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Evaluation of Antimicrobial Activity of Holopteliya Integrifolia

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Abstract: ***Medicinal plants have assumed greater importance in the recent days, due to the tremendous potential they offer in formulating new drugs which afflict humankind against many diseases. During the past one century there had been a rapid growth of allopathic system of medical treatment in Pakistan and India. There is now a growing focus on the importance of medicinal plants and the traditional health systems in solving the healthcare problems of the world. Most developing countries have viewed traditional medical practice as an integral part of their culture. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary metabolites in one or more parts of the plants. These metabolites according to their chemical skeleton are grouped as alkaloids, glycosides, corticosteroids, essential oil, etc. Plants like Holoptelea integrifolia (Roxb.) Planch (Family: Ulmaceae), is gaining greater importance due to various pharmacological effects for example: analgesic, anti-inflammatory and antibacterial activities in the treatment of diseases. This plant contains a variety of chemical compounds that have been considered for the treatment of cancer of bladder, convulsions, inflammation, topical ulcers, rheumatic inflammation, fever and dysentery. In this article the pharmacognostic characteristics of Holoptelea integrifolia, its medicinal significance and pharmacological effects have been presented.***

Keywords: Holoptelea integrifolia, Evaluation, Antimicrobial activity

1. Introduction

An **antimicrobial** is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic.[1]

The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials

Antibacterials are used to treat bacterial infections. Consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment, as for recurrent *Clostridium difficile* infections.[2]

Examples of antimicrobial agents:-
Tetracycline (one antibiotic used to treat urinary tract infections) Oseltamivir or Tamiflu (antiviral that treats the flu)

Terbinafine or Lamisil (antifungal that treats athlete's foot
The power of our microbe-inhibiting product protection is attributed to our lengthy lab and product testing during the technology development process.

In order to understand the value antimicrobials add to your products, it is important to understand how the technology works to fight damaging microbe growth

2. Objectives

Holoptelea integrifolia (Roxb) Planch (Ulmaceae) is commonly known as Indian elm, kanju. It is widely distributed throughout India in deciduous forests. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism.

Antimicrobial activity of holoptellia intigrifolia was determined by comparing with standard drug. And various strains of microorganism are used such as E.coli, Staph aureus, Bacillus subtillus.

Mechanism of action of antimicrobial[3]
The mechanisms by which compounds with antibacterial activity inhibit A large number of families and groups of antimicrobial agents are of clinical growth or cause bacterial death are varied and depend on the affected targets.

The bacterial cell wall-a unique structure in most bacteria that is absent in eukaryotic cells-can be affected in several ways at different stages of synthesis (fosfomycin, cycloserine) or transport (bacitracin, mureidomycins) of its metabolic precursors, or by a direct action on its structural organization (beta-lactams, glycopeptides).

The main drugs affecting the cytoplasmic membrane are polymyxins and daptomycin. Protein synthesis can be blocked by a large variety of compounds that affect any of the phases of this process, including activation (mupirocin), initiation (oxazolidinones, aminoglycosides), binding of the tRNA amino acid complex to ribosomes (tetracyclines, glycylcyclines) and elongation (amphenicols, lincosamides, macrolides, ketolides, streptogramins, fusidic acid).

The metabolism of nucleic acids can be altered at the DNA-dependent RNA polymerase or in the process of DNA coiling (quinolones); some compounds affect DNA directly (nitroimidazoles, nitrofurans). Trimethoprim and sulfamides (often used in combination) are examples of antimicrobial agents that block bacterial metabolic pathways.

Some compounds are unable to inhibit or kill bacteria in themselves, but can block bacterial mechanisms of resistance, enhancing the activity of other antimicrobials administered in combination. Among this group of agents, only certain beta-lactamase inhibitors are currently in clinical use.

Plant Profile

Nature has blessed mankind with a treasure of medicinal plants. Natural products have always remained a profile source for the discovery of new drugs and are used since Vedic period .Holoptelea ntegrifoliais a medium-sized large glaborous tree about 15-25 m in height with whitish or yellowish grey bark exfoliating in irregular flakes and possesses an offensive smell when cut freshly. It belongs to family Ulmaceae and is having 15 genera and 200.species.

Figure 1: Fruits of holoptellia intigrifolia

Vernacular Names:

Hindi- Chirmil, Chilbil, Chilla,
Dhamna, Kandru, Kanju, Karanji, Kumba, KunjanaliKunj;
Gujarati- Charel;
Marathi-Papara;
Sanskrit-Chirbilva;
Tamil-Ayi
Malayalam- Aval;
Punjabi-Arjan, Kacham, Khulen, Papri;
Telugu- Nemali,Nevili, Pedanevili; Uriya- Dharango(4)(5)

Distribution

It is widely distributed all over tropical and temperate regions of Northern hemisphere including Indian Peninsula to Indo China, Burma and Srilanka. It is abundantly found in sub Himalayan hills of Assam, Bihar, Ajmere,\ Bundelkhand

Pharmacognostic Studies

Holoptelia integrifolia is a large spreading glaborous deciduous tree about 15-18 m high having mucilaginous bark and elliptic leaves [4].Leaf is green in colour with slight aromatic odour.External margin of leaf is rough, with particulate venation acute apex and symmetrical base with curved petiole and broad alternate lamina. Leaf is broad approx 2-3 cm in size [5].

Leaves are 7.5-12.5 by 3.3-6.3 cm in size. These are elliptic, acuminate, glaborous having rounded base [4]. The upper epidermis of leaf consists of small barrel shaped parenchymatous cells.

Trichomes are present on both the surfaces of leaf and majority of them are present along the midrib and minimum are found along thelamina. Stomata are present on lower surface and represented by anomocytic type. The vascular bundleis ovoid in shape. Between the upper epidermis andthe vascular bundle, 6 to 7 layer of irregular shaped collenchyma cells are present. The vascular bundle iscollateral and open endark.

There occur few layers of cambium in between the xylem and phloem. The phloem consists of sieve tubes, companion cells and phloem parenchyma. Xylem consists of xylem vessels, tracheids and parenchyma. Xylem is seen onthe upper side whereas phloem is seen towards thelower side of the epidermis [5]. Stem is brown in colour having agreeable smell and smooth texture. The transverse section of stem is circular and covered with many unicellular uniseriate trichomes. The outermost multilayered periderm consists of cork cambium and secondary cortex. The cork layer is interrupted at many places due to the presence of lenticels. The cortex is multilayered and consists of parenchymatous cells. The primary phloem remains as patches of crushed tissue.

The secondary phloem consists of sieve tubes, companion cells, phloem, parenchyma and phloem rays. Vessels are present in broken conditions and crushed form. The xylem is represented by both primary and secondary xylem tissue. It consists of vessels and tracheids.

The primary xylem is towards the pith, while, the secondary xylem consists of large vessels and xylem parenchyma. Xylem is found in the form of continuous medullary rays. The pith is large and remains to the central part of the stem. It consists of thin walled parenchymatous cells having many intercellular spaces.

The pith regions have oil droplets. Flowers are sessile, male and bisexual,ciliate, and obtuse. Stamens are biseriate and anthers are slightly curved, hairy, introrse. Ovary is,compressed . ovate. Stalk is elongating in fruit which is dry, winged, compressed samara, reticulate. Seeds are flat and exalbuminous [5].

Chemical Constituents:

The plant has been reported to possess chemical constituents like terpenoids, sterols, saponins, tannins, proteins, carbohydrates and alkaloids [4] Flavonoids. The phytoconstituents isolated from stem bark are holoptelin- A and holoptelin-B, 2-aminonaphthoquinone, Friedlin, epifredlin, β-sitosterol, β-D-glucose, β-amyrin, hederagenin (heart wood), hexacosanol . 1, 4-naphthalenedione has been isolated from leaves of *Holopteleaintegrifolia* and is reported to possess antibacterial activity against Staphalococcusaureus.

Traditional Uses

Plant is useful in treatment of obesity, edema, and bronchitis. It has been known to be protease inhibitor. Mucilage and juice obtained from boiled bark has been reported to be useful in rheumatism, intestinaltumour when applied externally. Bark juice is applied to rheumatic swellings [6]. Bark juic is useful as oxytoxic in pregnancy. Paste of seeds andbark stem is externally useful in ringworm, eczemaand cutaneous affections. Paste of stem bark isapplied externally to treat inflammationn of lymphgland and common fever, scabies and ringworm.Paste of bark and leaf is applied externally for treatment of leucoderma[6,7]. It is used fororнamental purposes in Pakistan [6]. Bark boiled inoil of *Pongamiaglabra*and garlic is appliedexternally for the treatment of eczema[8]. Bark and leaves are astringent, bitter, antihelmintic, and areused for the treatment of diabetes, skin disease, intestinal disorder, leprosy, rheumatism andwound-healing in form of paste [9].It is an important pollenallergent plant of India [10, 11].

Phytochemical evaluation

Phytochemical examinations were carried out for all the extracts as per the standard methods[4].

Phytochemical evaluation

Phytochemical examinations were carried out for all the extracts as per the standard methods.

1) **Detection of alkaloids*:*** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.

b) Wagner's test

Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's test

Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

2) Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test

Filtrates were treated with 2 drops of alcoholic á-naphthol solution in a test tube and 2 ml of Conc. Sulphuric acid was added carefully along the sides of the test tube. Violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's test

Filtrates were treated with Benedict's reagent and heated on water bath. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3) Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) ModifiedBorntrager's Test:

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycoside

b) Legal's test

Extracts were treated with sodium nitropruside in pyridine and methanolic alkali. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4) Detection of saponins

a) Froth Test:

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam test:

Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5) Detection of phytosterols

a) Salkowski's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b) LibermannBurchard's test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

6) Detection of phenols

Ferric Chloride Test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7) Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

8) Detection of flavanoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9) Detection of proteins and aminoacids:

Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presence of proteins.

a)Ninhydrin test:

To the extract, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10) Detection of diterpenes

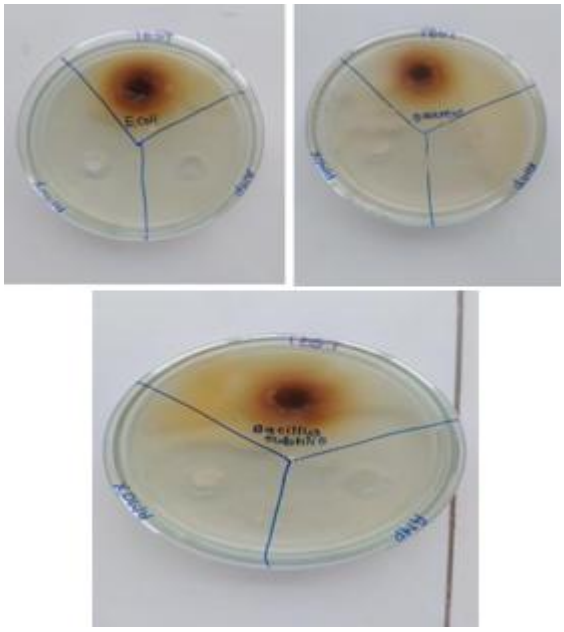
Copper acetate Test: Extracts were dissolved in water and treated with few drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

3. Materials and Methods [12]

Bacteria used	Ampicilline	Amoxicilline	Test drug
E.coil	9	7	17
Bacillus subtilus	10	8	12
Staph aureus	8	7	13

The antimicrobial assay was performed by agar well diffusion method .The disc was saturated with 100 µl of thetest compound, allowed to dry. For agarwell diffusion method, a well of 8 mm diameter was prepared in the plate. 100 µlof the extract was then introduced into the well. The plate was incubated at 37oC for 24 hrs. and then observed for presence of zone of inhibition

Zone of inhibition



4. Result and Discussion

The result of zone of inhibition which is the parameter observed for antimicrobial activity leaves extract of *Holoptellia intigrifolia* is as shown in table:

The our test drug is shows more antimicrobial activity than standard drug. .furhter study is required to explore that how much it is potent and efficacious as compared to a standard drugs antimicrobial agent like ampicilline,amoxicilline.

5. Conclusion

The results of our experiments showed that different bacterial species exhibited different sensitives towards the leaves extract of holoptellia intigrifolia, which have been a treat to human health it may be concluded from the present studies.

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