2 were 363.03 gm, 375.90 gm, and 418.07 gm respectively which was significantly($P<0.01$)higher in group T2 than T1 as compared to control.

It is evident from above study that *Tinospora cordifolia* stem powder had better growth performance at higher dose level and was indicating adaptogenic effect during extreme hot summer.

The normal body weight of broiler birds during 3rd to 6 weeks of age were ranged 560-1690gm in male and 515-1430 gm in female (Ensminger,1992). In our study overall body weight in group T0 was 1166.5 gm which was found within the normal body weight. Following treatment with Tinospora cordifolia at different dose level, the body weight was increased in respective treatment group. Devegowda *et al* (1990) also observed increased body weight, weight gain with *Livol* supplementation at 0.5 and 0.25 % level. Decrease in carcass weight due to reduced level of vitamin C during high ambient environmental temperature and heat

stress in poultry reported by Richards,1997 which was significantly improved by *T. cordifolia* as it is reach source of vitamin C (Donkoh, 1989)

4.7.2 Average weekly feed consumption

The average values of feed consumption in different treatment groups of broiler birds are given in Table 19. Fig. 8

In group T0 (control) the average weekly values of feed consumption per bird during 4^{th} 5^{th} and 6^{th} week were 770gm, 865gm and 650 gm respectively was significant differs ($P<0.01$) within weekly intervals. That was highly significant during 5^{th} week than 4^{th} and 6^{th} week.

In group T1 average weekly feed consumption during 4^{th} 5^{th} and 6^{th} week were 781gm, 1119gm and 835 gm respectively. These values showed significant increase in feed consumption during 5^{th} week than 4^{th} and 6^{th} week following treatment with *Tinospora cordifolia* @ 4 gm/kg feed for 21 days.

In the group T2 average weekly feed consumption per bird during 4^{th} 5^{th} and 6^{th} week were 775gm, 1200gm and 850 gm respectively. Significant increase in feed consumption during 5^{th} week was observed than 4^{th} and 6^{th} week.

Overall average weekly feed consumption during 4^{th}, 5^{th} and 6^{th} week were 775.33gm, 1061.3 gm and 778.33 gm respectively was significantly ($P<0.01$) different within weekly interval and was found highly significant during 5^{th} wee compared to 4^{th} and 6^{th}.

Comparing overall average of feed consumption per bird per week in the treatment groupsT0, T1, and T2 were 761.67gm, 911.67gm, and 941.67 gm respectively significantly ($P<0.01$) differs. Significantly higher feed consumption observed in group T2 than T1 as compared to T0.

Table 19. Average feed consumption per bird/week of different treatment groups of broiler fed with stem powder of *Tinospora cordifolia*

weeks / Treatment	Feed consumption (gm)			Treatment mean
	4th	5th	6th	
T0 (Ctrl.)	770 ± 16.22	865 ± 18.75	650 ± 9.27	761.67 ^{c} ± 14.74
T1 (@ 4gm/kg feed)	781 ± 9.06	1119 ± 21.93	835 ± 9.25	911.67 ^{b} ± 13.41
T2 (@ 8gm/kg feed)	775 ± 10.95	1200 ± 10.05	850 ± 15.09	941.67 ^{a} ± 12.03
Week mean	775.33 ^{b} ± 12.07	1061.3 ^{a} ± 16.91	778.33 ^{b} ± 11.20	871.67**

CD for treatment = 22.59**
CD for week = 22.59**
CD for treatment * week interaction = 39.12**
** significant at 0.01 percent level

Note : The means connected with same superscript do not differ significantly.

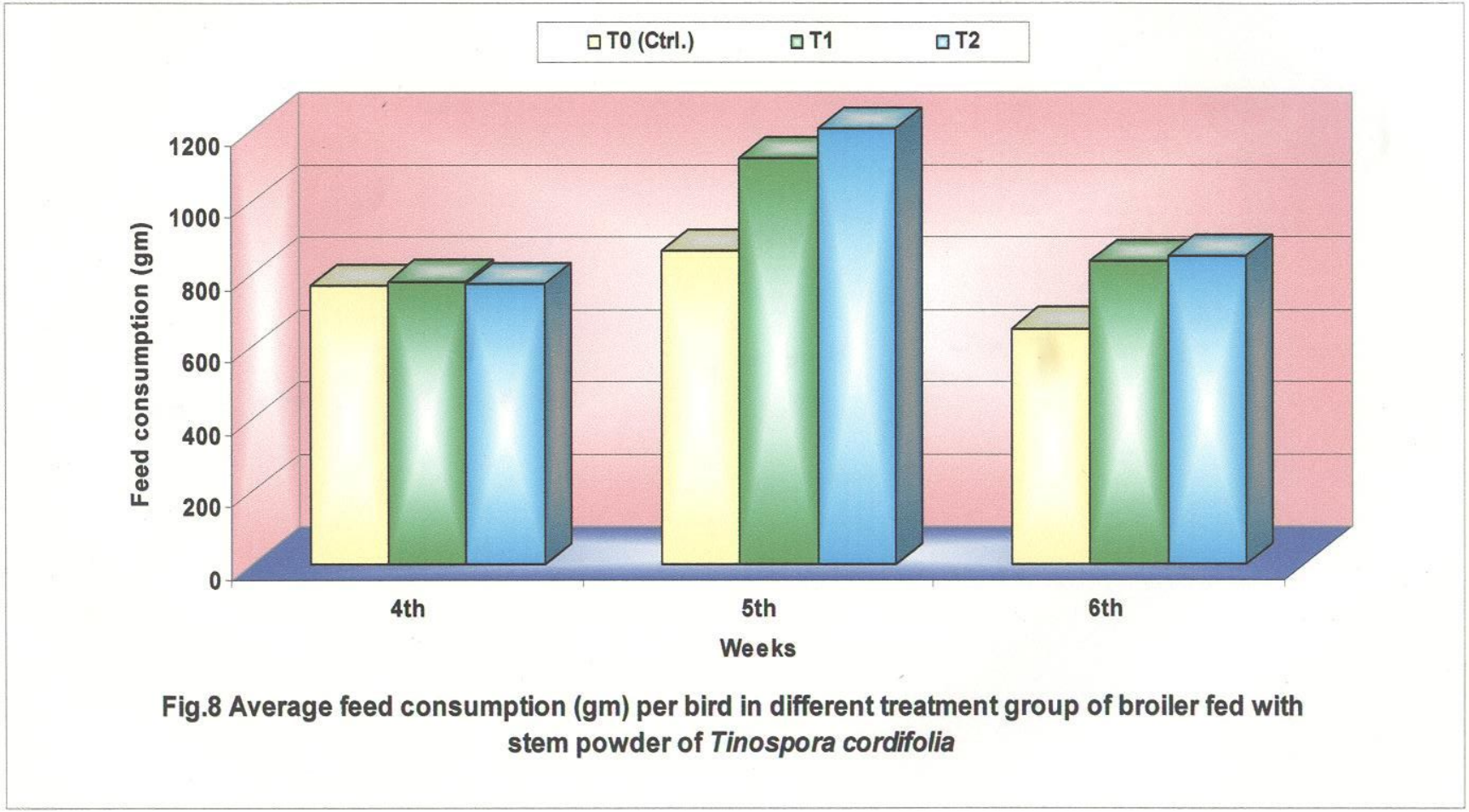

Fig.8 Average feed consumption (gm) per bird in different treatment group of broiler fed with stem powder of *Tinospora cordifolia*

Our findings are in accordance with the reports of Devegowda *et al* (1990) who observed increased in feed consumption following Livol supplementation at 0.5 and 0.25 % level in poultry.

4.7.3 Average weekly feed conversion ration

Average weekly value of FCR in different treatment groups of broiler bird is depicted in Table. 20. Fig. 9

Overall average weekly value of during 4^{th} 5^{th} and 6^{th} week were 2.77, 1.94 and 2.41 respectively. FCR found significantly lesser ($P<0.01$) during 5^{th} week than 4th and 6^{th} week was indicating lesser the feed consumption greater the body weight.

Comparing FCR values between different treatment groups T0, T1, and T2 were 2.19, 2.58 and 2.35 respectively. FCR values in group T1 and T2 were significantly higher ($P<0.01$) than control was indicating higher the feed consumption lesser the body weight gain might be due to heat stress.

4.7.4 Water intake

Table. 21 Fig. 10 depicted average water intake of broiler in different treatment group. The amount of water required of different treatment groups T0, T1 and T2 during 4^{th}, 5^{th}, 6^{th} week were ranged to 1412.9 to 2600, 1414.3 to 2606.4 and 1420.7 to 2615 ml per bird per week respectively.

In control group (T0) the water consumption was progressively increased from 4^{th}, 5th, and 6^{th} week were 1412.9 ml, 2001.4 ml and 2600 ml per bird per week which was significantly ($P<0.01$) increased at 6^{th} week than 4^{th} and 5^{th} week.

In group T1 the water consumption at 4^{th} week 1414.3 ml, 5^{th} week 2007.9ml and 6^{th} week 2606.4 ml .Significant increase ($P<0.01$) was observed weekly interval

Table 20. Feed conversion ratio per bird/week of different treatment groups broilers fed with stem powder of *Tinospora cordifolia*

weeks / Treatment	Feed conversion ratio			Treatment mean
	4th	5th	6th	
T0 (Ctrl.)	2.80± 0.075	1.74± 0.033	2.11± 0.10	2.19 ^{c}± 0.06
T1 (@ 4gm/kg feed)	2.76± 0.09	2.00±0.05	2.99± 0.12	2.58 ^{b}± 0.009
T2 (@ 8gm/kg feed)	2.82± 0.08	2.07± 0.02	2.15± 0.04	2.35 ^{a}± 0.004
Week mean	2.77 ^{c}± 0.008	1.94 ^{b}± 0.003	2.41 ^{a}± 0.008	2.37**

CD for treatment = 0.13**
CD for week = 0.13**
CD for treatment * week interaction = 0.22**
** significant at 0.01 percent level

Note : The means connected with same superscript do not differ significantly.

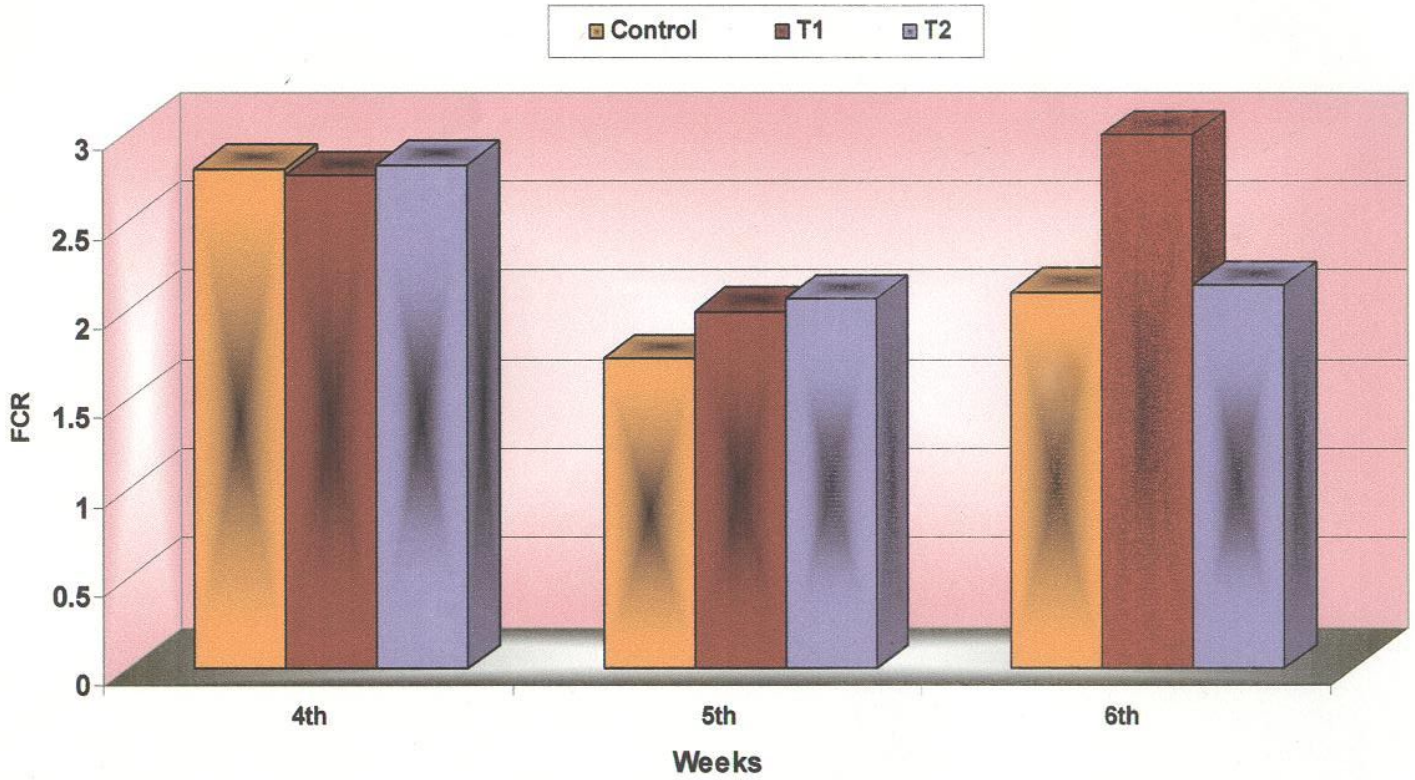

Fig.9 Average weekly feed conversion ratio per bird in different treatment groups of broilers fed with stem powder of *Tinospora cordifolia*

Table 21. Average water intake (ml) per bird/week of different treatment groups broilers fed with stem powder of *Tinospora cordifolia*

weeks / Treatment	Water intake (ml)			Treatment mean
	4th	5th	6th	
T0 (Ctrl.)	1412.9 ± 21.79	2001.4 ± 4.32	2600 ± 4.50	2004.8 [a] ± 10.20
T1 (@ 4gm/kg feed)	1414.3 ± 6.12	2007.9 ± 5.22	2606.4± 5.74	2009.5 [a] ± 5.69
T2 (@ 8gm/kg feed)	1420.7± 5.17	2015.00 ± 3.45	2615 ± 4.08	2016.9 [a] ± 4.23
Week mean	1416.00 [c]± 11.02	2008.1 [b]± 4.33	2607.1 [a]± 4.77	2010.4**

CD for treatment = 13.75 [NS]
CD for week = 13.75**
CD for treatment * week interaction =23.81 [NS]
** significant at 0.01 percent level

Note : The means connected with same superscript do not differ significantly.

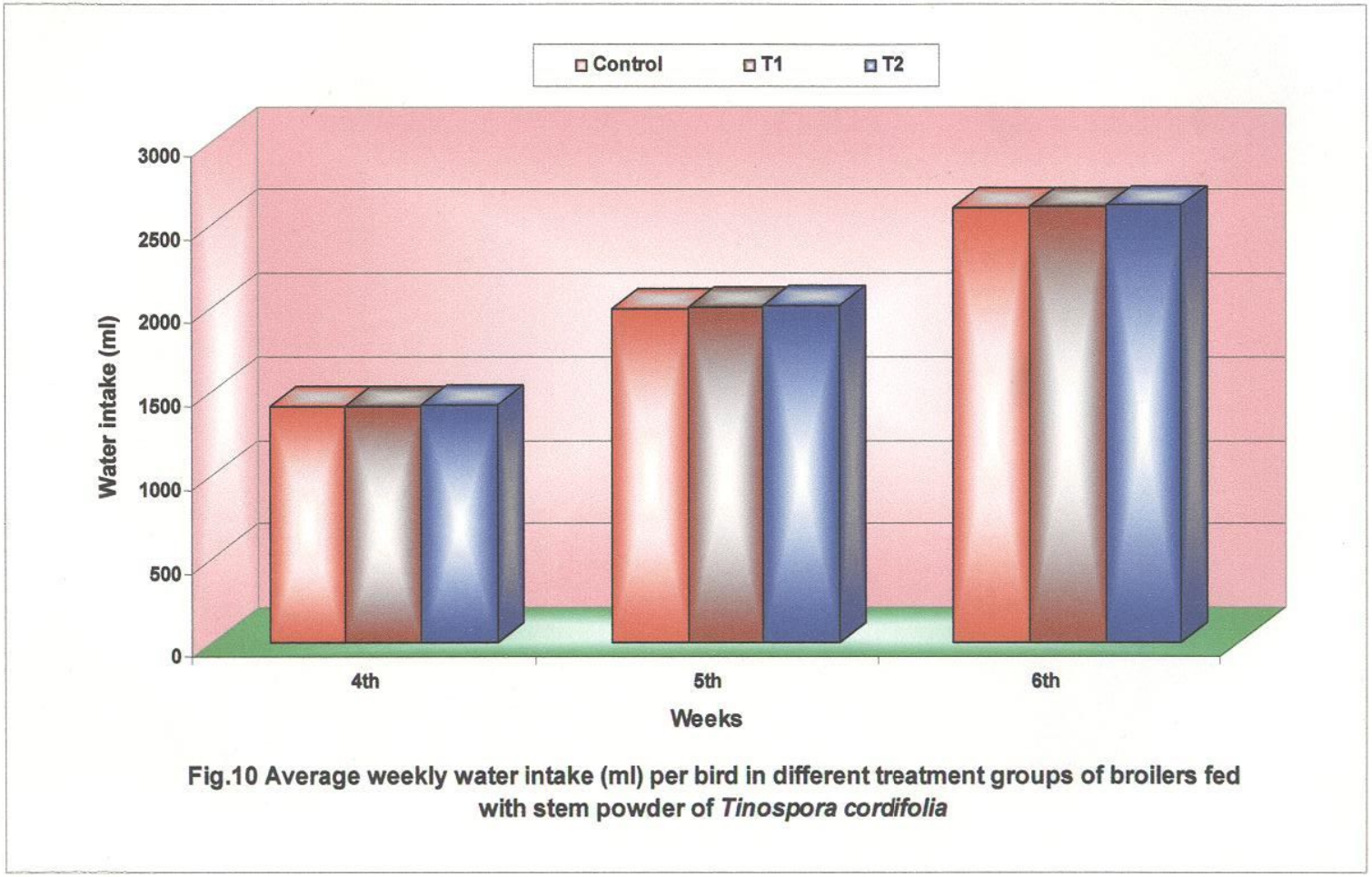

Fig.10 Average weekly water intake (ml) per bird in different treatment groups of broilers fed with stem powder of *Tinospora cordifolia*

were water requirement was considerably higher at 6th week compared to 4th and 5th week.

In group T2 the water consumption at 4th, 5th and 6th week were 1420.7, 2015 and 2615 ml respectively which was higher at 5th and 6th week than 4th week.

Comparing the consumption of water in different treatment groups there was no significant difference observed at 4th and 5th week however the water requirement in the group T2 and T1 was significantly ($P<0.01$) increased as compared to control .

The daily water consumption during 3rd to 6th week chickens of different ages are 90 to 260 litters per thousand birds (Ensminger, 1992). The observed values of water consumption are within the range given by Ensminger, (1992).

The water intake was significantly increased in T2 group than T1 as compare to control after 6th week and the difference in water intake might be due to increase metabolism and loss of water due to high environmental temperature.

4.7.5 DNCB Contact Skin Hypersensitivity Test (CMI)

Results of DNCB skin sensitivity test in terms of skin thickness and diameter of spreading lesion are mentioned in Table 22. Plate. XIV and XV

In control group skin thickness (mm) after challenge by DNCB at 0 hrs was 0.06 ± 0.003 which was increased to 0.31 ± 0.003 mm during first 24 hrs which was subsequently reduced to 0.20 ± 0.002 mm after 48 hr period. The increase in thickness during first 24 hr was significantly ($P<0.01$) higher. The thickness of skin after 72 hr was 0.11± 0.002 mm was found to be decreased than first 24 hour and 48 hour. similarly the diameter of spreading lesion was 3.17 ±0.03 cm during first 24 hr after challenging by DNCB was significantly to 2.66 ± 0.007 cm and 2.27 ± 0.01 cm followed by 48 hand 72 hour respectively after challenging.

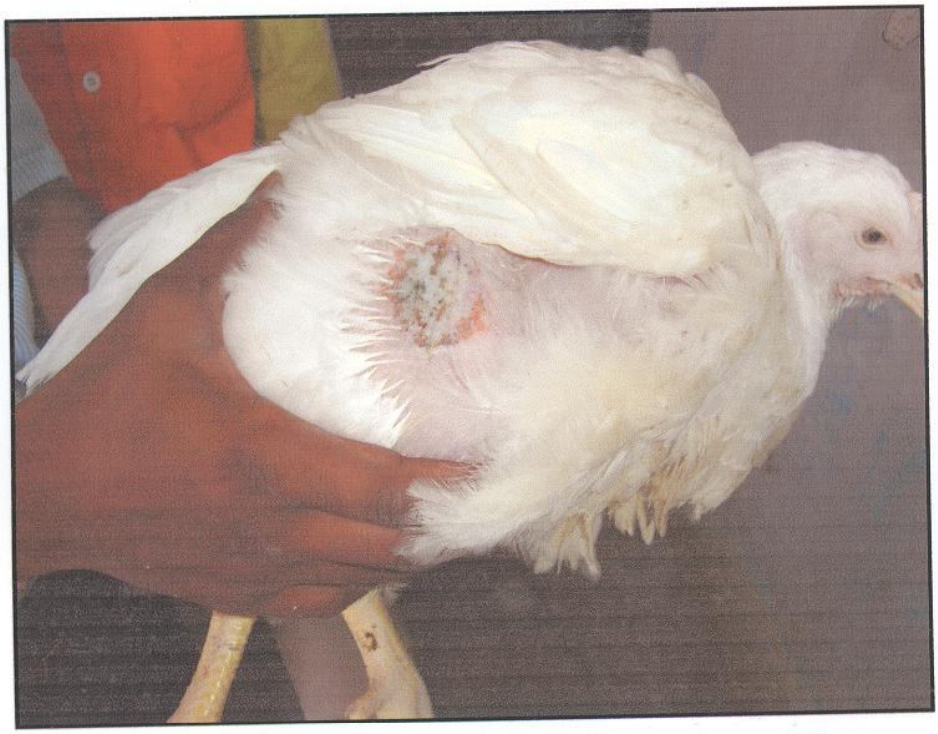

Plate. XIV: Skin lesion after challenge by DNCB

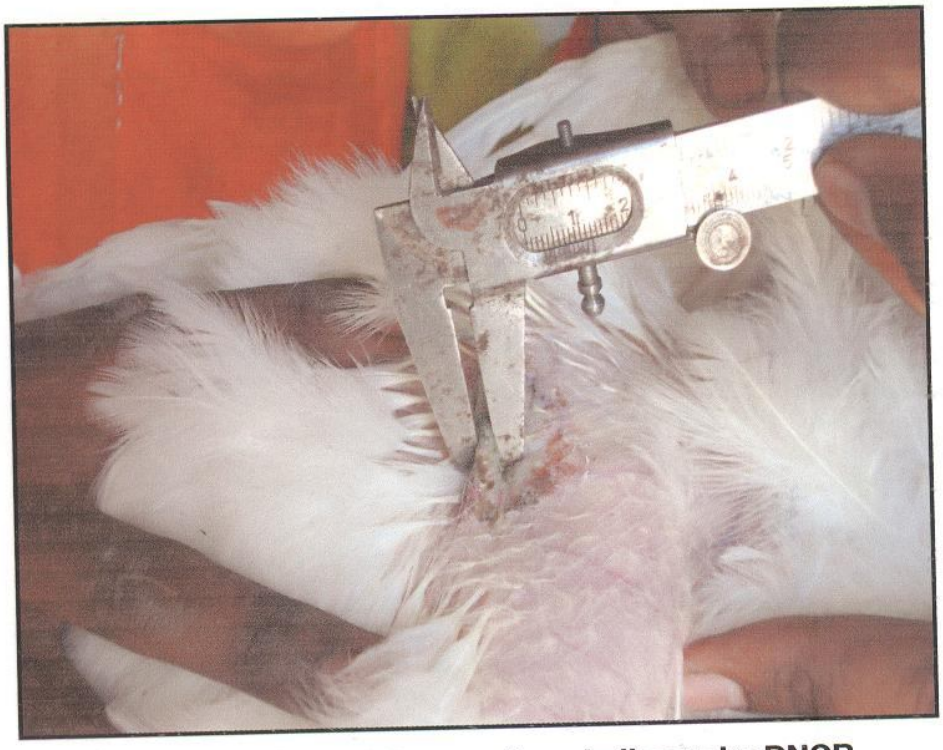

Plate. XV: Skin thickness after challenge by DNCB

Table 22. Delayed hypersensitivity reaction of DNCB post treatment intervals of different of broiler birds fed with *T. cordifolia* stem powder incorporated feed @ 4 gm/kg feed (T1) and 8gm/kg feed (T2)

Treatment groups	Thickness of skin (mm)				Hour mean	Diameter of spreading lesion(cm)			Hour mean
	0 hrs	24 hrs	48 hrs	72 hrs		24 hrs	48 hrs	72 hrs	
T0 (Ctrl.)	0.06 ±0.003	0.31 ±0.003	0.20 ±0.002	0.11 ±0.002	0.17^{b} ± 0.002	3.17 ±0.03	2.66 ±0.007	2.27 ±0.01	2.70^{c} ± 0.003
T1 (@ 4gm/kg feed)	0.10 ±0.001	0.35 ±0.002	0.23 ±0.003	0.13 ±0.002	0.20^{a} ± 0.002	3.36 ±0.01	2.85 ±0.005	2.52 ±0.04	2.91^{b} ± 0.003
T2 (@ 8gm/kg feed)	0.13 ±0.005	0.38 ±0.003	0.24 ±0.003	0.17 ±0.003	0.23^{a} ± 0.004	3.61 ±0.03	3.13 ±0.006	2.70 ±0.02	3.15^{a} ± 0.003
Treatment mean	0.09^{d} ± 0.003	0.35^{a} ± 0.003	0.22^{b} ± 0.003	0.14^{c} ± 0.002		3.38^{a} ± 0.002	2.88^{b} ± 0.005	2.50^{c} ± 0.005	

CD for treatment = 0.004**
CD for hour = 0.005**
CD for treatment * hour interaction =0.01**

CD for treatment = 0.03**
CD for hour = 0.03**
CD for treatment * hour interaction =0.06 ^{NS}

** significant at 0.01 percent level The means connected with same superscript do not differ significantly

In group T1 fed with *T.cordifolia* stem powder @ 4gm/kg feed showed skin thickness at 0 hrs was 0.10 ± 0.001 mm which was significantly($P<0.01$) increased after 24 hr only (0.35 ±0.002 mm) there after it was reduced to 0.23 ± 0.003 mm and returns nearly normal subsequent 72 hours (0.13 ± 0.002 mm). The reduction in thickness in this group was significant after 72 hours. The diameter of spreading lesion at 24 hrs 3.36 ±0.01 cm was significantly ($P<0.01$) higher than at 48 hours (2.85 ±0.005cm) and 72 hour (2.52±0.04cm). Diameter of spreading lesion after 72 hours was return nearly to normal at 0 hours (2.00±00cm).

In group T2 fed with *Tinospora cordifolia* stem powder at the dose rat 8gm/kg feed was showed skin thickness 0.13 ± 0.005 mm at 0 hrs which was significantly increased to 0.38 ±0.003 mm during first 24 hrs there after it was significantly decreased during subsequent 48 hrs and 72 hr respectively after challenging with DNCB. Diameter of spreading lesion in this group was 3.65±0.03 cm during first 24 hrs was significantly ($P<0.01$) higher than spreading lesion observed during subsequent 48 hrs (3.13 ±0.006 cm) and 72 hours (2.70±0.02 cm) respectively.

Comparing skin thickness between different groups T0, T1 and T2, It was observed that subsequently 24 hour of challenging the skin thickness significantly($P<0.01$)increased in both the *T. cordifolia* treatment groups than the control. The increase in skin thickness continued even after 72 hour in this group. Similarly the diameter of spreading lesion was significantly ($P<0.01$) move in T2 group 3.61 ±0.03 cm as compared to 3.36 ±0.01 and 3.17 ±0.03 cm in T2 and T0 groups respectively. The diameter of the spreading lesions T2 groups after 24 hours.

The diameter of inflamed area after 24 hour 3.61± 0.03 cm was maximum, increase in skin thickness 0.38± 0.003 after 24 hour as compared to 0.35 ±0.01 mm in T1 group was significantly ($P<0.01$) higher than T0 group its subsequent reduction in size after 48 and 72 hour was minimum in *T.cordifolia* treated group

(T1, T2) indicated that the *T. cordifolia* enhanced CMI in these birds which was initially responsible for profound inflammatory response that was subsequently continued for 72 hour.

The immunomodulatory potential of *Tinospora cordifolia* stem powder in various immune disorders and experimental investigations were reported by several authors. Atal, *et al* (1986) observed improved phagocytic function without affecting humoral and cell mediated immune response by ethanolic extract of *Tinospora cordifolia* in mice. Gross(1992) reported increase in inflammatory cytokines due to production of corticosteron and inhibit production of antibody which was improved by ascorbic acid during heat stress in broiler.The above reports are in consonant with the findings observed in our study.

Summary & Conclusion

CHAPTER V

SUMMARY AND CONCLUSIONS

Study was carried out to evaluate antibacterial activity and immunomodulatory potential of *Tinospora cordifolia* in poultry with the following objectives

1. To prepare aqueous and ethanolic hot extracts of stem of *Tinospora cordifolia* and to determine its percentage of extractability.
2. To study antibacterial activity of these extracts alone and potent extract in combination with reference drug ciprofloxacin against common pathogenic bacteria by disc diffusion and tube dilution method in vitro.
3. To determine minimal inhibitory concentration (MIC) of most potent antibacterial extract by tube dilution technique *in vitro.*
4. Phytochemical studies of these extracts (Qualitative analysis) for presence of active principles and
5. To evaluate immunomodulatory potential of *Tinospora cordifolia* stem powder employing DNCB skin sensitivity test in poultry.

The percentage of extractability of aqueous and ethanolic hot extracts was found to be 4.83 and 4.29 in the respective solvents.

These extracts at different concentrations viz. 100%, 50% and 10% were evaluated for antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* and *Staphylococcus aureus* by discs diffusion assay. The amount aqueous extract of 100%, 50% and 10% concentrations in each disc was found to be 22.67mg/disc, 17.17 mg/disc and 13.97 mg/disc respectively and ethanolic extract 14.53 mg/disc, 15.33 mg/disc and 18.30 mg/disc respectively.

The aqueous extract impregnated disc with 100% and 10% concentration were showed zone of inhibition (mm) 9.33, 7.67 mm and 7.67, 10.22 mm against

Escherichia coli and *Staphylococcus aureus*. 50% concentration was found to be ineffective against all test bacteria. 100% ethanolic concentration of extract was effective against *Staphylococcus aureus* (zone of inhibition 11.33 mm), 50% concentration against *E. coli* (9.67 mm), *Salmonella typhimurium* (11.33 mm) and *Staphylococcus aureus* (7.67 mm) and 10% concentration was effective against *E. coli* (9.00 mm), *Salmonella typhimurium* (10.00 mm) and Staphylococcus aureus (7.00 mm). Both aqueous and ethanol extract concentrations were found to be ineffective against *Pseudomonas auriginosa*. Zone of inhibition showed by all test extract concentrations was significantly lesser than the ciprofloxacin a reference drug this method. Sensitivity pattern of different bacteria to these extracts was less sensitive to sensitive.

Bacterial colony count evaluated by tube dilution method showed significant reduction in colony count by 50% ethanol extract (100±1.67 to 226±5.03) followed by 10% against *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* as compared to control bacterial colony count (197±4.36 to 266.67±10.14).

MIC of potent ethanol extract of *T. cordifolia* as determined by tube dilution method against *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* were 2.0, 1.0 and 1.5 mg/ml respectively.

Drug-extract interaction study assessed by disc diffusion method revealed antagonistic effect of 50% ethanolic extract with ciprofloxacin.

The glycosides, sugar, sterols, flavonoids and saponins were found in the aqueous and ethanol extract by qualitative phytochemical analysis.

Cell mediated immune response as immunological parameter was assessed in broiler birds exposed to extreme hot environmental conditions *in vivo*. Thirty broiler birds were grouped into three groups viz. T1 served as untreated control, T2 fed with T. cordifolia @ 4 gm/kg and T2 fed with 8 gm/kg feed incorporated T.

cordifolia stem powder. The body weight, weight gain, feed consumption and water intake was increased as compared to control.

Skin hypersensitivity test showed significant increase in skin thickness and size of spreading lesion in *T. cordifolia* supplemented groups compared to control. In all three groups the thickness of the skin at the challenge site was increased significantly within first twenty four hours. The increase in thickness was reduced subsequently and during subsequent 72 hrs it reaches nearer to normal in control group however in *T. cordifolia* fed groups T1 and T2 it remained still significantly increased.

The diameter of all spreading lesions was highest in *T. cordifolia* supplemented group was indicated that inflammatory process was more sever in these groups as compared to control.

The following conclusions were drawn from the present study.

1) Percentage extractability of aqueous and alcoholic extract were 4.83 and 4.29 respectively which was statistically non significant.
2) Both extracts of *T. cordifolia* had broad spectrum antibacterial activity which was concentration dependent and was comparatively much lesser than ciprofloxacin *in vitro*.
3) MIC values of potent ethanol extract were 2.0, 1.0 and 1.5 mg/ml against *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* respectively.
4) The glycosides, sugar, sterols, flavonoids and saponins were present in both aqueous and ethanol stem extract of T. cordifolia.
5) *T. cordifolia* stem powder supplementation in broiler significantly contribute to better body weight gain, feed consumption and water intake as compared to control and had persistence in sever inflammatory processes during summer was suggestive its adaptogenic potential of this plant.

Bibliography

BIBLIOGRAPHY

Adhila, A., K. Rani and R.S. Thakur (1991) Biosyntesis of the clerodane furanoditerpene lactone skeletone in *Tinospora cordifolia*. Phytochemistry. 30(8): 2573-2576.

Agraval, K., R. Jain and K.Sharma (2008) Efficacy of root extract of *Tinospora cordifolia* against seed born pathogens of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. Annals of Plant Protection Science, 16(1).

Agraval, S. S. and V. K. Singh (1999) Immunomodulator : a review of studies on Indian medicinal plants and synthetic peptides, Part I. Medinal Plant Pinsa B65 Nos. 3 and 4 : 179-204.

Ahmed, S. M., L. R. Manhas, V. Verma and R. K. Khanuja (2006) Quantitative determination of four constituents of *Tinospora* spp. by reversed phase HPLC- UV-DAD method. Broad based studies revealing variation in content of four secondary metabolites in the plant from different eco-geographical region of India. J.Chromatogr.Sci. 44(8):504-9.

Ahmed, L., Z. Mohammed and F. Mohammed (1998) Antimicrobial properties os some Indian medicinal plants. J. Ethanopharmacol ., 62 : 183-198

Ali, M (1990) Ayurvedic drugs in Unani Materia Medica. Ancient science of life IX (IV):191-201.

Ambasta, S.P. (1986) The useful plants of India. CSIR, New Delhi.

Atal, C.K., and B.M. Kapoor (1982) Cultivation and utilization of medicinal plants. Regional Res.Lab.CSIR, JammuTawi, PP.824.

Atal, C. K., M. L. Sharma, A. Kaul and A. Khajuria (1986) Immunomodulating agents of plant origin I: Preliminary screening, J. Ethanopharmacol.18:133-141.

Bapat R. D., N. N. Rege, R. S. Koti, N. K. Desai and S. A. Dahanukar (1995) Can we do away with PTBD. HPB Surg. 9(1):5-11.

Bauer, A.W., M.M. Kirby, T.C. Sherris and M. Truck (1966) Antibiotic susceptibility testing by standardized single disc diffusion method. Am. J. Clin. Pathol. 45:493-496.

Bhat G.A. and S.C. Gupta (2008) effect of antistress agent on performance of WHL hen during summer. Animal nutrition and feed technology, 8 : 259-264.

Bhatt, R. K. and B. K. Sabatta (1989) A furanoid diterpene glucoside from *Tinospora cordifolia*. Phytochemistry. 28 (9): 2419-2422.

Bhatt, R. K., B. Jampani and B. K. Sabatta (1988) A new clerodane derivative from *Tinospora cordifolia*. Phytochemistry. 27(4) :1212-1216.

Bhattacharya, S. K. and S. Ghosal (1994) Experimentation of antistress activity of a herbal formulation Zeetress. Indian J. Indg. Med. 10 (2) : 1-7

Bishayi, B. Roychowdhury, S. Ghosh and M. Sengupta (2002) Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl4 intoxicated mature albino rats. J. of Toxicol. Sci. 27(3):139-46.

Branter, A.E., and Grein (1994) Antimicrobial activity of plant used externally in traditional medicine. J. Ethnopharmacology, 4435-40.

Chatterjee, A. and S. Ghosh (1960) Tinosporin, the furonoid bitter principle of *T. cordifolia* Miers. Science and Cult. 23:140-141.

Chatterjee, A. and S.C. Pakrashi,(1991) The treatise of Indian Medicinal Plants. CSIR, New Delhi.

Chintalwar, G, A. Jain, A. Sipahimallani, A. Banergi, P. Sumariwalla, R. Ramkrishanan and K. Sainis (1999) An immunologically active arabinolactone form *Tinospora cordifolia*; Phytochemistry 52: 1089-1093

Chau P. Y., Y.K. Ceung and W.W.S.Ng (1986) Comparative In vitro antibacterial activity of Ofloxacin and Ciprofloxacin against some selected gram-positive and gram-negative isolates. J. of Infection (14): S237-S239.

Chopra, R. N. (1994) Indigenous drug of India. 2nd edn., Calcutta, India:Academic Publishers. In, Indian J. Pharmacol, 38(5):311-315.

Chopra, R.N., S.L. Nayar and I.C. Chopra (1956) Glossary of Indian medicinal plants. First .edn. PP.256-257, CSIR, New Delhi

Cowan, M. M. (1999) plant products as antimicrobial agents. Clin. Microbial. Rev. 2564-82.

Cruickshank, R.J., P. Duguid, B. P. Marmion and R.H.A. Swain (1975) Medical Microbiology. 12th edn. The English Book Society, E and S., Churchill, Livingstone,Edinburgh.

Dahanukar, S. A., U. M. Thatte, N. Pai, P. B. More and S. M. Karandikar (1988) Immunotherapeutic modification by *Tinospora cordifolia* of abdominal sepsis induced by caecal ligation in rats;Ind. J. Gatsroenterol. 7(1):21-23

Dahanurkar, S. A., R. A. Kulakarni, and N. N. Rege. (2000) Pharmacology of medicinal plants and natural products. Ind. J. Pharmacol. 32:S81-S118.

Dash, B. and B. K. Sharma (2001) Charak samhita, 7th Edn. Chaukhamba Sanskrit series office, Varanasi, India.

Desai, V. R., R. Ramkrishnan, G. J. Chintalwar and K. B. Sainis (2007) " G1-4A" , an immunomodulatory polysaccharide from *Tinospora cordifolia* modulates macrophase response and protect mice against lipopolysaccharide induced endotoxic shock.Int. Immunopharmacology. 7(10):1375-1386.

Devegowda, G., B.S.Ramappa and B.S. Barmase (1990) Livol supplementation in broiler diets improved there performance. Pashudhan. 5 : 25

Dhar, M.L, M. L. Dhar, B. N. Dhavan, B. N. Mahrotra and C.Ray (1968) Screening of Indian plants for biological activity: Part I, Ind. J.Experimental. Biol. 6:232- 245

Dhande, V. U., R. J. Kukde, and B. S. Thakur (1991) Effect of Livol on performance of broiler. Poultry Guide. 18 (17) : 35-36

Dhiman, A. (2006) Ayurvedic Drug Plants. PP. 144-146., Daya Publishing House, Delhi.

Dhuley, J. N, (1997) Effect of some Indian Herbs on macrophage functions in ochratoxin A treated mice, J. of Ethanopharmacol 58:15-20.

Diwanay, S., D. Chitre and B. Patwardhan (2004) Immunoprotection by botanical drugs in cancer chemotherapy. J. of Ethnopharmacology. 90(1):99-55.

Donkoh, A (1989) Ambient temperature : a factors affecting performance and physiological response of broiler chickens. Int. J. Biometeorology. 33 : 259-256.

Dubey, N.K., R. Kumar and P. Tripathi, (2004). Promotion of herbal medicines: India's opportunity. Curr. Sci., 86: 37-41

Ensminger, M. F (1992) Poultry science. Third edition International Book Distibuting Company, Lakhnow (India).PP. 144-153.

Franworth, N.R. and A.S. Bingel (1977) A new natural product and plant drug with pharmacological biological or therapeutical activity Editors. Wagner, H., Wolff, P. Springer-Verleg, Berlin, PP. 1

Fansworth, N.R.O., A.S. Akerela, D. Binger, D. Soejarta and Z. Eno (1986) Medicinal plants in therapy. Bulletin of the WHO 63 (6):965-981.

Gangan, V. D., P. Pradhan, A. P. Sipahimalani and A. Banerji (1995). Nor-diterpene furon glycoside from *Tinospora cordifolia*.Phytochemistry. 39(5):1139-1142.

Gangan, V. D., P. Pradhan, A. T. Sipahimalani and A. Banerji (1994) Cordifolioside ,A,B,C: nor-diterpene furon glycoside from *Tinospora cordifolia*. Phytochemistry. 37(3):781-786.

Girish, H.V. and S. Satish (2008) Antibacterial activity of important medicinal plants on human pathogenic bacteria-a Comparative Analysis. World Applied Sciences Journal 5 (3): 267-271

Gupta, A. K. and N. Mishra (2006) Ancient Indian Medicine and Medicinal Plants. In "Herbal drugs" Edrs. R. K. Shrama and A. Arora pp. 95 Jaypee Brothers Medical Publishers, New Delhi.

Gupta, K.C, and R. Vishwanath (1956) Antituberculus substances from plants; Antibiotic and Chemotherapy 6:194-5

Gross, W. B. (1992) Effect of short term exposure of chickens to coticosteron on resistance to challenge exposure with *Escherchia coli* and antibody response to sheep erythrocytes. Am. J. Vet. Res. 53 : 291-293

Hanuman, J. B., R. K. Bhatt and B. K. Sabata (1986) A di-terpenoid furanolactone from *Tinospora cordifolia*. Phytochemistry. 25(7) : 1677-1680.

Hanuman, J. B., R. K. Bhatt and B. K. Sabata (1986) A di-terpenoid furanolactone from *Tinospora cordifolia*. Phytochemistry. 25(7) : 1677-1680.

Harle, U. N. and N. J. Gaikwad (2004) Effect of *Mimordica charentia* and *Tinospora cordifolia* extract on intestinal drug transporter pump :P-glycoprotein:Indian Journal of Pharmacology 36(5) : 319-320.

Henken, A. M., G. R. Kleinhout, W. Wanderhel (1982) Effect of environmental temperature on immune response and metabolism of young chckens. Poultry Science. 61 : 503-509

Ikram, M., S. G. Khattak and S. N. Gilani (1987) Antipyretic studies of some indigenous Pakistani medicinal plants: II. J. of Ethnopharmacology 19(2):185-192.

Irwin, A. J. and N. Gopakumar (2004) Effect of *Tinospora cordifolia* in carrageenin induced changes of inflammation in albino rats. Abst., In IVth ISVPT conference held between 22-24 December, at Durg (Chhattisgarh) PP 22.

Jahfar, M and P. Azadi (2004) Glycosyl composition of polysaccharide from *Tinospora cordifolia* : II. Glycosyl linkage.Acta.Pharm.54(1) :73-8.

Jahfer, M (2003) Glycosol composition of polysaccharide from *Tinospora cordifolia* . Acta.Pharm. 53(1):65-9.

Jeyachandran R.,T.F. Xavier and S. P. Anand (2003) Antibacterial activity of stem extract of *Tinospora cordifolia* (wild) Hook. F and Thomson: Ancient Scinece of Life : XXIII (1) : 40-43

Kapil, A and S. Sharma (1997);Immunopotentiating compounds from *Tinospora cordifolia*, J. Ethanopharmacol. 58:89-95

Kapoor, L. D. (2005) Handbook of Ayuvedic Medicinal Plants. CRC Press, Boca Raton, Londan, New York, Washingtan DC. PP:324.

Katewa, S.S. and A. Arora (1997) Some Plants in Folk Medicine of Udaipur District (Rjastan) Ethnobotany. 9:48-51.

Khan, M. A., A. I. Gray and P. G. Waterman (1989) Tinospraside, an 18-norclerodane glucoside from *Tinospora cordifolia*. Phytochemistry, Vol. 28 (1): 273-275.

Kidwai, A.R., K.C. Salooja, V. Verma and S. Siddiqui (1949) Chemical examination of *Tinospora cordifolia*. J Sci. Ind. Res. 8B: 115.

Kirtikar .K.R and B.D.Basu (1935). Indian Medicinal plants periodicals experts' book agency Delhi- volumeIV 2nd edn. pp.2513

Khobragade, R.S., A. N. Sarag, D. H. Rekhate and A. P. Dhok (2005) Effect of herbal feed supplementation of medicinal plants *Tinpspora cordifolia* and *Leptadenia reticulata* on haemo-imuno-biochemical profile of broilers. Indian Vet. Med. Jour. Vol.29 : 280-282

Kirtikar, K. R. and B. D. Basu (1993) Indian Medicinal Plants. Second Edn. Vol. 1 PP. 77-80. Periodical Experts Book Agency, Delhi.

Kohli, K. and G. K. Jain (2006) Pharmacological studies of indigenous system of medicine. In "Herbal Drugs" Edtrs. R.K.Sharma and A. Arora, PP.95, Jaypee Brothers Medical Publishers, New Delhi.

Kumar, Y. and S. K. Jain (1998) A contribution to Ethnobotany of Surguja district in Madhya Pradesh (India). Ethnobotany. 10:89-96.

Mahesh, B., S. Satish (2008) Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences 4(S) : 839-843

Maurya, R., Dhark and S. S. Handa (1997). A sesquiterpene glucoside from *Tinospora cordifolia*, Phytochemistry. 44(4):749-750.

Maurya, R., L. R. Manhas, P. Gupta, P. K. Mishra, G. Sing and P. P. Yadav (2004) Amritosides A,B,C and D : clerodane furanoditerpene glucodside from *Tinospora cordifolia*. Phytochemestry. 65(14):2051-5.

Maurya, R., V. Wazir, A. Tyagi and R. S. Kapil (1995). Clerodane diterpenoids from *Tinospora cordifolia*. Phytochemistry. 38(3):659-661.

Morgan, G. W., P. Thaxton and S. W. Edens (1976) Reduced sympotoms of anaphylaxis in chickens by ACTH or heat Poultry Science., 55 : 1498-1504

Mohana , D.C., S. Satish , K.A. Raveesha (2008) Antibacterial evaluation of some plant extracts against some human pathogenic bacteria. Advances in Biological Research 2(3-4) : 49-55

Nagarkatti, D. S., N. N. Rege, N. K. Desai and S. A. Dahnukar (1994) Modulation of kupffer cell activity by *Tinospora cordifolia* in liver damage. J.of Postgrad. Med. 40:65-7.

Nair, P. K. R., S. J. Melnick, R. Ramchandran, E. Escalon and C. Ramchandran (2006) Mechanism of macrophase activation by (1,4)- alpha-d-glucan isolated from *Tinospora cordifolia*. Int. Immunology. 6(12,5):1815-1824.

Nair, P. K., S. Rodriguer, R. Ramchandran, A. Alamo, S. J. Melnick, E. Escalon, P. I. Jr. Garcia, S. F. Wnuk and C. Ramchandran (2004) Int. Immuno. Pharmacol. 4(13):1645-59.

Negi, K. S. and K.C. Pant (1994) Genetic Wealth of Agri-horticulture Crops, Their Wild Relatives, Indigenous and aromatic plants of UP, Himalaya. J Econ Taxon Bot., 18(1):17-41.

Newman, D. J., G. M. Cragg and K. M. Snader (2003) Natural products as sources of new drugs over the period 1981–2002. Phytochemistry. 66: 1022-37.

Parida, M. M., P.K. Das, P. Saxena, A.M. Jana and P. V. L. Rao (2006) Perspective and antimicrobial activity of natural plant products. In. Herbal Drugs-A twenty first century perspectives, Edtrs, Sharma, R.K. and Arora, R,Jaypee Brothers Medical Publishers Pvt. Ltd, New Delhi. PP. 484-485.

Patel, B. M. and P. S. Patel (1956) Fodder value of tree and vegetable leaves in Khaira district. The Indian Journal of Agriculture Science. XXVII (III): 307-315.

Peclzer, M. J., E.C.S. Chan and N. R. Krig (1986) Microbiology. 5th edn. PP. 535- Mc Grew Hills Book, Co. Singapore.

Patwardhan, B., D. Kalbag, P. S. Palki, B.A. Nagswapagi (1990) Search for immunomodulatory agents: A Review Indian Drugs. 28(2): 56-63

Prajapati, N. D., S. S. Purohit, A. K. Sharma and T. Kumar (2003) A Handbook of Medicinal Plants. pp:515. Agrobios, Jodhpur (India).

Rao, A.T., B. Pradhan, H.K. Mohapatra and B. C. Das (1997) Immune response due to Zeetress (herbal preparation) in infectious bursal disease vaccinated chicks. Pashudhan, 12 (9): 4-5.

Rege, N. N. and S. A. Dhanukar (1993) Qualitation of microbicidal activity of mononuclear phagocyte.: an *in vitro* technique. Postgrad. Med. 39(1):22-5.

Rege, N. N., U. M. Thatte and S. A. Dahanukar (1999) Adoptogenic preparation of six rasana herbs used in ayurvedi medicine. Phytotherpy Res. 13(4):275-91.

Rege, N. N., H. M. Nazareth, R. D. Bapat and S. A. Dahanukar (1989) Modulation of immunosuppression in obstructive jaundice by *Tinospora cordifolia*. Indian J. Med. Res. 90: 478-483.

Regnier, J. A., K. W. Kelly and C. T. Gaskin(1980) Acte thermal stressors and synthesis of antibodies in chickens. Poult. 59 : 985-990

Robert, B. and I. Henry, (2006) Medicinal Plants. Vol. I, PP. 48-50. Ajay Book service. New Delhi (India).

Rosenthaler, L. (1930) The chemical investigation of plant. 1st edn. Bell and Son's London, PP36.

Samy, R. P, (2005) Antimicrobial activity of some medicinal plants of India, Fitoterapia, 76(7-8):697-9.

Samy R.P., P.N. Pushparay and P. Gopalakrishna Kona (2008) a compilation bioactive compounds from ayurveda. Bioinformation, 3(3): 100-110.

Saaud I. Al-Mufarrej., Abdul Aziz A., Al-Bisher and mansour F. Hussein (1999) effect of short-term heat stress on the immune function of chickens: A Review. J. King Saud Univ., vol. 11, agric. Sci. (1), pp. 57-71, Riyadh (1419-1999)

Sharma, P.C., M.B. Yelne and T.J. Dennis (2005) Data Base on Medicinal Plants used in Ayurveda. Vol.3. PP.256-281.Central Council for Research in Ayurveda and Siddha, New Delhi

Snedecor, G. W. and W.G. Cochran (1967) Statistical methods. 6th edn.Oxford and IBH, New Delhi.

Sohni, Y. R. and R. M Bhatt (1996). Activity of crude extract of formulation in experimental hepatic amoebiasis in immunomodulation studies. J. of Ethnopharmacology. 54(2-3): 119-124.

Spelmam, K (2001) Traditional and clinical uses of *Tinospora cordifolia*, guduchi, Aust. J. Med. Herbalism 13(2): 49-57

Spleman, K., J. Burn, D. Nichols, N. Winters, S. Ottersberg and M. Tenborg (2006) Modulation of cytokine expression by traditional medicine: A review of herbal modulators. Altern. Med. Rev. 11(2):128-50.

Sreeramulu, P (1989) Evaluation of CMI response in lambs using 2, 4, DNCB, IVJ, 66:381-384

Stockwell, C. (1998) Natures Pharmacy Century Hatchinson, Ltd. London UK. In, Herbal Drugs-A twenty first century perspectives, Edtrs, Sharma, R.K. and Arora, R, Jaypee Brothers Medical Publishers Pvt. Ltd, New Delhi. PP. 484- 485.

Thatte, U. M, M. R. Kulkarni and S. A. Dahanukar (1992) Immunotherapeutic modulation of Escherrichia coli peritonitis and bacteremia by *Tinospora cordifolia*, J. Postgade. Med. 38(1):13-15.

Thatte, U. M., S. G. Rao and S. A. Dahanukar (1994) *Tinospora cordifolia* induces colon stimulatory activity in serum. J. Postgrad. Med. 40(4):202-3.

Trease, G. E. and W. C. Evans (1985) Text Book of Pharmacognosy, English Language Book Society, Bailliers Tindall, 12th edn., 483-484.

Umadevi , P ., S. Murugan, S. Suja , S.Selvi, P. Chinnaswamy and E. Vijayanand (2007) Antibacterial, in vitro lipid. per oxidation and phytochemical observation on Achyranthus bidentata blume. Pakistan Journal of Nutrition

Valsale, K.V., A. Rajan and M. Krishana Nair (1981). Evaluation of cutaneous hypersensitivity to 2-4-DNCB in ducks. Kerala J. vet. Sci. 12(2):332-336.

Zahraa, H. Al-Ghamdi (2008) effect of commutative heat stress on immunoresponses in broiler chickens reared in closed system. International Journal of Poultry Science 7 (10): 964-968, 2008

Appendix

APPENDIX

Table 2. Analysis of variance for amount of aqueous extract of *Tinospora cordifolia* stem impregnated on the blank disc/discs

Source	DF	Extract Impregnated disc		Extract in each disc	
		MSS	'F' value	MSS	'F' value
Treatment	2	22667	7.03*	56.94	1289.3**
Error	6	3.22		0.04	
Total	8				

** Significant P < 0.01
* Significant P < 0.05

Table 3. Analysis of variance for amount of ethanolic extract of *Tinospora cordifolia* stem impregnated on the blank disc/ discs

Source	DF	Extract Impregnated disc		Extract in each disc	
		MSS	'F' value	MSS	'F' value
Treatment	2	4247.4	530.2**	11.10	850.2**
Error	6	8.00		0.01	
Total	8				

** Significant P < 0.01

Table 10. Analysis of variance for comparative zone of inhibition of different extracts of *Tinospora cordifolia* stems and reference drug (ciprofloxacin) against different bacteria

Source	DF	*Escherchia coli*		*Salmonella typhimurium*		*Staphylococcus aureus*	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatment	6	262.63	262.63**	279.56	293.53**	425.42	3.65*
Error	14	1.00		0.95		4.36	
Total	20						

** Significant P < 0.01
* Significant P < 0.05

Table 14. Analysis of variance for zone of inhibition of ethanolic stem extract of *T. cordifolia*, ciprofloxacin and its combination against different bacteria

Source	DF	*Escherchia coli*		*Salmonella typhimurium*		*Staphylococcus aureus*	
		MSS	'F' value	MSS	'F' value	MSS	'F' value
Treatment	2	343.00	1029**	205.78	1852**	56.778	170.33**
Error	6	0.33		0.11		0.33	
Total	8						

** Significant P < 0.01

Table 17. Analysis of variance for Feed consumption of broilers at different age fed with different levels of stem powder of *Tinospora cordifolia*

S.V	D.F	MSS	'F' value
Treatment	2	279000	139.61**
Week	2	809470	405.06**
Treatment X Week	4	75445	37.75**
Error	81	1998.4	
Total	89		

** Significant P < 0.01

Table 18. Analysis of variance for body weight of broilers at different age fed with different levels of stem powder of *Tinospora cordifolia*

S.V	D.F	MSS	'F' value
Treatment	2	75406	168.48**
Week	3	8350900	18435**
Treatment X Week	6	20778	46.42**
Error	108	447.57	
Total	119		

** Significant P < 0.01

Table 19. Analysis of variance for body weight gain of broilers at different age fed with different levels of stem powder of *Tinospora cordifolia*

S.V	D.F	MSS	'F' value
Treatment	2	24861	29.21**
Week	2	597630	70.23**
Treatment X Week	4	13776	16.19**
Error	81	850.89	
Total	89		

** Significant P < 0.01

Table 20. Analysis of variance for Feed conversion ratio of broilers at different age fed with different levels of stem powder of *Tinospora cordifolia*

S.V	D.F	MSS	'F' value
Treatment	2	1.16	18.65**
Week	2	5.17	82.94**
Treatment X Week	4	0.79	12.70**
Error	81	0.06	
Total	89		

** Significant P < 0.01

Table 21. Analysis of variance for Water intake of broilers at different age fed with different levels of stem powder of *Tinospora cordifolia*

S.V	D.F	MSS	'F' value
Treatment	2	786.11	1.52^{NS}
Week	2	7449500	14367
Treatment X Week	4	27.77	0.05^{NS}
Error	54	518.52	
Total	62		

** Significant P < 0.01
NS at P < 0.01 and P < 0.05

Table 22. Analysis of variance for delayed hypersensitivity reaction of DNCB post treatment intervals of different groups of broiler birds fed with *T. cordifolia* stem powder incorporated feed

Thickness of skin

S.V	D.F	MSS	'F' value
Treatment	2	0.03	34.76**
Week	3	0.10	35.18**
Treatment X Week	6	0.13	9.64**
Error	108	0.001	
Total	119		

** Significant P < 0.01

Diameter of spreading lesion of skin

S.V	D.F	MSS	'F' value
Treatment	2	1.52	300.8**
Week	2	5.92	1169.8**
Treatment X Week	4	0.006	1.36 NS
Error	54	0.005	
Total	62		

** Significant P < 0.01

NS at P < 0.01 and P < 0.05

Vita

VITAE

The author Mr. Patil Mukesh Subhash was born on 20th May 1983 at his native place Kasoda in Erandol of Jalgaon district of Maharashtra State.

He has completed his primary education from Government Primary School, Kasoda of Jalgaon District. He has passed SSC board exam in 1999 with First class from Sadhana Madhyamik Vidyalaya, HSC in 2001 with First class with Distinction from F.M. Khandelwal Junior College of Science, Shirur District Dhule of Maharashtra State.

Being interested in animal welfare, he joined College of Veterinary Science and Animal Sciences, Parbhani and completed the B.V.Sc. and A.H. degree with first class in 2006.

He has joined M.V.Sc. degree course in the discipline of Veterinary Pharmacology and Toxicology at Post Graduate Institute of Veterinary and Animal Sciences, Akola to be complete.

Further he has Passed MS-CIT examination and participated in National Conference held at Mathura and extension activities undertaken by the institute.

Thesis Abstract

THESIS ABSTRACT

a)	Title of the thesis	:	**Studies on antibacterial activity and immunomodulatory potential of *Tinospora cordifolia* in poultry**
b)	Full name of student	:	**PATIL MUKESH SUBHASH**
c)	Name and address of Major Advisor	:	Dr. G.D. Ranvir , Associate Professor, Department of Pharmacolgy and Toxicology, College of Veterinary and Animal Sciences, MAFSU, Parbhani – 431 402 (M.S.)
d)	Degree to be awarded	:	M.V.Sc.
e)	Year of award of degree	:	2009
f)	Major subject	:	Pharmacology and Toxicology
g)	Total number of pages in the thesis	:	52
h)	Number of words in the abstract	:	692
i)	Signature of Student	:	Patil
j)	Signature, Name and address of forwarding authority (HOD/SH)	:	Dr.S.W.Hajare, Assistant professor, I/C Head Department of Veterinary Pharmacology and Toxicology, PGIVAS, Akola

ABSTRACT

Tinospora cordifolia herb is well known medicinal plant used as single drug entity in the Ayurveda. It is popular folklore herbal medicine used in the treatment of many ailments in man and animal. The present investigation was carried out to evaluate antibacterial activity and immunomodulatory potential of stem of *Tinospora cordifolia* in poultry with the following objectives

1. To prepare aqueous and ethanolic hot extracts of stem of *Tinospora cordifolia* and to determine its percentage of extractability.
2. To study antibacterial activity of these extracts alone and potent extract in combination with reference drug ciprofloxacin against common pathogenic bacteria by disc diffusion and tube dilution method in vitro.
3. To determine minimal inhibitory concentration (MIC) of most potent antibacterial extract by tube dilution technique *in vitro.*
4. Phytochemical studies of these extracts (Qualitative analysis) for presence of active principles and
5. To evaluate immunomodulatory potential of *Tinospora cordifolia* stem powder employing DNCB skin sensitivity test in poultry.

The percentage of extractability of aqueous and ethanolic extract was found to be 4.83 and 4.29 respectively.

The amount aqueous extract at 100%, 50% and 10% concentrations adsorbed in each disc was found to be 22.67mg/disc, 17.17 mg/disc and 13.97 mg/disc respectively. The ethanol extract in each disc adsorbed was found to be 14.53 mg/disc, 15.33 mg/disc,18.30 mg/disc. The amount of 10% ethanol extract adsorbed in each disc was found highest and lowest at 100% concentration.

Zone of inhibition (mm) of aqueous extract at 100%, and 10% concentration was found to be 9.33, 7.67 mm and 7.67, 10.22 mm against *Escherichia coli* and *Staphylococcus aureus* where 50% concentration was ineffective against all four test bacteria. Similarly zone of inhibition of ethanol extract at 100% concentration was effective against *Staphylococcus aureus* (11.33 mm) only. 50% concentration against *E. coli* (9.67 mm), *Salmonella typhimurium* (11.33 mm) and *Staphylococcus aureus* (7.67 mm) and 10% concentration were effective against *E. coli* (9.00 mm), *Salmonella typhimurium* (10.00 mm) and Staphylococcus aureus (7.00 mm). Both the extracts at different concentrations were ineffective against *Pseudomonas auriginosa*. Compared to ciprofloxacin both extracts concentration were showed lesser zone of inhibition that was indicative lesser antibacterial activity. Sensitivity pattern of different bacteria to these extracts was ranged from less sensitive to sensitive.

Colony count of susceptible bacteria was significant reduced by 50% ethanol extract (100±1.67 to 226±5.03) followed by 10% against *E. coli,*

Salmonella typhimurium and *Staphylococcus aureus* as compared to control bacterial colony count (197±4.36 to 266.67±10.14).

MIC of ethanolic extract against *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* were 2.0, 1.0 and 1.5 mg/ml respectively.

A drug interaction study was revealed antagonistic effect of 50% ethanolic extract with ciprofloxacin.

The glycosides, sugar, sterols, flavonoids and saponins were found in the aqueous and ethanol extracts (qualitative phytochemical analysis).

Immunomodulatory potential of *T. cordifolia* stem powder was evaluated in three different treatment groups of broiler birds were exposed to extreme hot environmental conditions. T1 group was untreated control, T2 fed with T. cordifolia @ 4 gm/kg feed and T2 group fed with 8 gm/kg feed incorporated *T. cordifolia* stem powder. Results of this investigation were showed increased body weight, weight gain, feed consumption and water intake was indicative of adaptogenic effect.

DNCB skin hypersensitivity test was performed to assess CMI was reveled significant increase in skin thickness and size of spreading lesion following different treatment levels as compared to control. The highest diameter of all spreading lesions in *T. cordifolia* fed group indicates inflammatory process that was more sever as compared to control.

The following conclusions were drawn from the present study.

1) Percentage extractability of aqueous and alcoholic extracts was found to be 4.83 and 4.29 respectively
2) *T. cordifolia* aqueous and ethanolic stem extracts had broad spectrum antibacterial activity which was comparatively lesser than ciprofloxacin in vitro.
3) MIC value of potent ethanolic extract was found to be 2.0, 1.0 and 1.5 mg/ml against *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* respectively.
4) The glycosides, sugar, sterols, flavonoids and saponins were present in both the aqueous and ethanolic extracts.
5) Supplementation of *T. cordifolia* stem powder in broiler feed contribute better body weight gain, feed consumption and water intake as compared control showed adaptogenic potential during hottest summer.

प्रबंध सारांश

प्रबंध सारांश

१) प्रबंध शिर्षक	:	"गुळवेल वनस्पतीची जिवाणूप्रती क्रियाशिलता व प्रतिकार क्षमता कोंबडयांमध्ये अभ्यासणे"
२) विद्यार्थ्याचे नांव	:	पाटील मुकेश सुभाष
३) मुख्य मार्गदर्शकाचें नांव	:	डॉ. जी. डी. रणवीर, सहाय्यक प्राध्यापक पशुवैद्यकिय औषध निर्माण शास्त्र व विषशास्त्र विभाग पशुवैद्यकिय व पशुविज्ञान महाविद्यालय परभणी (महाराष्ट्र)
४) प्रदान केली जाणारी पदवी	:	एम.व्ही.एस.सी. (औषध निर्माण शास्त्र व विषशास्त्र)
५) पदवी प्रदान करण्याचे वर्ष	:	२००९
६) मुख्य विषय	:	औषध निर्माण शास्त्र व विषशास्त्र
७) प्रबंधामधील एकूण पाने	:	५२
८) सारांश मधील एकूण शब्द	:	५३९
९) विद्यार्थ्याची सही	:	Patil
१०) प्रबंध कार्यवाहीस्तव पाठविणा-या अधिका-याची सही, नाव व पत्ता	:	(डॉ. एस. डब्ल्यु. हजारे) सहाय्यक प्राध्यापक पशुवैद्यकिय औषध निर्माण शास्त्र व विषशास्त्र विभाग स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था अकोला (महाराष्ट्र)

परिच्छेद

गुळवेल ही वनस्पती पुरातन काळापासून जनसामान्यास परिचीत असून आर्युवेदात या वनस्पतीचा उल्लेख व उपयुक्तता विषद करण्यात आली आहे. मनुष्य व प्राण्यांच्या विविध आजारात हिचा वापर करण्यात आला आहे. या वनस्पतीत विविध औषधी गुणधर्म असल्याने प्रस्तूत संशोधन गुळवेल वनस्पतीत असलेल्या जिवाणुप्रति कियाशिलता व कोंबडयातील प्रतिकार क्षमता या संशोधनातून पुढील उद्देशाने अभ्यासली आहे.

१) गुळवेल वनस्पतीच्या खोडाचा पाण्यातील अर्क तयार करून अर्काचे वजनी शेकडा प्रमाण ठरविणे.

२) गुळवेल अर्काची जिवाणूपती कियाशिलता तपासून त्याच्या सिप्रोफॉक्सासीन औषधीसोबतच्या सयुक्त अर्क मिश्रणाचा तुलनात्मक अभ्यास करणे.

३) जिवाणूची वाढ रोकण्याकरीता लागणारे अर्काचे कमितकमी तिव्रतेचे प्रमाण ठरविणे.

४) अर्कातील औषधी गुणधर्म विविध रासायनिक चाचण्यांव्दारे तपासून ठरविणे.

५) गुळवेल वनस्पतीच्या खोडाची भुक्टी कोंबडयांच्या खाद्यात मिसळून त्यांच्या प्रतिकार क्षमतेवर (डी. एन.सी.बी. स्कीन सेंसेटीव्हीटी टेस्ट) व शरीर धारणेवर होणारा परीणाम तपासणे.

गुळवेल वनस्पतीच्या खोडाच्या भुक्टीचे पाण्यातील व इथेनॉल द्रावणातील अर्काचे वजनी शेकडा प्रमाण ४.४३ ग्रॅम व ४.२९ ग्रॅम होते.

गुळवेल वनस्पतीच्या पाण्यातील अर्काचे १०० टक्के, ५० टक्के, १० टक्के तिव्रतेचे द्रावण कागदी टिकल्या (डिस्क) वर ओतून तयार केलेल्या कागदी टिकल्यांचे वनज अनुक्रमे २२.६७, १७.१७, १३.६७ ग्रॅम होते. तसेच इथेनॉल वजनी प्रमाण अनुक्रमे १४.५३, १५.३३ व १७.३० ग्रॅम प्रति डिस्क होते.

वरील प्रमाणे तयार केलेल्या अर्क मिश्रीत टिकल्यांची जिवाणुप्रती कियाशिलता तपासण्यात आली. १०० टक्के व १० टक्के पाण्यातील अर्काच्या टिकल्यांनी जिवाणूची वाढ रोकून निर्माण केलेल्या वर्तुळ परीघाचा व्यास अनुक्रमे इ.कोलाय (९.३३, ७.६७ मिमि) व स्टॅफायलोकोकस ऑरीअस (७.६२, १०.२२ मिमि) होता. तसेच इथेनॉलमधील १० टक्के अर्क इ.कोलाय (९.६७ मिमि), सालमोनेला टायफीम्युरीअम (११.३३ मिमि) आणि स्टॅफायलोकोकस ऑरीअस (११.३३ मिमि) तर १० टक्के अर्क इ.कोलाय (९.०० मिमि) सालमोनेला टायफीम्युरीयम (१०.०० मिमि)ा व स्टॅफायलोकोकस ऑरीअस (७.०० मिमि) प्रती परीणामकारक होता. पाण्यातील व इथेनॉल मधील विविध तिव्रता असलेले दोन्ही अर्क सुडोमोनास ऑरीजिनोसा जिवाणुवर प्रभावी नव्हते. दोन्ही अर्कांनी जिवाणूची वाढ रोखून निर्माण केलेले वर्तुळाचे परीघ हे प्रचलीत सिप्रोफलॉक्सासीन औषधीने निर्माण केलेले परीघापेक्षा सांख्यीकिय दृष्टया कमी प्रभावी होते.

टयुब डायल्युशन पध्दतीने तपासण्यात आलेल्या अर्कात ५० टक्के इथेनॉल अर्क जिवाणूची संख्या कमी करण्यात १० टक्के अर्कपिक्षा अधिक प्रभावी होता.

इथेनॉल अर्काचे जीवणूची वाढ रोकण्याकरीता असलेले कमीत कमी प्रमाण (इ.कोलाय सालमोनेला टायफीम्युरीअम व स्टॅफायलोकोकस ऑरीअस जिवाणूप्रती) २.०, १.० आणि १.५ मिग्रॅ प्रती मिली होते.

सिप्रोफलॉक्सासीन व इथेनॉल ५० टक्के अर्क यांच्या मिश्रणाची जिवाणूप्रती कियाशिलता अभ्यासली असता ५० टक्के अर्काचा सिप्रोफलॉक्सासीन सोबत विरोधात्मक परीणाम दिसून आला.

पाण्यातील व इथेनॉल अर्काच्या रासायनिक परीक्षणात ग्लायकोसाइड्स, शुगर, स्टेरॉल्स, फलवेनॉइडस, सॅपोनिन्स हे घटक असल्याचे दिसून आले.

गुळवेल वनस्पतीच्या खोडाची भूक्टी पक्षांच्या खाद्यात वापरून प्रतिकार क्षमतेवर होणारा परीणाम अभ्यासण्यात आला. नियंत्रीत गट १ व गुळवेल मिश्रीत खाद्य दिलेल्या गटात तुलनात्मक परीणाम दिसून आला. यात पक्षांच्या वजनात, पाणी व खाद्य खाण्याच्या क्षमतेत लक्षणीय वाढ दिसून आली. पक्षांच्या पेशी स्तरावरील प्रतिकार क्षमतेबाबत केलेल्या चाचणीत गुळवेल मिश्रीत खाद्य दिलेल्या गटात (टी १ आणि टी २) त्वचेची जाडी व जखमेचा व्यास यात साख्यीकिय दृष्टया नियंत्रीत गटाच्या तुलनेत वाढ दिसून आली त्यामुळे या. गटामध्ये सुजनप्रक्रिया चांगल्या प्रभावी होत्या. वरील अभ्यासावरून पुढील निष्कर्ष काढण्यात आले.

१) अर्काची पाण्यातील व इथेनॉल द्रावणातील वजनी शेकडा प्रमाण अनुक्रमे ४.८३ व ४.२९ होते.

२) पाण्यातील व इथेनॉल द्रावणातील गुळवेल अर्क जिवाणूप्रति क्रियाशिल होता. परंतू सिप्रोफलॉक्सासीन औषधीपेक्षा अर्काची क्रियाशिलता कमी होती.

३) जिवाणूची वाढ रोकण्याकरीता इथेनॉल अर्काचे कमीतकमी तिव्रतेचे प्रमाण अनुक्रमे २.०, १.० व १.५ मिग्रॅ प्रती मिली होते.

४) गुळवेल वनस्पतीच्या दोन्ही अर्कात ग्लायकोसाइड्स, शुगर, स्टेरॉल, फलवेनॉइडस आणि सॅपोनिन हे क्रियाशिल घटक होते.

५) गुळवेल वनस्पती मांसल कोबडयांच्या खाद्यामध्ये समाविष्ट केल्याने प्रतिकार क्षमतेत वाढ होउन पक्षांच्या वजनात, खाद्य व पाणी पिण्याच्या क्षमतेत लक्षणीय वाढ दिसून आली.