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# EVALUATION OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF *BARLERIA PRIONITIS* LINN AND *PHYLLANTHUS ACIDUS*

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## ABSTRACT

In this study, we assessed comparative antibacterial and antifungal effects of extracts from Barleriaprionitislinn and Phyllanthusacidus. Antimicrobial activity was done by agar well diffusion method. The study indicated that ethanolic extract of Barleriaprionitis was more effective against fungal and bacterial species showing highest inhibition such as 18mm against fungi A.niger and 15mm against C.albicans and 22mm against bacteria Streptomyces fulvissimus and 21mm against E.coli and Bacillus subtilis. The ethanolic extract of Phyllanthusacidus showed more activity against bacteria such as 15mm against B.subtilis and 13mm against Streptomyces fulvissimus and lowest activity fungus such as 12mm against C.albicans and 10mm against A.niger. The overall evaluation studies showed that the ethanolic extract of Barleriaprionitis showed highest degree of antibacterial and antifungal activity than ethanolic extract of Phyllanthusacidus.

**KEYWORDS:** Antimicrobial activity, Agar well diffusion method, Barleria prionitis, Phyllanthus acidus, Ethanolic extracts.

## 1. INTRODUCTION

In India medicinal plants and traditional medicine have been the basis of traditional healthcare especially in remote area where modern health facilities are inadequate. India being a largest producer of medicinal plants is rightly called the botanical garden of the world. Ayurveda, the system of medicine indigenous of India has thousands years of history with all its pride and glory. Ayurvedic pharmacopoeia comprises of both vegetable and

mineral products. (Mukherjee, 2002) India is one of the world's leading biodiversity center with the presence of over 45000 different plant species. India has 15000-18000 species of flowering plants, 2500 algae, 23000 Fungi, 1600 types of lichens and 1800 varieties of bryophytes and estimated 30 million types of microorganisms. These about 15000-20000 plants have good medicinal value. However, only traditional communities use about 7000-7500 for their medicinal values. Plant chemistry has been the basis for many synthetic drugs in modern pharmacopoeia (Chaudhri, 1996).

### 1.1 Importance of herbal medicine in health care system

Herbal medicines are prepared from a variety of plant material such as leaves, stems, roots, bark, etc. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. Herbal remedies can also be purchased in the form of pills, capsules or powders, or in more concentrated liquid forms called extracts and tinctures. They can apply topically in creams or ointments, soaked into cloths and used as compresses, or applied directly to the skin as poultice (Kokate CK., 1994).

### 1.2 Antimicrobial activity of plants

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural. In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years. Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re- emerging infectious diseases.

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated.

The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains. The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs. There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli are some of the important human pathogens that have developed resistance to antimicrobials.

**1.3 Role of Antibiotics in bacterial treatment**

Antibiotics are the mainstay of bacterial treatment. The goal of these drugs is to kill theinvading bacteria without harming the host. Antibiotic effectiveness depends on mechanism ofaction, drug distribution, site of infection, immune status of the host, and resistance factors ofbacteria.

Antibiotics work through several mechanisms. Some (such as vancomycin and penicillin) inhibit formation of bacterial cell walls. Erythromycin, tetracycline, and chloramphenicol interrupt protein synthesis. Still others inhibit bacterial metabolism (sulfa drugs) or interfere with DNA synthesis (ciprofloxacin, rifampin) and/or cell membrane permeability (polymyxin b).

When antibiotics were discovered in the 1940s, they were incredibly effective in bacterial infection treatment. Over time, many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance. Bacteria may be naturally resistant to different classes of antibiotics or may acquire resistance from other bacteria through exchange of resistant genes. Indiscriminate, inappropriate, and prolonged use of antibiotics

have selected out the most antibiotic-resistant bacteria. Antibiotic-resistant strains have emerged in hospitals, long-term care facilities, and communities worldwide.

### 1.4 Difference of Herbal and Conventional Drugs

Compared with well-defined synthetic drugs, herbal medicines exhibit some marked differences, namely:

1. The active principles are frequently unknown standardization, stability and quality control are feasible but not easy;
2. The availability and quality of raw materials are frequently problematic.
3. Well-controlled double-blind clinical and toxicological studies to prove their efficacy and safety are rare.
4. Empirical use in folk medicine is a very important characteristic.
5. They have a wide range of therapeutic use and are suitable for chronic treatments.
6. The occurrence of undesirable side effects seems to be less frequent with herbal medicines, but well-controlled randomized clinical trials have revealed that they also exist.
7. They usually cost less than synthetic drugs (Gesler WM., 1992).

### 1.5 Problems with modern Drugs

1. High cost and long time taken in development of a new drug.
2. Toxicity
3. Non-renewable source of basic raw materials.
4. Environmental pollution by the chemical industry.
5. Inadequate, especially in management of certain chronic diseases. (Trease and Evans, 1989).

## 2. LITERATURE REVIEW

### 2.1 LITERATURE REVIEW OF Barleria prionitis

1. Piush Sharma, et al., (2013) investigated and reported the phytochemical and ethanomedical values of Barleriaprionitis L. (Acanthaceae) which have been established against various disease models using modern scientific methodologies and tools.
2. Nidhi et al., (2013) evaluated the antibacterial activity of rhizome of Barleria prionitis in methanol extract.
3. Manider Karan et al., (2013) assessed topical anti-inflammatory activity of Barleria prionitis and Barleria Cristata against croton oil induced ear oedema in female rats.

4. Kuldeep Singh et al., (2013) evaluated the anti-inflammatory effect of methanol extract of Barleriaprionitis on carragennan induced rat paw oedema.
5. Manjusha et al., (2013) evaluated the gastro protective activity of methanolic extract of Barleria prionitis on ethanol and indomethacin induced ulcer models in rats.
6. Swathi Paul. et al., (2012) reported the potent antibacterial activity of different leaf extracts of Barleriaproinitis L.
7. ReemaDheer et al., (2011) studied the antibiotic activity of alcoholic extract of leaf and root of Barleria prionitis in normal and alloxan induced diabetic rats.
8. Chavan Chetan et al., (2011) investigated the antioxidant activity of ethanolic and aqueous extract of whole plant of Barleria prionitis.
9. Shabir A. Lone et al., (2011) reported the effect of different plant growth regulators on in-vitro propagation of barleriaprionitislinn.

**2.2 LITERATURE REVIEW OF Phyllanthus acidus**

1. M. Suriyavathana et al., (2011) reported the proximate analysis on biochemical study of Phyllanthus acidus, Phyllanthus emblica and Citrus limon.
2. R.C. Jagessar et al., (2008) reported the Selective Antimicrobial properties of Phyllanthus acidus leaf extract against Candida albicans, Escherichia coli and Staphylococcus aureus using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method.
3. A.Jagajothi et al., (2013) evaluated the antimicrobial Activity and phytochemical analysis of Phyllanthus acidus.
4. Raja Chakraborty et al., (2012) investigated the anti-inflammatory, antinociceptive and antioxidant activities of Phyllanthus acidus L. Extracts.
5. Ramachandra Y L et al., (2014) assessed the antioxidant potential of Phyllanthus acidus bark extracts.
6. Md. Razibul Habib et al., (2011) reported the evaluation of antioxidant, cytotoxic, antibacterial potential and photochemical screening of chloroform extract of Phyllanthus acidus.
7. Nilesh Kumar Jain et al., (2011) investigated the Protective effects of Phyllanthus acidus (L.) Skeels leaf extracts on Acetaminophen and thioacetamide induced hepatic injuries in Wistar rats.
8. Md. Mominur Rahmana et al., (2011) reportd the antibacterial, cytotoxic and antioxidant potential of Methanolic extract of Phyllanthus acidus.

9. Jorge A. Pino et al., (2008) evaluated the volatile compounds of grosella (Phyllanthus acidus [L.] Skeels) fruit.

## 3. AIM AND PLAN OF WORK

### 3.1 AIM OF WORK

The present study was conducted to compare the antimicrobial activity from two medicinal plants Barleriaprionitis and Phyllanthusacidus. The therapeutic utility and efficacy of these plants in various disease like diabetes, diuretic, stomach pain, gastric ulcer etc. is well documented among ethnic populations. Here we are using different stains to find out the antimicrobial activity such as S.fulvissimus, K.Pneumonia, S.flexneri, E.coli, B.subtitis, S.pyrogenes, P.auruginosa, P.mirabis, A.niger, C.albicans.

### 3.2 PLAN OF WORK

1. Selection of Plants.
2. Collection and authentication of plants.
3. Extraction of leaves of Barleria prionitis and Phyllanthus acidus.
4. Preliminary phytochemical studies.
5. Evaluation of Antimicrobial activity by agar well diffusion method.

## 4. PLANT PROFILE

### 4.1 PLANT PROFILE OF BARLERIA PRIONITIS

**General Information of *Berlaria prionitis linn***

A genus of herbs belongs to the family acanthaceae well distributed in India, Ceylon, Southasia etc.

**LATIN BINOMIAL & VERNACULAR NAMES**

Source :***Berlariaprionitislinn*.**

Family :Acanthaceae

**VERNACULAR NAMES**

English Name - Percupine flower

Tamil Name - Chemmulli, Korantam, Sullimalar

Sanskrit - Artagalah, Bana, Jhinti

Malayalam - Karimkurunni

Kannada - Mullugoranta

Hindi Name - Jhinti, Vajradanti, Parush

Marathi - Kolita, Pivalikorantiu

Gujarati - Pilokantasheriyo

**SCIENTIFIC CLASSIFICATION**

Kingdom - Plantae

Subkingdom - Tracheobionta

Division - Mangoliophyta

Subdivision - Spermatophytina

Class - Mangoliopsida

Subclass –Asteridae

Order - Lamiales

Super order - Asteranae

Family - Acanthaceae

Genus - Berlaria

Species - Prionitis

**Chemical constituents**

*Barleriaprionitis* consist of acabarlerin, barlerin, β- sitosterol, flavanol, glycoside, iridoids and scutellarein-7-neohesperidoside.

Aerial parts of *Barleria prionitis* consist of phenyl ethanol glycoside, barlerinoside along with six iridoid glycosides, shanzhiside methyl ester, 6-o-trans-p-coumaroyl-8-o-acetyl shanzhiside methyl ester, barlerin, acetyl barlerin, 7-methoxy diderroside, luplinoside. Also prinoside A, prinoside B, prinoside C.

**Traditional uses**

Traditionally the plant is used for asthma, whooping cough and the leaves and roots are used against toothache, rheumatism, cataract, leucoderma, scabies, liver ailments, piles treatment, diuretic, ulcers and also in cut, wounds, malaria, fever and irritation control.

**Pharmacological activity**

Anti-fertility activity, anti-oxidant activity, anti-inflammatory activity, hepatoprotective activity, anti-microbial activity, anthelmintic, anti dental decay activity, anti-diabetic activity, anti-diarrhoeal activity, diuretic activity, besides these it has got AChE inhibitory activity, anxiolytics. (Banerjee D., 2012).

## 4.2 PLANT PROFILE OF PHYLLANTHUS ACIDUS

**General Information of *Phyllanthus acidus***

A genus of herbs belongs to the family phyllanthaceae well distributed in throughoutAsia and also in the Caribbean region, Central and South America etc.

**LATIN BINOMIAL & VERNACULAR NAMES**

Source : ***Phyllanthusacidus*.**

Family : Euphorbiaceae (or) Phyllanthaceae

**VERNACULAR NAMES**

English Name - Goos-berry

Tamil Name - Araineelikai

Sanskrit - Lavali, Sugandhamula

Malayalam - Arinelli, Nellikkapuli

Kannada - Kirnelli, Nallikai

Hindi Name - Chillimilli

Marathi - Rayawala

Gujarati - KhaatiAawala, Raayaamali

**SCIENTIFIC CLASSIFICATION**

Kingdom - Plantae

Subkingdom - Tracheobionta

Division - Mangoliophyta

Class - Mangoliopsida

Subclass –Rosidae

Order - Lamiales

Super order - Malpighiales

Family - Euphorbiaceae or Phyllanthaceae

Genus - Phyllanthus

Species - Phyllanthusacidus

**Parts used**

Leaves and seeds

**Phytoconstituents**

The important chemical constituents present in the Phyllanthus acidus such as lignin, terpenes, sterols, polyphenolic compounds, tannins, flavanoids, glycosides and alkoloids. The root bark contains saponins, gallic acid, tannins and a crystalline substance, lupeol, 4-hydroxybenzoic acid, caffeic acid, adenosine, kaempferol and hypogallic acid.

**Medicinal use**

In India, the fruit are taken as liver tonic, to enrich the blood. The syrup is prescribed as stomachic and the seeds are cathartic. The leaves, with added pepper, are poulticed on sciatica, lumbago, rheumatism. The acrid latex of various parts of the tree is emetic and purgative. (Siow-Ping Tan., 2020)

## 5. METERIALS AND METHODS

### 5.1 COLLECTION AND AUTHENTICATION OF PLANT

The leaves of Barleriaprionitis L and Phyllanthus acidus L were collected from the surrounding areas of Erode district, Tamilnadu, India. The samples were identified and

authenticated (Reg.No: BSI/SRC/5/23/2022/Tech/583 and 587) at Botanical Survey of India, Southern circle, Coimbatore.

**Extraction procedure**

The leaves were dried under shade and then made in to a coarse powder with a mechanical grinder. Powdered dried leaves were passed through sieve No. 40 and stored in an airtight container for further use. Both petroleum ether and ethanol extraction were carried out by using Soxhlet extractor.

**1) Preparation of petroleum ether extract**

The dried powder material of leaves (100gm) were first extracted with petroleum ether (60-80$^{o}$) in a Soxhlet apparatus and after complete extraction (48 hrs), the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator.

**2) Preparation of ethanolic extract**

After the extraction with petroleum ether the same plant material were dried and again extracted with ethanol (99.9% v/v) in Soxhlet apparatus and after complete extraction (48 hr) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator. All the extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

**5.2 PHYTOCHEMICAL SCREENING OF Barleria prionitis AND Phyllanthus acidus:**

The plant extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to establishing and proving the identity of a substance. The active ingredients, after isolation, can be incorporated into the modern medicine for the development of newer formulation for therapeutic ailments.

**Qualitative phytochemical analysis**

The petroleum ether and ethanolic extracts of Barleria prionitis and Phyllanthus acidus were subjected to qualitative tests for the detection of various plant constituents (Kokate CK.,), (Krishnaswamy NR.,), (Surekhayarnalkar.,), (Khandelwal KR.,), (Rajgopal G.,).

**1. DETECTION OF CARBOHYDRATE**

Small quantity of extract was dissolved in 4ml of distilled water and filtered. The filtrate was collected and subjected for the following tests.

**a. Molisch's test:** 1 ml of filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown to violet ring, indicate the presence of carbohydrate.

**b. Fehling's test:** To the Fehling solution A and B extract was added and boiled. The formation of brick red precipitate indicates the presence of reducing sugar.

**c. Benedict's test:** 1 ml of extract was added to 5ml of Benedict's reagent, was added and boiled for 2 mins and cool. Formation of a red precipitate shows the presence of sugars.

**d. Tollen's test:** 1ml of extract was added with 2ml of Tollen's reagent was added and boiled. A silver mirror is obtained inside the wall of the tube which indicates the presence of aldose sugar.

**2. TEST FOR GLYCOSIDES**

**a. Legal's test**

The filtrate was hydrolyzed with dilute hydrochloric acid and heated on water bath. Then added 1ml of pyridine and few drops of sodium nitroprusside solution, made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

**b. Borntrager's test:** Filtrate was hydrolyzed with dilute hydrochloric acid on water bath, then treated with chloroform and shake well. After that separates the chloroform layer and added equal volume of dilute ammonia solution. If ammonia layer acquire pink or violet colour, indicates the presence of glycosides.

**c. Baljet test:** 1ml of extract was added to 1ml of sodium picrate solution and the yellow to orange colour shows the presence of glycosides.

**d. Keller killiani test:** The ethanol extract 0.5ml of strong solution of lead acetate was added and filtered. The filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cool residue in 3ml of glacial acetic acid containing 2 drops of ferric chloride solution carefully transferred the solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer form at the junction of the 2 liquid and the upper layer slowly becomes bluish green, darkening the withstanding.

### 3. DETECTION OF ALKALOIDS

Small quantity of extract was treated with few drops of dilute hydrochloric acid and filtered it. The filtrate was collected and subjected for tests with following reagents.

**a. Mayer's reagent:** To the filtrate potassium mercuric iodide was added. The formation of cream colour precipitate, it shows the presence of alkaloids.

**b. Dragendroff's reagent:** To the filtrate potassium bismuth iodide was added. If it shows reddish brown precipitate, indicates the presence of alkaloids.

**c. Wagner's reagent:** To the filtrate iodine in potassium iodide solution was added. If it shows reddish brown precipitate, indicates the presence of alkaloids.

**d. Hager's reagent:** To the filtrate saturated aqueous solution of picric acid was added. Formation of yellow precipitate indicates the presence of alkaloids.

**e. Tannic acid test:** 1ml of extract was added to 1ml of 10% tannic acid solution, buff coloured indicates the presence of alkaloids.

### 4. DETECTION OF PHYTOSTEROL AND STEROIDS

Small quantity of extract was dissolved in 5ml of chloroform and then subjected to the following tests.

**a. Salkowski test:** To the above solution 1 ml chloroform and few drops of concentrated sulphuric acid was added. The test tube was shaken for few minutes. The development of red colour in chloroform layer indicates the presence of steroids.

**b. Liebermann- Burchard reaction:** To the above solution 1 ml of chloroform and few drops of concentrated sulphuric acid and 1-2 ml of acetic anhydride were added. Development of red colour first, then blue and finally green colour, indicates the presence of steroids.

**c. Liebermann's reaction:** To 3 ml of extract in a test tube, 1 ml of acetic anhydride was added and gently heated. The contents of test tube were cooled. Few drops of concentrated sulphuric acid was added from the side of test tube. Appearance of blue shows the presence of steroids.

### 5. DETECTION OF PROTEINS AND AMINOACIDS

Small quantity of the extract was dissolved in few ml of water and filtered. The collected filtrate was used for following tests.

**a. Millon's test:** To the filtrate Millon's reagent was added. If white precipitate slowly turns to red colour, indicates the presence of proteins.

**b. Biuret test:** Filtrate was treated with 5% sodium hydroxide and few drops of 1% copper sulphate solution. Formation of violet or pink colour indicates the presence of proteins.

**c. Ninhydrin test:** To the filtrate Ninhydrin reagent was added. Development of violet or purple colour indicates the presence of amino acids.

**d. Xanthoproteic test:** 1ml of extract was treated with 1ml of concentrated nitric acid. A white precipitate formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange colour indicates the presence of aminoacid.

## 6. DETECTION OF TANNINS

The test extract was dissolved in water, warmed and filtered. The filtrate was used for the following tests.

**a. Ferric chloride test:** 5 ml of filtrate was allowed to react with 1 ml of 5% ferric chloride solution. If dark green or deep blue colour is obtained, tannins are present.

**b. Lead acetate test:** 5 ml of filtrate was treated with 1 ml of 10% lead acetate solution. Yellow colour precipitation, indicates the presence of tannins.

**c. Potassium dichromate test:** 5 ml of filtrate was treated with 1 ml 10% aqueous potassium dichromate solution. If yellowish-brown precipitate formed it suggest the presence of tannins.

**d. Gelatin solution test:** 1% w/v solution of gelatin in water, containing 10% sodium chloride was prepared. A little of this solution was added to the filtrate. If white precipitate is obtained, tannins are present.

**e. Vanillin hydrochloride test:** 1ml of extract was added with vanillin hydrochloride. Formation of purplish red colour indicates the presence of tannins.

## 7. DETECTION OF FLAVONOIDS

**a. Shinoda test:** The small quantity of extract was dissolved in alcohol, to that piece of magnesium followed by concentrated hydrochloric acid were added by drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

**b. Pew's test:** A pinch of zinc powder and about 5 drops of 5N hydrochloric acid were added to the test solution. The formation of deep purple red or cherry red colour indicates the presence of flavonoids.

c. Extract was treated with aqueous sodium hydroxide solution. Development of yellow colour indicates the presence of flavonoids.

d. Extract was treated with sulphuric acid. Development of yellow to crimson shows the presence of flavonoids.

**8. DETECTION OF SAPONINS**

**a. Foam test:** The extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

**b. Heamolytic test:** To the extract or dry powder added one drop of blood placed on glass slide. If heamolytic zone appears shows the presence of saponins.

**c. Lead acetate test:** 1ml of sample solution was treated with 1% of lead acetate solution, formation of a white precipitate indicates the presence of saponins.

**9. DETECTION OF TERPENOIDS**

**a. Knoller's Test:** In a test tube, 2 or 3 granules of tin was added and dissolved in 2 ml of thionyl chloride solution. Then, test solution was added. A deep purple colour that changes to red indicates the presence of terpenoids.

**b. Salkowsky's Test:** 5ml of extract was mixed in 2ml of chloroform and concentrated sulphuric acid was carefully added to form a layer. Formation of reddish brown coloration of the interface indicates the presence of terpenoids.

**10. TEST FOR FIXED OIL AND FATS:**

**a. Spot test:** Pressed a small quantity of extract between the two filter papers, the strain on the filter paper indicates the presence of fixed oils.

**b. Saponification test:** Added a few drops of 0.5N of alcoholic potassium hydroxide to small quantity of various extract along with a drop of phenolphthalein separately and heat on a water bath for 1 to 2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

**11. TEST FOR GUMS AND MUCILAGE**

10ml of ethanol extract was slowly added 25 ml of absolute alcohol with constant stirring filter the precipitate and dried in air. The precipitate for its swelling property indicates the presence of carbohydrates.

**5.3 ANTI MICROBIAL ACTIVITY**

**Test Microorganisms**

Gram-positive organisms (Staphylococcus aureus, streptomycesfulvissimus, Bacillus subtilis*)*, Gram-Negative organisms (Klebsiellapneumoniae, Pseudomonas aeruginosa, Escherichia coli, Shigellaflexneri, proteus mirabilis) and a fungal yeast Candida albicanswere chosen based on their clinical and pharmacological importance (McCracken WA, 1983).

They were maintained on Mueller-Hinton Agar medium. Twenty-four hour old pure cultures were prepared for use each time.

**Experimental Procedure**

**Antibacterial study (plate hole diffusion method)**

Antibacterial study (plate hole diffusion method or agar well diffusion) assay was used to determine the growth inhibition of bacteria by plant extracts. Bacteria was maintained at $4^0$C to nutrient agar plate before use. Nutrient agar medium was prepared and each universal containing 20ml was poured. Broth were inoculated with different bacteria species and incubated at $37^0$C for 24 hours. A total 25ml of Molten Hinton (MH) agar was poured into sterile universals. Each universals was inoculated with 0.2ml of different bacterial species mixed well with MH into sterile petridishes and allow to set. A well was prepared in the plants with the help of cork-borer (6mm) four holes per plates were made into the set agar containing the bacteria culture. A total of 0.2ml of plant extract were poured into the wells with concentrations as 1000µg/ml, 500µg/ml, 200µg/ml, 125 µg/ml and 62.5µg/ml. The plates were incubated overnight at $37^0$C. The result were obtained by measuring the zone diameter. The result was compared with standard antibiotic Oxytetracycline. (Jagessar R.C., *et.al.*, 2008 & Bapat U.C., *et.al.*, 2014).

**Antifungal study (plate hole diffusion method)**

Antifungal study (plate hole diffusion method or agar well diffusion) assay was used to determine the growth inhibition of fungal by plant extracts. Fungi were maintained at $4^0$C on Sabourd's dextrose agar plate before use. Sabourd's dextrose agar medium was prepared and each universal containing 20ml was poured. The universals with broth were inoculated with different fungal species and incubated at 28-$30^0$C for 24 hours. A total 25ml of Sabourd's dextrose agar was poured into sterile universals. Each universals was inoculated with 0.2ml of different fungal species mixed well with the Sabourd's dextrose agar into sterile petridishes and allow to set. A well was prepared in the plants with the help of cork-borer (6mm) four holes per plates were made into the set agar containing the fungal culture. A total of 0.2ml of plant extract were poured into the wells with concentrations as 1000µg/ml, 500µg/ml, 200µg/ml, 125 µg/ml and 62.5µg/ml. The plates were incubated overnight at 28-$30^0$C. The result were obtained by measured the zone diameter. The result was compared with standard antifungal Ketoconazole. (Jagessar R.C., *et.al.*, 2008 & Bapat U.C., *et.al.*, 2014).

## 6. RESULTS AND DISCUSSION

The leaves of *Barleriaprionitis* and *Phyllanthusacidus* were selected for the detailed study to evaluateits phytochemical and biological properties.

Literaturesurveyrevealedsofornoworkhasbeendoneonthisfernclaimingmaximumthera peuticuses. Sowefeltworthwhiletovalidate scientifically, the folkclaim for its therapeutic activity. We have alsotakenitsdetailed preliminary phytochemical investigations to prove its appropriate identification andrationalizeits use as drug oftherapeuticimportance.

### 6.1 QUALITATIVEPHYTOCHEMICALANALYSIS

In the phytochemical studies, petrolium ether, ethanolic extracts of leaves of *Barleriaprionitis and Phyllanthusacidus*showedthe presencevarious phytoconstituents.

Preliminaryphytochemicalscreening of *Barleriaprionitis*revealedthepresenceofbioactive compounds such as glycosides, alkaloids, tannins, flavonoids, phenols and saponins inethanolic extract and fixed oil, gums and mucilage in petroleum ether extract. On the other handthe petroleum ether extract of *Phyllanthusacidus*contained fixed oil, fats, gums and mucilage. While the ethanolic extractshowed the presenceofglycosides, alkaloids, flavonoids, steroidsand phenols. Different phytochemicals have been found to possess a wide range of activities, which may help inprotection against chronicdiseases. Theresults aregivenin Table-1.

**Table 1: Data showing the preliminary phytochemical screening of the leavesextracts of *Barleria prionitis and Phyllanthus acidus.***

| Phytoconstituents | Phyllanthusacidus | | Barleriaprionitis | |
|---|---|---|---|---|
| | Petroleum Ether | Ethanol (99.9%v/v) | Petroleum Ether | Ethanol (99.9%v/v) |
| Carbohydrate | Absent | Absent | Absent | Present |
| Glycosides | Absent | Present | Absent | Present |
| Alkaloids | Absent | Present | Present | Present |
| Steroids | Absent | Present | Present | Absent |
| Tannins | Absent | Absent | Absent | Present |
| Flavonoids | Absent | Present | Absent | Present |
| Phenols | Absent | Present | Absent | Present |
| Saponins | Absent | Absent | Absent | Present |
| Terpenoids | Absent | Absent | Absent | Present |
| Fixedoilandfats | Present | Absent | Present | Absent |
| Gumsand mucilage | Present | Absent | Present | Absent |

### 6.2 ANTIMICROBIALACTIVITY

Antimicrobialpropertiesofmedicinalplantsarebeingincreasinglyreportedfromdifferent parts of the world. The World Health Organization estimates that plant extract or theiractive constituents are used as folk medicine in traditional therapies of 80% of the world'spopulation. In the present work, the extracts obtained from *Barleriaprionitis and Phyllanthusacidus*show strong activity against most of the tested bacterial and fungal strains. The result swere compared with standard antibioticdrugs.

**Table 2: Antibacterial study of ethanolic extract of *Barleria prionitis.***

| Microorganisms | 1000µg/ml (mm) | 500µg/ml (mm) | 250µg/ml (mm) | 125µg/ml (mm) | 62.5µg/ml (mm) | Oxytetracycline (1mg/ml) (mm) |
|---|---|---|---|---|---|---|
| Escherichiacoli (MTCC 1687) | 21 | 14 | 10 | 06 | 0 | 24 |
| Klebsiellapneumonia (MTCC 7162) | 16 | 10 | 06 | 03 | 0 | 22 |
| Proteus mirabilis (MTCC9242) | 19 | 14 | 11 | 06 | 02 | 20 |
| Shigellaflexneri (MTCC 1457) | 18 | 15 | 12 | 07 | 0 | 25 |
| Stephylococcusaureus (MTCC 96) | 18 | 12 | 08 | 02 | 0 | 22 |
| Streptomyces Fulvissimus (MTCC 7336) | 22 | 16 | 12 | 07 | 0 | 26 |
| Bacillus subtilis (MTCC736) | 21 | 14 | 11 | 07 | 0 | 21 |
| Pseudomonas Aeruginosa (MTCC 2488) | 12 | 04 | 0 | 0 | 0 | 24 |

**Table 3: Antifungalstudyof ethanolic extract of *Barleri aprionitis.***

| Microorganisms | 1000µg/ml (mm) | 500µg/ml (mm) | 250µg/ml (mm) | 125µg/ml (mm) | 62.5µg/ml (mm) | Ketaconazole (1mg/ml) (mm) |
|---|---|---|---|---|---|---|
| Candida albicans (MTCC 282) | 15 | 08 | 05 | 02 | 0 | 18 |
| Aspergillusniger (MTCC 183) | 18 | 11 | 07 | 05 | 02 | 20 |

**Table 4:Antibacterial study of ethanolic extract of *Phyllanthus acidus.***

| Microorganisms | 1000µg/ml (mm) | 500µg/ml (mm) | 250µg/ml (mm) | 125µg/ml (mm) | 62.5µg/ml (mm) | Oxytetracycline (1mg/ml) (mm) |
|---|---|---|---|---|---|---|
| Escherichiacoli (MTCC 1687) | 11 | 06 | 05 | 0 | 0 | 24 |
| Klebsiellapneumonia (MTCC 7162) | 07 | 04 | 03 | 0 | 0 | 22 |
| Proteus mirabilis (MTCC9242) | 08 | 06 | 04 | 0 | 0 | 20 |
| Shigellaflexneri (MTCC 1457) | 12 | 08 | 06 | 03 | 0 | 21 |
| Stephylococcusaureus (MTCC 96) | 08 | 05 | 04 | 0 | 0 | 20 |
| Streptomyces Fulvissimus (MTCC 7336) | 13 | 10 | 06 | 03 | 0 | 26 |
| Bacillus subtilis (MTCC736) | 15 | 12 | 08 | 05 | 0 | 23 |
| Pseudomonas Aeruginosa (MTCC 2488) | 10 | 05 | 03 | 0 | 0 | 21 |

**Table 5: Anti fungal study of ethanolic extract of *Phyllanthusacidus.***

| Microorganisms | 1000µg/ml (mm) | 500µg/ml (mm) | 250µg/ml (mm) | 125µg/ml (mm) | 62.5µg/ml (mm) | Ketaconazole (1mg/ml) (mm) |
|---|---|---|---|---|---|---|
| Candidaalbicans (MTCC282) | 15 | 08 | 05 | 02 | 0 | 18 |
| Aspergillusniger (MTCC183) | 18 | 11 | 07 | 05 | 02 | 20 |

In the present study, the antimicrobial activity of leaves of *Barleriaprionitis*and *Phyllanthusacidus*were performed by using agar diffusion method. The antimicrobial activity of the extractswere studied in different concentrations (62.5, 125, 250, 500, and 1000 µg/ml) against Eightpathogenicbacterialstrains, three Gram-positive (Staphylococcusaureus, Streptomycesfilvissimus, Basillussubtilis), and five Gram-negative (Escherichiacoli, Pseudomonasaeruginosa, Klebsiella pneumonia, proteus mirabilis, shigellaflexneri) and two fungal strains(Aspergillusniger & Candida albicans ). These strains have been selected for the basis of itsapplicationpurposeoffurther formulation study.

The antibacterial and antifungal properties of two different plant extracts were identified. Theresults indicated that, the extracts obtained from the plants showed inhibition of growth againsttested microorganisms. Successful prediction of extracted compounds from plant material largelydependent on the type of solvents used in the extraction procedure. The

traditional practitionersmake use of water as a primer solvents, but on the first observation ethanol was better solvent forextracting antimicrobial substance, ethanolic extract of *Barleriaprionitis*reported to be moreeffective against fungal and bacterial species showing highest inhibition such as 18mm againstfungi *A.niger*and 15mm against *C.albicans*and 22mm against bacteria *Streptomyces fulvissimus*and 21mm against *E. coli*and *Bacillus subtilis*. The ethanolic extract of *Phyllanthusacidus*showedmoreactivityagainstbacteriasuchas15mm against *B. Subtilis* and 13mm against *Streptomyces fulvissimus*and lowest activity fungus such as 12mm against *C.albicans*and 10mmagainst *A.niger*. The overall evaluation studies showed that the ethanolic extract of *Barleriaprionitis*showed highest degree of antibacterial and antifungal activity than ethanolic extract of *Phyllanthusacidus*.

The antimicrobial effect of ethanol extracts against these organisms may be due to the abilityof the ethanol to extract some of the active properties of these plants glycosides, alkaloids, tannins, flavonoids, saponins and other secondary metabolites which are reported to beantimicrobial (CowanMM., 1999), (OkwuDE., 2006). The results how that the extract of *Barleria Prionitis*was found to be more effective against all the microbes tested compared to *Phyllanthusacidus*extract.

## 7. SUMMARYAND CONCLUSION

The term medicinal plants include plants have a medicinal activities. Medicinal plantsused in drug development and synthesis, because of its rich resource of ingredients. Thereare about half million plants around the world and they have medicinal properties and itcould be decisive in the treatment of future or present studies. In developed countries, plant drug constituent as much as 25% of the total drugs, while in fast developing countries, the contribution is as much as 80%. The economic importance of medicinalplants is much more to countries such as India than to the rest of the world. Only arelatively small fraction of the world's green pharmacy has been investigated for possible pharmacological and the rapeuticproperties. This holds the promise of investigation important phytoconstituents from plant origin.

The leaves of *Barleria prionitis* and *Phyllanthus acidus* were selected for the detailed study to evaluate its phytochemical and biological properties. Literature survey revealed so for no work has been done on this fern claiming maximum therapeutic uses. So we felt worth while to validate scientifically, the folk claim for its therapeutic activity. We have also taken its

detailed preliminary phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

The plant materials were collected from the surrounding areas of Erode district and authenticated. The leaves were powdered and extracted with petroleum ether and absolute ethanol using Soxhlet apparatus separately. The extracts were concentrated to dryness under reduced pressure and controlled temperature. Qualitative phytochemical screening was carried out to identify the phytoconstituents.

Preliminary phytochemical screening of *Barleria prionitis* revealed the presence of bioactive compounds such as carbohydrates, glycosides, alkaloids, tannins, flavonoids, phenols and saponins in ethanolic extract. On the other hand the ethanolic extract of *Phyllanthusacidus* showed the presence of glycosides, alkaloids, flavonoids, steroids, phenols.

## 8. CONCLUSION

In the present study, the herbal extracts of *Barleria prionitis* and *Phyllanthus acidus* leaves examined for antimicrobial activity.Theethanolic extract of *Barleria prionitis* was found to be active on most of the clinically isolated microorganism and fungi, as compare with standard drugs. The activities of these extracts are found to be quiet comparable with the standard antibiotics screenedunder similar conditions. So these extracts can be used as an external antiseptic in prevention and treatment of bacterial infections. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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