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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF STEM OF PHYLLANTHUS AMARUS PLANT

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ABSTRACT:
Present study reports phytochemical screening and the microbial activity of crude extracts of leaf of Phyllanthus amarus on some human pathogenic bacteria and fungi. Phyllanthus amarus has been used in the traditional medicines of various cultures, including Amazonian tribes for the treatment of gallstones and kidney stones; in Ayurvedic medicine for bronchitis, anemia, diabetes; and in Malay traditional medicine for diarrhea, kidney ailments and gonorrhea. stem extracts were prepared with different solvents and tested for the presence of primary and secondary metabolites which are pharmacologically active compounds. Ethanol, ethyl acetate and hexane extracts revealed the presence of more constituents including alkaloid, tannins, anthroquinones, glycosides, phenols, flavonoids etc. As many of the active ingredients in chemically manufactured drugs were originally derived from plant compounds, extracts are also used to screen the antimicrobial activity. The zone of inhibition was determined utilizing the well diffusion method. Ethanol was found to be highly sensitive against Gram positive S-aurous and gram-negative E-coli. More recently there have been preclinical and clinical studies looking into the plants supposed liver- protective abilities and effect on hepatitis B. The inhibitory activity of these extract confirmed the potential use of the plant in the treatments of microbial induced ailments.

Keywords: - Phytochemical screening, Phyllanthus amarus, pharmacologically, Antimicrobial activity.

INTRODUCTION :

The most common name of Phyllanthus amarus is Bhuiamla. Bhuiamla is a very common broad spectrum magical medicinal herb native to India. It grows as weed throughout the hotter part of country. During rainy season, you can find it growing abundantly in gardens, waste lands, parks , along roadsides, open areas, etc. You can identify the medicinal herb by looking underside if its leaves. There you will notice a small round structure which looks like amla. There are many Medicinal use of Bhui Amla.

In modern medicine, there is no effective specific therapy is available for viral hepatitis but Tamalki has shown clinical efficacy, in viral Hepatitis B. Phyllanthus amarus is a leafy herbal plant found in tropical regions in America, Africa, India, China and South East Asia. Common name for this plant include gale of the wind, carry me seed on the leaf, pick-a-back, Bhuiavala (Hindi), Bhuiamla (Bengali), stonebreaker, dukung anak (Malay). Phyllanthus amarus is a small, annual plant that grows to a height of 30-60 cm. Its thin branches spread out, and each branches has two rows of small, elliptic oblong leaves of 5-10mm long that are arranged alternately. Its radial flowers are star-shaped and of about 2mm in size. It grows well in soil of high moisture with light shade, and reaches maturity in 2-3 months.

Phyllanthus amarus has been used in the traditional medicines of various cultures, including Amazonian tribes for the treatment of gallstones and kidney stones; in Ayurvedic medicine for bronchitis, anaemia, diabetes; and in Malay traditional medicine for diarrhea, kidney ailments and gonorrhea. More recently there have been preclinical and clinical studies

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looking into the plant's supposed liver-protective abilities and effect on hepatitis B. The roots is stout and woody and stems are often branched at base and angular. Leaves are numerous and sub-sessile, distichthious, stipulate and paripinnate with small leaflets. The leaflets are oblong, having nerve obscure and base rounded. Root is stout tortuous and woody. Flowers are veryminute, shortly pedicelled numerous and axillary and yellowish in colour. Sepals are 5-6, ovate-oblong outer acute, coriaceous with pale margins; disk in both sexes of glands; male flowers 1-3 pedicelled; female flowers are solitary, larger and erect. Stamens are 3 sessile on a short column didynamous, styles minute, reflaxed very short. The fruit is capsule, minute, globose and dehiscent. Seeds are with strong parallel and transverse growth.The plant is widely used to tone-up sluggish liver and also given in chronic liver condition and jaundice.In unani medicine, the plant is used in jaundice as deobstruent, diuretic, cooling and astringent.The herb and its root have exhibited antiviral actions on Hepatitis -B.Phyllanthus amarus is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhea, menorrhagia and other genital affections. It is useful in gastropathy, diarrhea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds.The Bhuiamla plant is diuretic plant. It has the ability to clear or open the natural ducts of the fluids and secretions of the body. The plant has astringent properties and causes contraction of the skin cells and body tissues. This plant is used as single drug to treat jaundice. The plant has antiviral activity against hepatitis B virus. It has the liver protecting activity which is supported by lab studies. It effectively repair CCl_4 induced the liver damage in rats. Bhuiamla is also indicated in Gonorrhea, Frequent menstruation, Diabetes, disorders of the skin such as sores, swelling and itchiness.

Bhui amla Plant has the following medicinal properties:

- Lipid Lowering Activity
- Anti-Diabetic Activity
- Anti-Malarial Activity
- Hypertensive and hypoglycemic activity
- Antiviral activity against hepatitis B virus
- HIV (human immunodeficiency virus) Replication inhibition effect
- Hepato-protective effect /the liver
- Analgesic, Antibacterial, Antifungal, Astringent
- Ability to expel and prevent stone formation in the body

The plant exhibits medicinal properties due to the presence of various bioactive substances. For the medicinal purpose whole plant,leaves , and roots are used.Bhuiamla is effective medicinal herb for treating the liver diseases including Hepatitis B.The plant shows marked anti-hepatitis B virus surface antigen activity in in-vivo and in-vitro lab studies.In a clinical study, 37 patients with chronic viral hepatitis B, were treated with daily dose of 600mg of Bhuiamla for 30 days. 59% of the patients lost HBsAg two weeks after the end of the treatment. HBsAg is the surface antigen of the hepatitis B virus (HBV). It indicates current hepatitis B infection. From this study, the authors postulated that Phyllanthus niruri might inhibit proliferation if the virus by inhibiting replication of the genetic material of the virus.Phyllanthus amarus or Bhui amla is used traditionally for treating a variety of diseases. The hot water extract of whole plant on oral intake has fever reducing and laxative effect. The decoration is also useful in the fever reduction. In treatment of jaundice whole plant is dried and pulverised to get powder that is taken with buttermilk. in

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this case of loose motions, dysentery 0dried leaves infusion is given. In diabetes, hot water extract is useful. Fresh leaf juice or fresh root juice are taken orally for sexually transmitted disease STDs. The plant is also used externally for skin diseases, cuts, bruises the leaves juice is applied tropically on the affected part. The fruit is used externally for tubercular ulcers scabies and ringworm.

The seed under leaf can help to treat high blood pressure and a range of other conditions. Phyllanthus amarus is broad spectrum medicinal plant that has received world-wide recognition . Phyllanthus amarus is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, as anti-helminthes to expel intestinal worms and act as mild Laxative. the plants of the genus Phyllanthusare widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver disease.The traditional uses of Phyllanthus amarus for kidney stones and gall bladder stones have been validated, where Phyllanthus amarus extract was found to exhibit a potent and effective non-concentration dependent inhibitory effect on calcium oxalate crystal formation, the building blocks of most kidney stones. This response was present even at high concentration. This may explain why it has been used traditional medicine as prevention against kidney stone formation. Phyllanthus amarus has been found be 94% successful in eliminating stones.

MATERIALS AND METHOD:

Plants Collection:

The present work was carried out at Department of Chemistry, J.M.V. Chandrapur, Gondwana University. The plant named Phyllanthus amarus collected from Chandrapur forest region. Their botanical identity of plant was determined and authenticated from literature available in Department of Botany, J.M.V. Chandrapur. The leaves of Phyllanthus amarus plant was thoroughly washed with water and dried under shade for about ten days. The dried plant sample was grinding well into a fine powder in a mixture grinder. The powder was stored in air sealed polyethylene bag at room temperature before extraction.

Preparation of Ethanol extract:

100 g of the dried and powdered Phyllanthus amarus leaves were extracted at room temperature with 500 ml absolute ethanol for 72 h. extraction was done using the soxhlet apparatus briefly 100 gm of powder leaf was stored in a air sealed polyethylene bag & placed in soxhlet& extracted with absolute ethanol The extraction was done until the solvent in the soxhlet turned colourless. The extract was concentrated by recovering the solvent using the soxhlet apparatus until the extract became just pourable. It was poured into a beaker & this was then used for the analysis.

Phytochemical Analysis:

The extracts were analyzed for the presence of Alkaloids, Terpenoids, Tannine, Saponin, Flavonoid, Phlobatannin, Anthraquionone, Reducing Sugar, Glycoside and Cardiac glycoside

1. Alkaloid: About 0.2 g of the extracts was warmed with 2% H2SO4 for two minutes. It was filtered and few drop of Dragencloffs reagent were added. Orange red precipitated indicates the presence of alkaloids.

2. Tannine: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicate the presence of tannins.

3. Anthraquinones: About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allow to cool. Equal volume of CHCl3 was added to the filtrated .Few drop of 10% NH3 were added to

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the mixture and heat. Formation of rose-pink colour indicates the presence of anthraquinones.

4. Glycoside: The extracts was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drop of Fehling's solution A and B were added. Red precipitate indicates the presence of glycoside.

5. Reducing Sugars: The extracts was shaken with distilled water and filtered. The filtrate was boiled with drop of Fehling's solution for minutes. An orange red precipitate indicates presence of reducing sugar.

6. Saponin: About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

7. Flavonoids: Extracts of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless, indicates the presence of flavonoid, .Phlobatannins: The extracts (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2%HCl solution. Red precipitated show the presence of Phlobatannins.

8. Terpenoids (Salkowski test): 0.2 g of extracts was mixed with 2 ml Chloroform (CHCl3) and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

9. Cardiac glycosides: Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H2SO4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brow ring, while in acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Preparation of aqueous extract:

100 g of the dried and powdered Phyllanthus amarus leaves were extracted at room temperature with 500 ml aqueous solution for 72 h. extraction was done using the soxhlet apparatus briefly 100gm of powder steam was stored in air sealed polyethylene bag & placed in soxhlet & extracted with absolute ethanol The extraction was done until the solvent in the soxhlet turned colourless. The extract was concentrated by recovering the solvent using the soxhlet apparatus until the extract became just pourable. It was poured into a beaker & this was then used for the analysis.

Preparation of extracts:

The microorganism used in the study: Gram-negative E-coli, Gram-positive S-aurous and Nizer fungus Aspergillus were obtained from stock culture in the Department of Microbiology, J.M.V. Chandrapur.

Antimicrobial screening of extracts:

Susceptibility test were carried out. The modified agar well diffusion method to test the antimicrobial activity of the extracts. The medium employed was diagnostic sensitivity agar. The culture were prepared in triplicate and incubated at 370C for 24 to 72 h. 0.2 ml of the broth culture of the test organism was put in a sterile Petri-dish and 18 ml of sterile molten diagnostic sensitivity agar, was added. Well were bored into the medium using 0.1 ml of the extracts. Streptomycin and Chloramphenicol were used as the standard antimicrobial agents at a concentration of 10 mcg/disk, 30 mcg/disk respectively. The plates were kept in sterilized inoculation chamber for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37o C for 24 h and the diameter of zone of inhibition of microbial growth were measured in the plates in millimeters.

RESULT:

Phytochemical screening of ethanol and hexane extracts of Phyllanthus amarus leaves is shown in table 1. The susceptibility of test

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microorganism to the crude extracts of Phyllanthus amarus leaves is shown in table 2.

DISCUSSION:

The qualitative analysis of extracts from ethanol and leaves of Phyllanthus amarus. Showed the presence of photochemical constituents such as

1.Ethanol extract present in alkaloid, tannine, terpenoid, reducing sugar, flavonoid,Cardiac glycoside and absent in anthraquinone, saponine and glycoside.

2.Hexane extract present in alkaloid, tannine, saponine, flavonoid, terpenoid, cardiac glycoside.Absent in anthraquinone, reducing sugar and glycoside.

This result are summarized in table 1.1 and table 1.2. The above result indicates that, the stem of plant extract in ethanol solvent investigated are rich in alkaloid, tannine, reducing sugar, flavonoid, terpenoid, cardiac glycoside. The Ethanol extract have showed absence of anthraquinone, saponine and glycoside. Extract of stem were tested against Gram positive S-aurous and Gram negative E-coli (with zone of inhibition above 13mm for gram +ve and 16mm for gram -ve means highly sensitive). Ethanol extract was showed more antimicrobial activity than standard antibiotics and chloramphenicol. The inhibitory activity of these extract confirmed the potential use of the plant in the treatment of microbial induced ailments.

The qualitative analysis of extracts from hexane and steam of Phyllanthus amarus showed the presence of photochemical constituents such as presence of alkaloid, saponine, flavonoid, terpenoid and cardiac glycoside. The results are summarized in table 2. The above results indicates that, the stem of plant is rich in alkaloid, tannine, flavonoid, terpenoid, saponine and cardiac glycoside. The extract shows the absence of anthraquinone, glycoside and reducing sugar. Extract of stem were tested against Gram positive S-aurous and Gram negative E-coli. Gram positive S-aurous and Gram negative E-coli (with zone inhibition above 21mm for gram +ve and 25mm for gram -ve means highly sensitive). Ethanol extract was showed more antimicrobial activity than standard antibiotics streptomycin and chloramphenicol. The inhibitory activity of these extract confirmed the potential use of the plant in the treatment of microbial induced aliments.

The plant studied here can be seen as a potential source of drugs. Further studies are going on this plant in order to isolate, identify, characteristics and elucidate the structure of bioactive compounds.

CONCLUSION:

The plant Phyllanthus amarus is rich in alkaloid, flavonoid, saponine, etc. The antimicrobial activity of the extracts can be co-related to their specific contents loke alkaloids, flavonoids and terpenoids. The ethanol extract was found to be antimicrobially more effective than the hexane extract. The antimicrobial activity of S-aurous is more effective than E-coli. Phyllanthus amarus and its compound Phyllanthin, as well as, investigate if these natural products could modulate. The plant summarizes information concerning the morphology , ecology and most importantly phytochemical constituents and antimicrobial activity. Phyllanthus amarus herb is widely used Tropical countries including India. It has significant traditional uses, some of them have been experimentally established and an attempt has been made to isolate potential chemical constituents and their mechanism of action.

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Table 1.1 Phytochemical tests of ethanol stem extract of Plant Phyllanthus amarus

Chemical composition	Ethanol Extract
Alkaloid	Present
Tannine	Present
Anthraquinone	Absent
Glycoside	Absent
Reducing sugar	Present
Saponine	Absent
Flavonoid	Present
Terpenoid	Present
Cardiac glycoside	Present

Table 1.2 Phytochemical tests of hexene stem extract of Plant Phyllanthus amarus

Chemical composition	Hexene Extract
Alkaloid	Present
Tannine	Present
Anthraquinone	Absent
Glycoside	Absent
Reducing sugar	Absent
Saponine	Present
Flavonoid	Present
Terpenoid	Present
Cardiac glycoside	Present

Table 2.1-Antimicrobial activity of stem in ethanol

1	Gram +ve s-aureus	Gram -ve E-coli
2	16mm	13mm

Table 2.2- Antimicrobial activity of stem in hexane

1	Gram +ve s-aureus	Gram -ve E-coli
2	9mm	10mm

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