CHAPTER 5

ANTIINFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF THE EXTRACTS AND COMPOUNDS ISOLATED FROM THE ROOTS OF T. ARJUNA AND T. ALATA
General Introduction to Pharmacology and Biological Activity

The life of a cell is an ever-changing process. It is dependent upon compounds of definite molecular structure for growth and reproduction. Cell function can be modified by the introduction of compounds of similar or different structures into the system. The process of life may be altered by trauma, degeneration, or the invasion of viruses or bacteria. Illness is the distortion of the normal cell life or function. Frequently it is possible to restore the process to a norm by introducing a chemical agent of designed structure into the system. Such an agent is a drug and its application is pharmacology.

Pharmacology is the study of the effects of pharmacon or biologically active substances on man or other animals system. It is not restricted to therapeutic agents or drugs, because it is also applicable to all active agents; that is, bacteriocides, fungicides, amoebicides, nematocides, toxins, etc. which affect the living body. Pharmacology may be defined most simply as the study of drugs. It embraces all the scientific knowledge of drugs such as the name, source, selection, physical and chemical properties, and mixing or preparing of drugs in the form of medicine.

The pharmacologist on the basis of experimental work on a variety of species of laboratory animals must predict an effective human dose which hopefully will produce a minimum of side effects. The pharmacologist designs and operates model system for detecting and evaluating the activity of compounds for control of diseases such as those of the central nervous system, the gastrointestinal tract, the cardiovascular bed, the endocrine organs, etc.

The biochemist or physiologist who uses drugs as tools for the advancement of his own discipline or the toxicologist who is concerned with the potential hazards of
drugs. The physician is interested only in drugs that are useful in the prevention, diagnosis and treatment of human disease. The relationship of pharmacology to other disciplines has been indicated by the following diagram:

![Diagram of the relationship of pharmacology to other disciplines]

The word pharmacology is derived from the Greek word *Pharmacon* means *drugs* and *logos* means a *discourse* or *knowledge* and hence includes such allied fields as pharmacy, pharmacodynamics, pharmacognosy, chemotherapy, pharmacodynamic agents, posology, materia medica and toxicology.

The pharmacology activity of a compound is an involved function of the structure and very small changes may profoundly modify the pharmacologic effects. These structural modifications may involve replacing one group with another at a specific point in the molecule, shifting the same group from place to place in the parent molecule, saturating valence bond or modifying the acidity or basicity. This correlation between the pharmacological action and the structure in a series of compounds, is its structure activity relationship. A slight alteration in the structure might totally change a particular effect observed in the parent molecule.
Drugs that are in use today were obtained either as a result of planned studies or mainly by change observation. The exact mode and mechanism of some substances have been worked out, but in many cases the efforts have failed. It is the science of experimental pharmacology which is trying to rationalise the empirical therapy. The detailed pharmacological study of the compounds with a view to investigate the possibility of their being included in the category of drugs and their possible clinical applications, come within the realm of experimental pharmacology with all drugs and new substances. Therefore, a systematic study is concluded to elucidate their pharmacological action, mechanism of action, absorption, fate, excretion and toxic effects etc. Such studies, if carried out in human beings with new substances, are not free from risks and dangers and not always possible even with established drugs. Therefore, recourse a new moiety must be experimented on animals. Experiments are conducted either in vivo in healthy animals or diseased animals or animals after producing conditions stimulating disease in human beings or in vitro with isolated tissues, microorganisms, parasites etc. The results of such experimental studies are, in the end reevaluated in human beings before undertaking the final therapeutic trials.

It is therefore, quite evident that experimental pharmacology forms one of the most important pillars of medical science. Much of the credit goes to research, along with development, production and distribution facilities of the pharmacological industry. Chemists and physical scientists have been predominantly responsible for synthesis, isolation and characterization of medicinal agents. However, biological scientists have played equally essential and organising meaningful screening and testing models. This idea has encouraged me to take up the pharmacological and biological activities of the present work.

This chapter has been divided into three sections:
Section – I: This section includes the antiinflammatory activity of the ethanolic extract and the new compound-D isolated from the roots of *Terminalia arjuna*.

Section – II: This section describes the antibacterial activity of the ethanolic extract and new isolated compounds - E, F, G and H respectively from the roots of *Terminalia alata*.

Section – III: This section includes the antifungal activity of -

(i) The ethanolic extract and the compounds - E, F, G and H isolated from the roots of *Terminalia alata*.

(ii) The ethanolic extract and the compound-D isolated from the roots of *Terminalia arjuna*. 
SECTION – I

GENERAL INTRODUCTION OF ANTIINFLAMMATORY ACTIVITY

The inflammatory phenomenon is extremely complex and not completely understood but there are certain feature of the process that are generally agreed to be characteristic. These include perforation of small blood vessel with associated leakage of blood cell into the intestinal spaces and migration of leucocytes into the inflammed tissue. This is usually accompanied by the familiar sign of oedema, erythema, heat, hyperalgesia and pain. Inflammation is a response of the tissue to an infection, destruction, irritation or foreign substance. It is a part of the host's defense but when the response becomes too great, it may be for worse than the disease state which is counteracted and is extreme case it may be fatal.

In other words, inflammation is the process by which the body fights the energy of any living or non living foreign body and characterized by certain in the body. These conditions may be attributed to vasodilation, leakage of plasma into tissues, increase of blood supply and stretching of the tissues respectively. Rheumatism is a non-specific term embracing a diverse group of diseases and syndromes which have in common, disorder or diseases of connective tissue and hence it is usually presented with pain or stiffness or swelling of muscles and joints. The inflammatory response consists of three successive phase.

(i) Increased vascular permeability with resulting edema and swelling.
(ii) Cellular infiltration and phagocytoses.
(iii) Proliferation of the fibroblasts synthesising new connective tissue to repair the injury.
The presence of sodium in the *salix alba* (bark) give an idea to use synthetic salicylates such as acetyl salicylic acid and methyl salicylates as antiinflammatory agents. The antiinflammatory activity of phenylbutazone exceeds that of salicylates, antipyrine and aminopyzine. The analgesic and antipyretic activity of phenylbutazone is however, much less than that of salicylates\textsuperscript{235}. Antiinflammatory agents act on various systems responsible for inflammation such as plasmin, cloting, arachidonic acid or complement system. The antiinflammatory agent may also reduce the inflammation of fabrinolysis that is by inhibition of platelets or by mixed lymphocyte reaction or by inhibition of complement. Interruption of arachidonia acid cascade is one of the mechanism of antiinflammatory action. This can be achieved by inhibiting membrane phospholipids, blocking of cyclooxygenase pathway on lipoxigenase patheay. The antiinflammatory drugs are found to be regulate leukocyte function by inhibition of macrophages, phagocytes and release of lysosomal hydrolases. These drug also act by inhibiting various enzyme systems like protease, histidin decarboxylase, elastase, etc.

The antiinflammatory activity of aspirin was discovered in 1875. Aspirin and salicylates have been the therapeutic agents most widely used in treatment of inflammation and rheumatic disease. The mechanism by which salicylates produce their antiinflammatory effects are not known but numerous theories have been proposed. Aspirin like non steroidal antiinflammatory agents, have been active in almost every biological system in which they have been tested because inflammation is a complex process that can be induced by a variety of means and can be mediated by a number of only partly known mechanisms with multiple manifestations. It would be surprising if antiinflammatory agents would exert their effect through a spectrum of different biochemical mode of action. The dramatic effect of salicylates is the antiinflammatory effects of rheumatic fever is the time honored and even with the
development of the corticosteroids, these drugs are still of great value in this respect. It has been reported that the steroids are no more effect than the salicylates in preventing the cardiac complications of rheumatic fever\textsuperscript{236,237}.

Antinflammatory agents are divided into two classes:

1. **Steroidal Antiinflammatory Agents**: The steroidal agents are useful in inhibition of the release of phospholipids in lipoxygenase pathway which inhibit the release of arachidonic acid from membrane, example dexamethasone, etc.

2. **Nonsteroidal Antiinflammatory Agents**: These agents are said to inhibit biosynthesis of prostaglandin at cyclooxygenase pathway. They also believed to inhibit oxidative phosphorylation, which derived inflammed tissue to need metabolic energy in the form of adenosine triphosphate for example indomethacin, aspirin, ibuprofen, naproen, etc.

**Screening Methods**

The screening methods for antiinflammatory activity have been classified as follows:

1. Nonimmunological method
2. Immunological method and
3. Miscellaneous method

1. **Nonimmunological Method**

   This have been further divided in three types:

   (a) **For evaluation of acute inflammation**
   (i) Carrageenan induced hind paw oedema method\textsuperscript{238}
   (ii) 5-hydroxy tryptamine induced hind paw oedema method\textsuperscript{239}
   (iii) Formalin induced hind paw oedema method\textsuperscript{240}
   (iv) Hyaluronidase hind paw oedema method\textsuperscript{241}
   (v) Histamin induced hind paw oedema method\textsuperscript{241}
(vi) Terpentine oil induced arthritis in knee joints method\textsuperscript{242}

(b) For evaluation of subacute inflammation

(i) Carrageenan granuloma pouch technique\textsuperscript{243}

(ii) Cotton pellet granuloma technique\textsuperscript{244}

(c) For evaluation of chronic inflammation

(i) Formaldehyde induced arthritis method\textsuperscript{245}

2. Immunological Method

This method is of two types:

(i) Adjuvant arthritis method\textsuperscript{238} and

(ii) Tuberculin sensitivity test method\textsuperscript{237}

3. Miscellaneous Method

It is of two types:

(i) UV erythema method\textsuperscript{237} and

(ii) Urate crystal induced synovitia method\textsuperscript{246}
EVALUATION OF ANTIINFLAMMATORY ACTIVITY

Antiinflammatory activity was carried out with adult albino rats (weighing 150-180 g) of either sex. The ethanolic extract and the new constituent, 2α,19α-dihydroxy-3-oxo-12-oleanen-28-oic acid-28-O-β-D glucopyranoside (compound-D) isolated from the roots of *Terminalia arjuna* were separately administerted orally at a dose of 100 mg/kg body weight i.p. in aqueous suspension and the standard drug (acetyl salicylic acid) was administered at the dose of 100mg/kg body weight in aqueous suspension. ALD$_{50}$ values were determined employing albino rats as test animals. The acute toxicity was determined in rats by oral administration of the extract and compound-D at graded doses and recording the mortality after 24 hours. The extract and compound-D were found to be relatively less toxic as the ALD$_{50}$ values >1000 mg/kg i.p.

The extract and compound-D was examined for antiinflammatory activity by rat paw oedema test as described by Winter, Riseley and Nuss$^{238}$ utilising carrageenan as the phlogistic agent. Antiinflammatory activity was determined by measuring the change in the volume of inflammed foot produced by injection of 0.05 ml of 1% freshly prepared carrageenan suspension. The volume was measured by plethysmograph. Initial volume of right hind paw of albino rats were measured plethysmographically without the administration of extract and compound-D.

Albino rats were divided into three groups, each group consisting of six rats. A group of rats were treated orally with 100 mg/kg body weight of the aqueous suspension of the ethanolic extract and compound-D. Another group was administered orally 100 mg/kg body weight of aqueous suspension of acetyl salicylic acid (standard drug) and the third control group was fed with the same volume of distilled water. One hour after the drug administration the animals were injected with
0.05 ml of freshly prepared 1% carrageenan suspension in normal saline in the right hind paw planter apponeurosis. The measurement of the paw volume were take using Harris and Spencer mercury displacement technique with the help of plethysmometer, immediately before and 1, 2 and 3 hours after the carrageenan injection. The percent inhibition (I) of inflammation after 3 hours was calculated by the formula given by Newbould:

\[
I = 100 \left(1 - \frac{(a-x)}{(b-y)}\right)
\]

Where:

\(x\) = Mean foot volume of rats before the administration of carrageenan injection in the test and the standard drug.

\(a\) = Mean foot volume of rats after the administration of carrageenan injection in the standard group.

\(y\) = Mean foot volume of rats before the administration of carrageenan injection in the control group.

\(b\) = Mean foot volume of rats after the administration of carrageenan injection in the control group.

The results have been shown in Table-27.
TABLE-27

Antiinflammatory Activity of the Extract and Compound-D Isolated from the Roots of T. arjuna

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Dose mg/kg per os</th>
<th>Volume of paw (ml) after carrageenan administration (Mean ± S.E)</th>
<th>Total increase in paw volume after 3 hours</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 hour</td>
<td>1 hour</td>
<td>2 hours</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>-</td>
<td>0.53±0.05</td>
<td>0.64±0.03</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>2.</td>
<td>EtOH Extract of roots of T. arjuna</td>
<td>100</td>
<td>0.55±0.02</td>
<td>0.59±0.03</td>
<td>0.63±0.01</td>
</tr>
<tr>
<td>3.</td>
<td>Compound-D</td>
<td>100</td>
<td>0.51±0.03</td>
<td>0.54±0.02</td>
<td>0.59±0.01</td>
</tr>
<tr>
<td>4.</td>
<td>Std.</td>
<td>100</td>
<td>0.50±0.04</td>
<td>0.54±0.02</td>
<td>0.56±0.05</td>
</tr>
</tbody>
</table>

Std (Standard drug) = Acetyl salicylic acid.

Conclusion

The ethanolic extract and compound-D were found to exhibit 65.21 and 68.48% respectively inflammation in comparison with standard drug acetyl salicylic acid which showed 71.73% inhibition in the same condition on rats.
SECTION – II

GENERAL INTRODUCTION OF ANTIBACTERIAL ACTIVITY

The antimicrobial drug occupy a unique niche in the history of medicine. Many remedies have been used against microbial infections and research still continues, which would lead one to conclude that the ideal antimicrobial agent has not yet been found. The bacterial infection involve mainly urinary, respiratory and digestive system. The modern era of chemotherapy of microbial infection started with the clinical use of sulphonamide in 1936. The golden age of antimicrobial therapy began with the production of penicillin in 1940, discovered by Fleming in 1929.

The sulphonamide drugs were the first effective chemotherapeutic agents to be employed systemically for prevention and cure of bacterial infection in man. An enormous literature exists on the antimicrobial properties of sulphonamides. Sulphonamides have a wide range of antimicrobial activity against both gram-positive and gram-negative organisms. With a few exception, there is a direct correlation between their efficacy in vitro and in vivo. In general, the sulphonamides exert only a bacteriostatic effect in the body, cellular and humoral defense mechanism of the host are essential for the final eradication of the infection. Under some circumstances, however the drugs are actually bactericidal; for example the very high concentration of sulphonamide in the urine can result in the death of certain organism causing urinary tract infection. On a weight basis sulphonamide is much less potent than the clinically employed antibiotics.

The sensitivity of bacteria to various antimicrobial agents are usually determined by microbiological methods. The most accurate technique for use in the routine diagnostic laboratory involves inoculation of the organism being tested in a liquid culture medium in test tubes containing serial dilution of the drug. After a
suitable period of incubation, the lower concentration of antibiotic inhibiting growth of
the bacteria is expressed as the sensitivity. A more rapid method involves the use of
commercially available filter paper discs infiltrated with specific quantities of the drugs.
These are placed on the surface of the plates over which a culture of the organism
being examined has first been streaked.

The large number of antibiotic available today, the physician often faces of
difficult problem in selecting the agent most suitable for a specific situation. In order to
provide maximal benefit for the patient, it is essential for the physician to have a
working knowledge of the common pathogenic organisms. In some cases, the
responsible bacteria, although of different species, may be sensitive to a single
antimicrobial agent, while in others they have distinctly different drug susceptibilities.
This emphasises the need for determination of the drug sensitivity of each of the
components of a mixed flora.

New approved drugs reported between 1983 and 1994, drugs of natural origin
(derived from natural product source) predominate (78%) in the area of
antibacterials. The antibacterial drugs from the plants have been largely investigated
and antibacterial activity of the plant extract have been reported by many
workers. However, recent researches have shown that plant product could be of
better use against these diseases because plant products can easily be metabolized
into animal system along with their curative effect. Many constituents from plants have
been studied thoroughly against a number of microorganisms by Smith and
Katoom. To extend our knowledge in this field, the screening of the ethanolic
extract and new isolated constituents, 2α, 3β, 19α-trihydroxy-olean-12-en-28-oic acid
methyl ester-3β-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (compound-E);
2α, 3β, 19α-trihydroxy-olean-12-en-28-oic acid-3-O-β-D-galactopyranosyl-(1→3)-β-D-
glucopyranoside (compound-F); 8-methyl-5, 7, 2', 4'-tetramethoxy-flavanone
(compound-G) and 2, 4, 4', 6'-tetramethoxy-chalcone-2-O-β-D-glucopyranoside (compound-H) from the roots of *Terminalia alata* for antibacterial activity has been performed. The antibacterial activity of these compounds and extract have described in this section.
EVALUATION OF ANTIBACTERIAL ACTIVITY

Various methods are available for the screening of antibacterial activity\(^{252-254}\). However, the most widely used method consists in determining the antibacterial activity of the drug by adding it in varying concentration to the cultures of the test organism. In the present work the antibacterial activity of the extract and new compounds - E, F, G and H isolated from the roots of *Terminalia alata* was evaluated by filter paper disc method\(^{255}\).

The extract and compounds - E, F, G and H (in ethanol) have been screened *in vitro* against the following bacteria using streptomycin as a standard drug.

(i) *Escherichia coli*

(ii) *Streptococcus aureus*

(iii) *Shigella dysentriae* and

(iv) *Shigella flexneri*

This method consists of the following steps:

(i) Preparation of the medium, its sterilization and tubing.

(ii) Treatment of the glass apparatus and its sterilization.

(iii) Pouring of the needed medium into sterilized petridishes.

(iv) Preparation of the required concentration of the compound and dipping the sterilized Whatman filter paper disc (6 mm) into it.

(v) Incubation of the zone of inhibition.

(vi) Measurement of zone of inhibition.

Out of these steps the most important is the selection of the suitable medium and its preparation because it is the composition of the medium which exerts greatest influence upon the activity of the compound. The other factors which influence *in vitro* test are:

(i) The kind and the condition of the test organisms.
(ii) Concentration of the drug and the dilution of the drug at the site of action (incubation period).

(iii) Environment factor which may augment or counteract of the intraction of the drug and parasite.

(iv) Temperature of the incubation because, for each bacteria there is an optimum temperature and for most of the pathogenic bacteria this temperature is $37^\circ$.

(v) pH of the medium which is usually in the range of 6-7.

In the present work the medium which is employed has the following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat extract</td>
<td>100 ml</td>
</tr>
<tr>
<td>Peptone</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.5 g</td>
</tr>
</tbody>
</table>

For the preparation of the meat extract, 500 g fat free minced meat was placed in 1000 ml of distilled water for 24 hours in cold. The mixture was strained through a muslin cloth. The residue was discarded and the filtrate obtained having red in colour. The surface fat was removed by skimming with filter paper and boil for 15 minutes. The insoluble protein coagulated. The fluid was filtered through a chesse cloth and make up the original volume by the addition of distilled water. This gave the clear extract yellow in colour.

**Nutrient Broth**

As the meat extract is deficient in nitrogenous materials, the heat resistant protein derivatives in the form of peptone was added. Peptone is the principal source of nitrogen. It may also contain some vitamins and some times carbohydrates depending upon the kind of proteinous material digested. Sodium chloride is added to increase the salt content.
All the ingredients, peptone (1 g), sodium chloride (500 mg) and meat extract (100 ml) were mixed and heated till they dissolved. It was then filtered through a filter paper by adjusting the pH at 7.4 by using normal sodium hydroxide solution. The medium so obtained was poured into the sterile flask and plugged with sterile cotton plug which was then sterilize into an autoclave at the 15 lb pressure for 15 minutes.

**Nutrient Agar**

Agar is a complex carbohydrate obtained from certain marine algae and is used as a solidifying agent for media. It is not considered as a source of nutrition for the bacteria. It has the following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar powder</td>
<td>2 g</td>
</tr>
<tr>
<td>Nutrient broth</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

This mixture was boiled in running steam for about half an hour which dissolved completely agar particles. It was then filtered through a glass wool in hot medium by adjusting the pH at 7.4. The medium was then kept in the flask and heated in an autoclave at 15 lb pressure for 15 minutes and then transferred into the sterilized petri dishes.

**Inoculation of Test Plates**

At least four or five well isolated colonies having the same morphological type were selected from an agar plate culture with a wire loop. The top of each colony was touched and transferred the growth to a tube containing 4 to 5 ml of a suitable broth medium. The broth culture was incubated at 37° for 8 hours. The sterile cotton swab on wooden application was dipped into the inoculum and excess of inoculum was removed from the swab by rotating several time firmly inside the wall of the test tube above the final level. The dried surface of a Miller Hinto agar plate was inoculated by streaking the swab over the entire sterile agar surface. The streaking was repeated two or three times so as to ensure an even distribution of the inoculum.
Application of Disc

Within 15 minutes after the plates were inoculated, antimicrobial impregnated disc were applied on the surface of the inoculated plates with sterile forceps. Aseptically each disc was pressed on the medium to ensure complete contact. The spatial arrangement of the discs must be such that they are no closer from the edges of the plates and for enough apart to prevent overlapping of the zone of inhibition. Since some diffusion of test compound is almost instantaneous, a disc was not moved once it came in the contact with the agar surface.

Readings and Interpretation

After 24 hours of incubation, the plates were examined and the diameter of the zone of complete inhibition was measured to the nearest whole milimeter with a sliding calipers. In the present work the activity of the extract and the compounds are measured by +, ++, +++ and ++++ (given in table), depending upon the diameter and clarity of the zones of inhibition.

The results of antibacterial activity are presented in Table-28.
TABLE-28

Antibacterial Activity of the Extract and Isolated Compounds - E, F, G and H

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract and Compounds</th>
<th>E. Coli 25 ppm</th>
<th>E. Coli 50 ppm</th>
<th>S. aureus 25 ppm</th>
<th>S. aureus 50 ppm</th>
<th>S. dysentriae 25 ppm</th>
<th>S. dysentriae 50 ppm</th>
<th>S. fluuxeni 25 ppm</th>
<th>S. fluuxeni 50 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>EtOH extract</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>Compound-E</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Compound-F</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>Compound-G</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>5.</td>
<td>Compound-H</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td>Std.</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Std : Streptomycin; inhibition zone diameter in mm; (+) = 12-15 mm; (++) = 16-20 mm;
(++) = 21-26 mm; (+++) = 27-30 mm.

**Conclusion**

The ethanolic extract and compounds - E, F, G and H were found to be effective against most of the above tested bacteria.
SECTION – III

GENERAL INTRODUCTION OF ANTIFUNGAL ACTIVITY

Previously fungal infection was regarded as a rare disease. Now in recent years fungal infections have been common and known throughout the world. So naturally, tremendous work was expected to be done to find various antifungal agents. Many remedies have been used against fungal infections and research still continues which would lead one to conclude that the ideal topical antifungal agent has not yet been found. However, now practically the whole spectrum of fungus diseases can be successfully treated.

The human disease caused by fungi are though less important than bacterial or protozoal disease yet are not uncommon. Clinically, it is to divide fungal infections into two main groups:

(i) Superficial fungal infections affecting skin, hair and nails.

(ii) Deep or systemic fungal infections affecting internal organs like lungs, liver, intestines, brain, etc.

The superficial fungal infection are mostly due to various species of trichophyton, epidermophyton, microsporon, etc. and on the skin they mainly on the horney layer of the epidermis. External application of the antifungal agents are mostly used in these cases and a high drug concentration can be achieved directly at the local side of disease. The systemic fungal infections are mainly due to moniliasis, histoplasmosis, torulosis, aspergillosis, etc. and they mostly need internal administration of drugs for their control. Although the recently discovered antifungal atibiotics have revolutionised the treatment of all types of mycosis infections yet the drug therapy of this group of the diseases is less satisfactory than the former.
The commonly used antifungal agents are mostly fungistatic and only few are fungicidal. These drugs can be divided into the following classes:

1. **Local Antifungal Agents**: The drugs which are used as local fungal infection are
   
   (i) Fatty acids e.g. undecylenic acid, caprylic acid, propionic acid and their salts.
   
   (ii) Phenols e.g. phenol, resorcinol, etc.
   
   (iii) Hydroxyquinolines e.g. iodochlorohydroxyquinoline, di-iodohydroxyquinoline, etc.
   
   (iv) Heavy metal containing salts e.g. phenyl mercuricnitrate, ammoniated mercury, silver nitrate, etc.
   
   (v) Miscellaneous e.g. benzoic acid, salicylic acid, sulphur, iodine, chlorophenesin, etc.

2. **Systemic Antifungal Agents**: The drugs which are used as systemic fungal infection are
   
   (i) Antibiotics, e.g. nystain, griseofulvin, amphoterecin B. penicillin, etc.
   
   (ii) Aromatic diamidines, e.g. stibamidine, hydroxystibamidine, pentamidine, etc.
EVALUATION OF ANTIFUNGAL ACTIVITY

There are several methods available for recording the antifungal activity. In the present work antifungal activity of the ethanolic extracts and compounds - E, F, G and H isolated from the roots of T. alata and compound-D isolated from the roots of T. arjuna was evaluated by filter paper disc method\textsuperscript{255}. Griseofulvin was used as a standard drug for comparison. The extracts and compounds - D, E, F, G and H were screened for their antifungal activity \textit{in vitro} against the following fungi.

(i) \textit{Candida albicans}
(ii) \textit{Crysosporium pannical}
(iii) \textit{Aspergillus niger}
(iv) \textit{Rhizopus oryzae}

This method consists of the following steps:

\textbf{Sterilization of the Apparatus}

All the glass apparatus were cleaned with chromic acid followed by washing with distilled water and sterilized by heating at 200\textdegree in a hot air oven.

\textbf{Preparation of the Medium}

Sabouradus glucose agar medium was used for antifungal screening which consist of

\begin{align*}
\text{Glucose} & : 40 \text{ g} \\
\text{Peptone} & : 10 \text{ g} \\
\text{Agar} & : 20 \text{ g} \\
\text{Streptomycin} & : 0.2 \text{ g}
\end{align*}

Streptomycin was used to check the growth of undesirable bacteria. The above mentioned ingredients were weighted and dissolved in a 500 ml of distilled water. After the ingredients were dissolved completely, more distilled water was
added to make the solution upto one litre and pH of the medium was kept at 7.6±0.01. The medium was heated in a autoclave at 15 lb pressure for half an hour and then transferred into sterilized petri dishes. The broth has prepared in 100 ml by using glucose and peptone.

The spore suspension of each test organism (72 hours culture) was kept in a broth at 35-40°. The petri dishes were incubated in the same manner as described for antibacterial activity. These petri dishes were incubated, at 30° for 48 hours. The zone of inhibition was considered as an indication for the antifungal activity. In the present work the activity of the extracts and the compounds are measured by +, ++, +++ and ++++ (given in table), depending upon the diameter and clarity of the zones of inhibition.

The results of antifungal activity are presented in Table-29.
### TABLE-29

Antifungal Activity of the Extracts and Isolated Compounds - D, E, F, G and H

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract and Compounds</th>
<th>C. albicans</th>
<th>C. pannical</th>
<th>A. niger</th>
<th>R. oryzae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 ppm</td>
<td>50 ppm</td>
<td>25 ppm</td>
<td>50 ppm</td>
</tr>
<tr>
<td>1.</td>
<td>EtOH extract (T. alata)</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>EtOH extract (T. arjuna)</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Compound-D</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>Compound-E</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>5.</td>
<td>Compound-F</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>6.</td>
<td>Compound-G</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>7.</td>
<td>Compound-H</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>8.</td>
<td>Gf.</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Gf: Griseofulvin; inhibition zone diameter in mm: (+) = 10-13 mm; (++) = 14-19 mm; (+++) = 20-25 mm; (++++) = 26-32 mm.

**Conclusion**

The ethanolic extracts and compounds - D, E, F, G and H exhibited antifungal activity against the above selected fungi.