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Studies On *in vitro* Culture and Secondary Metabolite Production of *Phyllanthus reticulates* Poir.

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Abstarct

Medicinal plants have been used as remedies for human diseases for centuries. In Indian Ayurvedic medicine, various herbaceous species of *Phyllanthus* are together known as *bhuiamla*, which is used to treat a wide variety of diseases such as jaundice, gonorrhea and more. A more common species of the genus, *Phyllanthus reticulates*, widely found in southern parts of India is studied here for its potential medicinal properties. The study tries to quantitatively and qualitatively analyse the properties and effectives of ethanol and aqueous of the plant sample.

Keywords: *P.reticulates*, anti-microbial, antioxidant, anti-cancer

I. INTRODUCTION

Traditional medicine has long been associated with nature, extensively medicinal plants and their properties. The modern allopathic medicine however does not see the traditional medicine as efficient enough the cure various ailments (Balick and cox, 1996). It is to be noted that many modern medicines have originated from traditional medicinal plants. (Cotton, 1996). At this time, it is highly essential to gain knowledge of the medicinal properties of plants and the compounds causing them. It is essential because, many modern drugs have various side-effects and microorganisms have developed resistance to a lot of drugs. Thus, the plant kingdom with2, 50,000 floral species is now viewed as a potential source of active compounds for novel medicines (Rahmatullah, *et al*., 2012).

Many medicinal plants are used in modern medicine where they occupy a very significant place as raw materials for important drugs and plants used for traditional medicine contain a wide range of substances that can be used to treatchronic as well as infectious diseases (1). Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics (2). The development of resistance in microorganisms due to the excessive use of conventional antibiotics and the increase in the emergence of infectious diseases has led to the search of new antimicrobial compounds with increased efficiency in terms of their mechanisms of action (3). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher plants appears rewarding as it willlead to the development of phytomedicine to act against microbes (4). Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections (5). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced in vivo, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing, etc. (6, 7). Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals (8). Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and are used for industrial processing but they possess potential health risk due to toxic properties and should be replaced with natural antioxidants (9).

II.RELATED WORK

Plants belonging to the genus *Phyllanthus* are well known in traditional medicine. It has a history of well documented folk remedies in many countries. *Phyllanthus* has more than 700 species in at least 10 sub-genera. (Holm – Nielsen, 1979). It grows as shrubs, trees and biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Taylor, 2003). Pharmacologically important compounds have been isolated from many species of Phyllanthus.

Phyllanthus reticulates (Family: *Euphorbiacae*) is a large, often scandent, shrub. The plant grows throughout the tropical areas of India, Bangladesh, China and the Malay Islands (Shruthi, *et al*, 2010).

In Indian system of medicine *Phyllanthus reticulates* is an important medicinal plant and is popularly known as 'Potato-bush'. It is distributed throughout India, in hedges or waste places near villages and along streams and canals (Orient Longman, 2003).

The plant with lanticellete branches grows from 8-10 ft in height. The fruiting and flowering season of the plant is from July to March. Leaves of the plant contain tannic acid and gum; fruit of the plant becomes purplish black when ripe. The leaf juice of the plant is diuretic and is used to cure diarrhea in infants (Nadkarni, *et al*, 2009). The plant is also used for ailments like smallpox, syphilis and asthma (Nadkarni, 1982). It is extensively used as a cooling agent to treat inflammation, eye sore, burns, and chafing of the skin (Chopra *et al*, 1956).

The plants of this genus were found to contain lignins, flavonoids, triterpenoids, alkaloids and polyphenolic compounds (Toshida *et al*, 1964). However, Phyllanthus reticulates is yet to be widely examined for its chemical and biological properties.

Microorganisms are increasingly showing resistance to conventional antibiotics. The over use of conventional

antibiotics led to the development of what is called as 'Super bugs'. The emergence of infectious diseases is also on a rapid increase. This recent trend had led to the search of new antimicrobial compounds that have better mechanisms of action against microorganisms, thus showing increased efficiency against resistance (Baucer *et al*., 2003).

Researchers identified 119 secondary metabolites isolated from plants that are being used globally as drugs (Ragu *et al*., 2010). Hence plant sources are viewed as being immensely potential due to their therapeutic effects (Bandow *et al*., 2003). Plant based antimicrobials may be used to treat infectious diseases while mitigating the side effects of synthetic antimicrobials (Twu *et al*, 1999). With further research, they can be used as lead compounds in drug development (Aiyoro *et al*., 2008).

Free radicals are fundamental to any biochemical process and form an essential part of metabolism, The excessive presence of free radicals cause oxidative stress which is mainly linked to majority of diseases (Gutteridgdem JMC,1995;Tiwari A., 2001).

Many disease states such as arthritis, connective tissue disorders, carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (Joyce, 1987). In the treatment of these diseases, antioxidant therapy is being widely used. Compounds that delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions are called as antioxidants (Velioglu, 1998). The phenolic components such as flavonoids, phenolic acids and phenolic diterpenes contribute to the antioxidative effect (Pietta PG, 1998; Shahidi *et al*, 1992).

The plants of the Euphorbiaceae family contain polyphenols, flavonoids glycosides and many such phytochemicals in abundance (Sio-Hang Lam, 2007). Thus the plants of the *Phyllanthus* species possess significant antioxidant capacities that can be used to cure various ailments. It can even be associated with lower incidence and lower mortality rates of cancer in several human populations (Velioglu, 1998).

Many plant species in the *Phyllanthus* genus have been screened and reported to have anti-tumor activity. *Phyllanthus emblica* has been used for the treatment of various disorders such as scurvy, cancer and heart diseases (Khopde *et al*, 2000). Plants are seen as a rich source of novel chemotherapeutic agents. Their further study will lead to the discovery of efficient drugs against cancer.

Hepatitis B virus (HBV) in both its acute manifestations and its chronic consequences is a major public health problem throughout the world (Sherlock, 1981). Even with the advancement of medical technology, the need to find an effective cure to various liver ailments is still prevalent. Hence scientists are now increasingly looking at medicinal plants as a potential source of drugs (Satyavati *et al.* 1987). Many plants in the Euphorbiaceae family are observed to be beneficial for the treatment of liver ailments. Plants such as *Phyllanthus niruri, Phymmanthus emblica Phyllanthus urinaria,* etc., have been reported to be effective (Satyavati *et al*. 1987).

The aqueous extract of the plant *Phyllanthus niruri* was found to inhibit DNA polymerase of hepatitis B virus and also to bind to the surface antigen of hepatitis B virus in vitro (Venkateswaran *et al*, 1954). Due to the presence of phytochemicals such as flavonoids, polyphenolic compounds etc., in *Phyllanthus reticulates* it can be effective against the hepatitis B virus responsible for hepatotoxicity causing fatal liver diseases (Yoshida *et al*., 1982). Though primitive study regarding the Hepatitis B viral activity of *Phyllanthus reticulates* (Biplap Kumar Das., 2011) has been carried out, it further needs to be validated.

Hence the purpose of this study was to evaluate the antimicrobial, antioxidant, anticancer and anti hepatitis activity of *Phyllanthus reticulatus.*

III.MATERIALS AND METHODS

a.QUALITATIVE PHYTOCHEMICAL ANALYSIS: (Raman, 2006)

1. TEST FOR TANNINS:

1ml of sample was taken, 0.1 % ferric chloride was added drop-wise. A dark green colour indicates the presence of Tannins.

2. TEST FOR SAPONINS:

1000 µl of concentrate was mixed with 2000µl of water. After 15 minutes, the tube was shaken and observed for any foam formation which indicates the presence of Saponins.

3. TEST FOR FLAVONOIDS:

1000 µl of concentrate was added with Hydrochloric acid and magnesium chloride was added to 1 ml of extract. Tomato red colour formation is positive.

4. TEST FOR ALKALOIDS:

1000 µl of concentrate was mixed with few drops of Dragendorff's reagent. The test indicated positive by a prominent yellow precipitate formation.

5. TEST FOR PROTEINS:

1000 µl of concentrate was added with few drops of Bradford's reagent and observed for blue colour formation.

6. TEST FOR STEROIDS:

Few drops of 10% concentrated sulphuric acid were added to 1 ml of sample .A prominent green colour indicates the test as positive.

7. TEST FOR ANTHRAQUINONES:

1000 µl of concentrate treated with aqueous ammonia. Anthraquinones presence was indicated by the presence of pink colour change.

ANTIMICROBIAL ACTIVITY

1.Antibacterial Activity Assay:

Number of samples: 1sample x 2 extracts

Extracts: 2

1. Ethanol
2. Aqueous extract.

Number of Microorganisms tested: 5

1. *Staphylococcus aureus,*
2. *Bacillus subtilis,*
3. *E. coli,*
4. *Pseudomonas aeroginosa,*
5. *Salmonella typhi.*

Standard: Ampicillin

2.Preparation Of Inoculum:
The test organisms were inoculated into a fresh broth and incubated at 37ºC. Young cultures (8 hrs of incubation) were taken for the antimicrobial study by disc diffusion method.

b.Antibacterial Assay: (Bauer *et al*., 1966)
Antibacterial assay was carried out by disc diffusion method. The test organisms were swabbed on the plates. Samples were diluted for 3 different concentrations viz., 1000 microgram/mili litre, 750 microgram/mili litre and 500 microgram/mili litre. Ampicillin was taken as positive control. 20 µl of diluted samples and positive control were added in sterile discs and placed in MHA plates. The treated plates were kept at 37ºC for 24 hrs. After incubation the plates were observed for any clear area around the disc and the diameter of the zone was recorded to determine the antibacterial activity.

ANTIFUNGAL ACTIVITY ASSAY:
Number of samples: 1 sample x 2 extracts
Extracts: 2
1. Ethanol
2. Aqueous extract.

Number of Microorganisms: 4
1. *Candida albicans,*
2. *Aspergillus Niger,*
3. *Trichoderma viridae,*
4. *Rhizopus microsporus.*

Standard: Amphotericin-B

Preparation of inoculum:
The test organisms were enriched by inoculating into SDA broth and kept at room temperature. Young cultures were taken for the antimicrobial study.

1.Antifungal assay:
Antifungal a ctivity of the samples was carried out by disc diffusion method. The test organisms were swabbed on the plates. Samples were diluted for 3 different concentrations viz., 1000 microgram/mililitre, 750 microgram/mililitre and 500 microgram/mililitre. Amphotericin-B was taken as positive control. 20 µl of diluted
samples and positive control were added in sterile discs and placed in SDA plates. The treated plates were kept at room temperature for twenty four hrs. The inhibition area was measured to determine the antifungal activity.

ANTIOXIDANT ASSAY
Dpph assay: (Molyneux, 2004)
DPPH assay is a simple and sensitive method to find out the antioxidant capacity of a test sample. DPPH is a purple coloured stable and commercially available organic compound. It has the maximum absorption at 517nm. The basic principle of the assay is the number of hydrogen atoms donated by the test sample was received by DPPH and in turn get reduced. Due to that the primary purple colour of the compound is turned to yellow colour. By measuring the absorption decrease at 517nm the antioxidant activity of a test sample can be determined.

Requirements:
1. DPPH,
2. Dimethylsulphoxide,
3. BHT (standard) - 1.6 mg/ml in methanol,
4. Samples desired concentration from 1 mg /ml – max of 5mg / ml (in DMSO)

S.NO.	REAGENTS	BLANK	STANDARD	TEST
1	Methanol	3.8ml	3.7ml	3.7ml
2	BHT	-	100µl	-
3	Sample	-	-	100µl
4	DPPH	200µl	200µl	200µl
Kept under dark for 30 mins				
Absortion at 517 nm				

Procedure for DPPH Assay

Procedure:
Aliquot absolute methanol of 3.7 ml to all the tubes except blank and add absolute methanol of 3.8 ml to the blank tube.
Add 100µl of BHT to standard tube & 100µl of respective samples to all remaining tubes except blank. 200µl of DPPH was added to all the test tubes including blank under dark and kept for thirty minutes at room temperature. Absorbance was read at 517nm.

Calculation:
% Antioxidant activity = $\frac{\text{(O.D of the Blank) - (O.D of the Test)}}{\text{(O.D of the Blank)}} \times 100$

Ferric Ion Reduction Potential (FRAP): (Benzie and Strain, 1999)
FRAP assay (Ferric reducing ability of plasma) is an inexpensive, simple and novel technique to determine the antioxidant capacity of a sample. The results are highly reproducible. The principle is based on the conversion of ferric tripyridyltriazine to ferrous tripyridyltriazine by a reductant at low pH.The reduced compound develop strong blue colour and was measured at 593 nanometer. FRAP values were calculated by comparing the absorbance change at 593 nm from 0 to 4 minutes interval.

Requirements:
- Reagent A - Acetate Buffer (300mM, pH 3.6)
  - 16 ml of glacial acetic acid was added to 3.1g of sodium acetate trihydrate; solution was then made up to 1L using distilled water. pH of the solution was checked.
- Reagent B - TPTZ (2,4,6- tripyridyl-s-triazine) Solution
  - 0.031g of TPTZ was added to 10ml of 40mM HCl.
- Reagent C - Ferric Chloride Solution
  - 0.054g of Ferric Chloride was dissolved in 10ml of distilled water.

Reagents B and C were prepared freshly every time when the assay was performed.

Preparation of FRAP Reagent:
2.5 ml of Reagent B and 2.5 ml of Reagent C were added to 25 ml of Reagent A to make 30 ml of the FRAP reagent. This was placed in a 37ºC water bath for minimum of 10 minutes.
Standard-Ascorbic acid: 1.76 mg of Ascorbic acid was dissolved in distilled water of 100 ml

FRAP Assay Procedure:

1. 100µl of sample was added with 3 millilitre of working FRAP reagent and absorbance (593nm) was measured at zero minute after vortexing.
2. Samples are kept at 37°C in water bath and absorbance was measured after 4 minutes.
3. Ascorbic acid standards (100 µM-1000 µM) were also processed in the same way.

Procedure for FRAP Assay

Solutions	Blank	Standard	Test
Sample	-	-	100µl
Standard (Ascorbic acid)	-	100µl	-
Working FRAP Solution	3000µl	3000µl	3000µl

Calculation:

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute/change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000µM)

ASSAY OF CATALASE

The activity of Catalase was determined by the method of Sinha (1979). Dichromate in acetic acid was converted to per chromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620nm. The Catalase preparation was allowed to spit H_2O_2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined calorimetrically.

PROCEDURE

A 20 % homogenate of the plant samples were prepared in plant extract. Phosphate buffer (0.067M, pH 7.0) and the homogenate were employed for the assay. The samples were read against a control without homogenate, but containing the H_2O_2-phosphate buffer.

To 0.9ml of phosphate buffer, 0.1mL of supernatant and 0.4mL of hydrogen peroxide was added. The reaction was arrested after 60 seconds by adding 2.0mL of dichromate-acetic acid mixture. The tubes were kept in boiling water bath for 10 min, cooled and the colour developed was read at 620nm.

Catalase activity was expressed as mol of H_2O_2consumed/min/mg of protein.

ASSAY OF SUPEROXIDE DISMUTASE (SOD) (Kakkar et al., 1984)

Principle:-

The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm.

Reagents:-

1. Sodium pyrophosphate buffer (pH-8.3) - 0.025M
2. Phenazine methosulphate (PMS) - 186µM
3. Nitroblue tetrazolium (NBT) - 300 µM
4. NADH - 780 µM
5. Glacial acetic acid
6. n-butanol
7. Potassium phosphate buffer

pH – 6.4

PROCEDURE

The different samples, namely leaves, stolon and roots (0.5g), were ground with 0.3ml of Potassium phosphate buffer, centrifuged at 2000g for 10 minutes and the supernatants were used for the assay.

ASSAY

The assay mixture contained 1.2 ml if Sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2 ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.2 ml of NADH. The mixture was incubated at 30^0c for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer (Genesys 10-S, USA). One unit of enzyme activity is defined as the amount of enzyme that gave 50 % inhibition of NBT reduction in one minute.

CALCULATION:

%INHIBITION = A_{560nm} of blank - A_{560nm} of test / A_{560nm} of blank X100

SCAVENGING OF NITRIC OXIDE RADICALS

Principle:

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce Nitrite ions, which can be measured at 550nm by spectrophotometer in the presence of Griess reagent (Kumar S *et al*., 2008).

Procedure:

Plant extract was dissolved in distilled water for this quantification. Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) was incubated with 100 mg/ml of sample and tubes were incubated at 29ºC for 3 hours. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagent. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthyl ethylene diamine hydrochloride was observed at 550nm on spectrophotometer. **Calculations**

$$\% \text{ inhibition} = \frac{\text{O.D. of control - O.D. of Test x 100}}{\text{O.D. of control}}$$

ANTICANCER ACTIVITY

MTT assay: (Mosmann, 1983)

The 24 well plates seeded with Cells were taken out and observed under an inverted microscope for the growth. Once the cell reaches the confluence in the well, the growth

medium was removed aseptically using sterile pipettes. Then 500 µl of various concentrations of the samples and L-ascorbic acid were added and incubated at 37^0C for 24hrs in 5% CO_2 condition. 500 µl of growth medium was added in to the Control well. After incubation, samples were removed carefully and washed with MEM without serum twice. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added in all the wells and incubated for four hours. After incubation, 1000µl of DMSO was added in all the wells. Colour changes from yellow to purple was measured at 570 nm using DMSO as the blank. IC_{50} values were calculated by graphical method.

The % cell viability was calculated by the following formula:

% cell viability = $\frac{\text{A570 of treated cells} \times 100}{\text{A570 of control cells}}$

Graphs are plotted using,

1. Percentage of Cell Viability at Y-axis &
2. Concentration of the sample in X-axis.

WOUND HEALING ASSAY

This assay is done to check the cell migration.

PROCEDURE:

- The VERO cell lines were used for wound healing assay.
- The cells were seeded into the plate and incubated for 24 hrs.
- After incubation, the cells were observed for growth and assay was proceeded.
- The samples were weighed and dissolved in DMSO.
- It was serially diluted at different concentrations and the concentration was chosen based on IC_{50} value obtained from MTT Assay.
- The medium was discarded and the plate was kept under microscope.

A sterile tip was used and wound was created.

- The desired concentration (125 to 62.5µg/ml) were added to the respective wells and incubated.
- After 4 hrs incubation, the plate was observed for the growth of cells.

ANTI HEPATITIS B VIRAL ACTIVITY

The aim of this study was to evaluate the antiviral activity of *Phyllanthus reticulatus* plant against hepatitis B virus (HBV) using HBsAg positive serum sample from hepatitis B affected patients. The results obtained showed that *Phyllanthus reticulates* showed good properties against HBV.

HEPATOPROTECTIVE EFFECT

The in-vitro hepatoprotective effect of *Phyllanthus reticulates* was studied using liver cell line. The hepatoprotective effect against acetaminophen-induced hepatotoxicity was assessed by the percentage of cell viability. From this study it can concluded that *Phyllanthus reticulates* exhibited hepatoprotective effect.

RESULTS:

PHYTOCHEMICAL SCREENING:

Individual fractions were analyzed for its qualitative phytochemical. Standard procedures were adopted for screening (Raman, 2006). The major phytochemicals tested were tannins, saponins, flavonoids, alkaloids, proteins, steroids and anthraquinones

The phytochemicals tannins and proteins were found in both the ethanolic and aqueous extract, while saponins, flavonoids and alkaloids were found only in the aqueous extract. Steroids were present only in the ethanolic extract while anthraquinones were absent both in the ethanolic and aqueous

TEST	Sample	
	Aqueous	*Ethanol*
TANNINS	+	+
SAPONINS	+	-
FLAVONOIDS	+	-
ALKALOIDS	+	-
PROTEINS	+	+
STEROIDS	-	+
ANTHROQUINONES	-	-

Table: Phytochemical studies of the Ethanol and aqueous extract
(+) = Positive
(-) = Negative

ANTIBACTERIAL:

At a concentration of 1000µg/ml of the ethanolic extract, *Pseudomonas aeroginosa* showed maximum zone of inhibition at 20mm. *Staphylococcus aureus* showed 15mm while *Salmonella spp*.

showed 11mm, *E.coli* showed the least zone

of inhibition at 9mm. At 750µg/ml and 500µg/ml concentration, *P.aeroginosa* again showed the maximum zone of inhibition at

20mm. *S.aureus* and *Salmonella spp.* showed 15mm and 10mm respectively. *E.coli* again showed the least of 8mm.

For the aqueous extract at 1000µg/ml concentration, *P.aerogenosa* showed the maximum of 25mm of zone of inhibition. *S.aureus* showed 20mm while *E.coli* and *Salmonella spp* both showed the least zone of inhibition of 8mm. At 750µg/ml, *P.aerogenosa* showed 18mm concentration,

while *S.aureus* showed 15mm. *E.coli* and *Salmonella spp* again showed the least zone of inhibition of 8mm. At 500µg/ml, *P.aerogenosa* showed 15mm zone of inhibition while *S.aureus* showed 13mm zone of inhibition. *E.coli* and *Salmonella spp* showed the least zone of inhibition at 7mm.

Organisms	Zone of Inhibition(mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Staphylococcus aureus	20	15	13	25
E. coli	8	8	7	12
Salmonella spp.	8	8	7	9
Pseudomonas aeroginosa	25	18	15	34

ANTIFUNGAL:

Ethanol extract

During the determination of the antifungal activity at 1000µg/ml concentration, the maximum zone of inhibition was showed by *Trichoderma viride* at 30mm. *Rhizopus spp* showed 12mm while *Candida albicans* showed 10mm zone of inhibition. *Aspergillus niger* showed the least zone of inhibition at 9mm. At both 750µg/ml and 500µg/ml concentration of the ethanolic extract, *T.viride* showed 10mm, *Rhizopus spp.* showed 8mm and *C.albicans* showed 7mm zone of inhibition. *A.niger* showed 8mm at 750µg/ml concentration while the zone of inhibition was found to be 7mm at 500µg/ml.

Aqueous Extract

In the aqueous extract, at a concentration of 1000µg/ml, *Rhizoppus spp*. showed the maximum zone of inhibition at 45mm. *T.viride* showed 35mm while *A.niger* showed 10mm. *C.albicans* showed the least of 8mm. At a concentration of 750µg/ml, *Rhizoppus spp* again showed a maximum at 40mm while *C.albicans* showed a minimum at 8mm. *T.viride* showed 33mm and *A.niger* showed 10mm zone of inhibition. At the concentration of 500µg/ml, the maximum was recorded at 35mm by *Rhizoppus spp*. and minimum by *C.albicans* at 8mm. *T,viride* showed a zone of inhibition of 25mm and *A.niger* showed 9mm.

S.NO	SAMPLE	Concentration (µg/ml)	O.D I	II	III	Average	DPPH activity (%)
1	Ethanol Extract	1000	0.303	0.319	0.308	0.310	83.72
2	Aqueous Extract	1000	0.488	0.405	0.469	0.454	76.16
3	Standard-BHT	1000	0.002	0.002	0.002	0.002	99.8

DPPH:

The Antioxidant activity of given samples using DPPH Assay method

Blank O.D: 1.905

The DPPH activity of the ethanolic extract was found to be 83.72% while that of the aqueous extract was found to be 76.16%

FRAP:

The FRAP value of the ethanolic extract was measured as 109µM and the aqueous extract was found as 130µM.

S.NO.	NAME OF THE SAMPLE	FRAP(µM)
1.	Ethanol Extract	109
2.	Aqueous extract	130

CATALASE:

The Catalase activity of the ethanolic extract was 5.3 H_2O_2 consumed/min while that of the aqueous extract was 0.825 H_2O_2 consumed/min.

SOD:

S.NO.	NAME OF THE SAMPLE	SOD
1.	Ethanol Extract	90.68
2.	Aqueous Extract	71.56

SOD of the ethanol extract was 90.68 and aqueous extract was 71.56

Nitric Oxide:

S.NO.	NAME OF THE SAMPLE	INHIBITION %
1.	Ethanol Extract	26.94
2.	Aqueous Extract	80.120

The inhibitory percentage for ethanol extract was 26.94% and aqueous extract was 80.120%

ANTICANCER ACTIVITY:

Anticancer effect of Ethanol extract on *HT29* cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.051	2.90
2	500	1:1	0.138	7.86
3	250	1:2	0.228	12.99
4	125	1:4	0.332	18.92
5	62.5	1:8	0.493	28.10
6	31.2	1:16	0.883	50.34
7	15.6	1:32	1.043	59.46
8	7.8	1:64	1.236	70.46
9	Cell control	-	1.754	100

The 50% inhibitory concentration of the ethanolic extract was 31.2µg/ml.

Anticancer effect of aqueous extract on *HT29* cell line

S.No	Concentration(µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.032	1.82
2	500	1:1	0.120	6.84
3	250	1:2	0.298	16.98
4	125	1:4	0.386	22.00
5	62.5	1:8	0.499	28.44
6	31.2	1:16	0.609	34.72
7	15.6	1:32	0.872	**49.71**
8	7.8	1:64	1.019	58.09
9	Cell control	-	1.754	100

The 50% inhibitory concentration of the aqueous extract was 15.6µg/ml.

CONCLUSION:

The study so conducted on aqueous and ethanol extracts of the plant *Phyllanthus reticulates* has shown considerable medicinal potential in treating various ailments. The presence of tannins, saponins, flavonoids, alkaloids and proteins, in aqueous extract of the plant sample is more suitable for medicinal exploration and can be used to extract bio chemicals or develop important drugs. The plant was found to show considerable anti-oxidant, anti-microbial and anti cancer activities. Present study had revealed its pharmacological properties and chemical constituents present, which can be of further use in developing new drugs and extracting medicinally useful biochemicals.

REFERENCES:

1. Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI(2008). Antibacterial activities of crude stem bark extracts of Distemonanthus benthamianus Baill; J. Biol.Sci.8 (2): 356 – 361.
2. Balick, J.M., and Cox, P.A. (1996). Plants, people and Culture: the science of Ethnobotany, New York: Scientific American Library.
3. Bandow JE, Brotz H, Leichert LIO, Proteomic approach to understanding antibiotic action. Antimicrob Agent Chemother. 2003; 47:948 – 955.
4. Chopra, R.N., NAyer, S.L., and Chopra, I.C. 1956. Glossary of Indian Medicinal plants. CSIR, New Delhi, India, 2nd edition. P.191.
5. Cotton, C.M. (1996). Ethnobotany: Principle and Application. New York: John Wiley and sons.
6. Gutteridgdem JMC. Free radicals in disease processes: A complication of cause and consequence. Free Radic. Res. Comm. 1995; 19:141-158.
7. Holm-Nielsen, L.B. 1979. Comments on the distribution and evolution of the genus Phyllanthus (Euphorbiaceae), p. 277-290. In: K. Larsen and L.B. Holm- Nielsen (Eds.). Tropical botany. Academic Press, New York.iosci.Biotechnol.Biochem (2000),64:503-509.
8. Iwu MW, Duncan AR, and Akunji CO. 1999. New antimicrobials of plant Rigin In; JAnick, J.(Ed.), perspectives on New crops and new uses. ASHS press, Alexandria, VA, 1999; 457 – 62.
9. Joyce DA. Oxygen radicals in disease. Adv. Drug Reac. Bull. 1987; 127:476-79.
10. Khopde S.M, Priyadarshini K.I, Guha S.N , Satav J.G, Venkatesan P and Rao M.N.A
11. Nadkarni KM, Nadkarni AK. Indian Materia medica.vol. One. Mumbai: popular prakashan; 2009, p.943-944.
12. Nandkarni K.M., Indian Maretia Med vol. 2 (1982), p. 948.of Food Science & Nutrition 1992; 32: 67–103.
13. Orient Longman, Indian edicinal plants, a compendium of 500 species, 2003; 4:Phyllanthus Emblica Linn, IJPSR/Vol. I/ Issue I/July-September,2010/Pg.30-33.
14. Pietta PG. Flavonoids in medicinal plants. In CA Rice (ed. Evans and Packer L), New York: Dekker, 1998: 61–110.
15. Raghu H.S and Ravindra. P. 2010. Antimicrobial activity and Phytochemical study of
16. Rahmatullah, Zubaida Khatun, Abid Hasanm Waheda Parvin, Md. Moniruzzaman, Asha Khatun, Mostafi Jumrut Mahal, MD. Shaiful Alam Bhuiyan, Sadia Moin Mou, Rownak Jahan (2012). Survery and scientific evaluation of Medicinal plants used by the pahan and Teli Tribal communities of Natore District, Bangladesh. Tradit Complement Altern Med. (2012): 9(3): 366-373.
17. Sathyavati G.V. and Gupta A.K. 1987. Medicinal plants of India. New Delhi, **2**, 345.
18. Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants. Critical Reviews
19. Sherlock S. 1981. Diseases of the liver and Biliary system. Blackwell Scientific publication, Oxford, London, Edinburgh, Boston, Melbourne, 6th edition, pp.244.
20. Shruthi SD, Padmalatha Rai S, Ramachandra YL (2010). *In vitro* antibacterial activities of Kirganelia reticulata baill. against methicillin-resistant Staphylococcus aureus. *Pharmacophore* 2010, Vol. 1 (2), 123-131.