

Ayurvedic preparation from *Azadirachta indica*, *Terminalia chebula*, *Hemigraphis colorata* extracts and Its Antimicrobial investigation

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Abstract: Medicinal plants with proven wound healing capacity and antimicrobial contents are analysed based on previous studies. Finally *Terminalia chebula* extract, *Azadirachta indica* seed oil, *Hemigraphis colorata* extracts were selected for the preparation of Ayurvedic formulation. Different concentrations were tried on a trial and error basis and the content ratio was finalised. Formulation was prepared with utmost care under expert supervision. The physical and chemical properties of wound healing cream were investigated, High power thin layer chromatography investigations were done to separate the chemical contents. Inhibition zone diameters were measured to study antimicrobial activity against pathogenic bacteria like *Eshcherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* etc.

Keywords: *Azadirachta indica*, Antibacterial, Ayurvedic preparation, *Hemigraphis colorata*, *Terminalia chebula*.

I. INTRODUCTION

We use plants for food, clothing, shelter, flavours and fragrance etc. But more than that medicinal systems like Ayurveda, Unani and Chinese medicines which mainly make use of plant based products provide the solutions for the threatening diseases of present world [1]. Plants have been the basis of many traditional systems throughout the world for thousands of years and continue to provide mankind with new remedies. The use of plants as medicines has evolved to the isolation of active compounds[2]. Isolation of morphine from opium in the early 19th century subsequently led to the isolation of early drugs such as cocaine, codeine, digitoxin and quinine of which some are still in use[3]. Scientific community is always in search of classes of molecules and secondary metabolites that have led to the development of these pharmacologically active extracts[4]. The efficacy and quality of phyto-medications were not proven in olden days but things have changed with the advancement of new tools of characterization techniques. A single plant contains a large number of bioactive compounds, indicating their potential as a source of new drugs[5]. Plant derived antioxidants are a subject of great interest among manufactures, food scientists, and consumers. Many spices and culinary herbs provide phenolic compounds which shows excellent antioxidant capacity in fruits, cereals, and nuts[6].

The concept of developing drugs from plants used in indigenous medical system is much older, while in some cases the direct links between a local and biomedical use exists, in other cases the relation ship is much more complex. Action of pathogenic bacteria on wounds is a major concern for patients and clinicians alike[7]. Chronic wounds affect a large number of patients and seriously affect their quality of life. All over the world around 6 million people suffer from wounds of one type or other. The reactive Oxygen species (ROS) are harmful to wound healing due to their effects on cells and tissues[8]. Absorbable synthetic biomaterials are considered to be degraded through ROS (Aliyeva et al., 2004). Free radical scavenging enzymes (FRSE) plays an essential role in the reduction, de-activation and removal of ROS as well as regulating wound healing process[9]. Another acute response, inflammation results in a coordinated influx of neutrophils at the wound site. The cells through their characteristic 'respiratory burst' activity produce a lot of free radicals[10]. Other than this wound related non-phagocytic cells also produce free radicals by involving non-phagocytic oxidase mechanism (Griendling, 2000). Therefore the wound site rich in both Oxygen and Nitrogen centered reactive species along with their derivatives. These radicals produces large oxidative stress leading to lipid peroxidation, DNA leakage, and enzyme inactivation including free radicals scavenger enzymes. Pathogenesis of many diseases suggests that anti oxidants may be of therapeutic use in these conditions. Therefore compounds with free radical scavenging properties show improved wound healing and protection of cells from oxidative damage[11].

Balick and Cox (1996) reported that only 1-3 % of drugs listed in Western pharmacopia are intended for use in the skin and for wounds. But for herbal remedies are concerned at least one third of it is intended for

use in skin and on wounds. Research on wound healing agents is one of the developing areas in modern biomedical research[12]. Traditional practitioners across the world particularly in countries like India and China with age-old traditional practices have valuable information of many lesser known hitherto unknown wild plants used by the traditional healers for treating wounds and burns[13]. Several drugs of plant, mineral, animal origin are described in the traditional texts of Indian systems of medicine like Ayurveda for their healing properties under the term 'Vranaropaka'. Other than classical system of Indian medicine the tribal and folk medicine also employs many plants for the treatments of cuts, burns and wounds. *Azadirachta indica*, *Terminalia chebula*, *Hemigraphis colorata* are the botanical names of three such plants which are extensively studied by many researchers in the past decade and their wound healing capability is proved beyond doubts. This work explains the preparation of an Ayurvedic cream working against pathogenic bacteria active on wound sites, from the above mentioned herbal extracts and their physicochemical and bactericidal studies.

II. MATERIALS AND METHOD

2.1 Hemigraphis colorata: *Hemigraphis colorata* is a tropical perennial herb chiefly grown as an ornamental indoor and outdoor plant because of its attractive and vivid foliage. In folk medicines the leaves are ground in to paste and applied on fresh cuts and wounds to promote wound healing and used to treat anaemia. This is a prostrate herb with rooting branches, opposite broad cordate and toothed leaves and terminal heads of small white flowers[14]. The leaves are 6 to 10 cm long and shimmering silvery violet underneath red purple. This plant is adapted to India and is a native of Java. *H. colorata* leaf paste when applied on the wound site promotes the wound healing in mice but oral administration was ineffective. In mice, the leaf paste provides faster wound contraction and epithelialisation. The excision and incision wound model studies revealed that methanolic extract is comparable to standard reference Vokadine[15]. Subramoniam et al., have first reported the wound healing properties of the leaf of *Hemigraphis colorata* and they have suggested that this plant leaf extract can be employed in wound healing in folklore medicines. The plant is known by several names such as Aluminium plant, Cementary plant, Metal leaf, Red flame Ivy, Waffle plant, Java Ivy etc. In south India the plant is known as Murikooti or Murianpacha due to its incredible potency to heal wounds. The leaves of *Hemigraphis colorata* found to contain the constituents such as saponin, flavanoids, terpenoid, total phenols etc all having excellent pharmacological activity.

To prepare the formulation, fresh plant leaves thoroughly cleaned in water and ground in to a paste and the leaf juice is extracted. 100 gm leaf juice is used for the preparation of Ayurvedic formulation. The water content of the leaf is estimated around 80 %.

2.2 Azadirachta indica: *Neem* (*Azadirachta indica*) is a widely prevalent and esteemed wonder tree, mainly cultivated in Indian subcontinent. Every part of this tree is traditionally used as household remedy for many diseases. The importance of this medicinal plant is more evident from the report of US National Academy of science publication in 1992 with a title called "Neem-a tree for solving global problem"[16]. It is established in many scientific studies that neem seeds contain chemical compounds to control more than 100 species of insects and microorganism[17]. Three different forms of neem products such as aqueous extract, seed oil, seed powder have been used extensively against microorganisms. The excellent bioactivity of this neem products are attributed to the chemical compounds such as Nimbin, Nimbidin, and Salanin[18]. But the most important of the above compounds is the triterpenoid, *Azadirachtin*. *Neem* leaf contains several valuable components such as isoprenoids that include terpenoids containing limonoids, *azadirone* and its derivatives etc.[19]. Among nonisoprenoids aminoacids, polysaccharides, polyphenolics like flavanoids. These compounds possess insecticidal, ovicidal, antifeedant and growth inhibiting effects against micro organisms. *Neem* leaves are reported as the main sources of the active compounds obtainable from the plant. The fruits and seeds appear to be more important[20]. *Neem* seed consists of 30-40% of oil and it is extracted by means of organic solvents such as acetone, ethanol, methanol, and petroleum ether. Traditionally the oil is extracted by kneading and wetting with hot water until the oil in the dough-like material begins to ooze out. For the preparation of Ayurvedic cream 20 mL of neem seed oil extracted from the selected seeds of neem is used.

2.3 Terminalia chebula: The compounds present in the *Terminalia chebula* were shown to have anti-cancer, antimicrobial, anti-inflammatory, antimutagenic, antifungal, antiviral, antidiabetic and antianaphylactic activities[21]. It is a strong antioxidant and can be used in the treatment of neurodegenerative diseases by inhibiting the production of reactive Oxygen species (ROS). Fruits largely used in the treatment of several diseases of mouth such as dental caries, spongy and bleeding gums, gingivitis, and stomatitis[22]. In Ayurveda and *Sidha* this plant is extensively utilised for constipation dyspepsia, hemorrhoids, parasites, intermittent fever, candidiasis, parasites, urinary discharges, rheumatism, epilepsy, arthritis, cardiovascular diseases, memory loss, depression, anorexia and wounds[23].

For the preparation of wound healing cream the drug is obtained from local market and identified by experts and quality is ensured. 100 gm powdered drug is taken and it is mixed with 200 ml water, after boiling around 30 minutes concentrated and dried in to 15 gm solids.

2.4 Ayurvedic Formulation

A unique combination proven to be effective for healing wounds of different types with bactericidal activity is formulated on trial and error basis. The Ayurvedic cream is prepared with *Hemigraphis colorata*, *Terminalia chebula*, *Azadirachta indica seed oil* and *Boric acid*.

Composition

1. Hemigraphis colorata water extract (20 g from 100 g Leaf juice)
100g leaf juice concentrated and dried to 20 gm solids.
2. Terminaliachebula(15 g from 100 g powdered drug)
100g powdered drug is mixed with 200ml water, boiled for 30 minutes and concentrated and dried to 15 gm solids.
3. Boric acid 2 g
4. 20 mL Neem seed oil
5. 43 gram Vaseline (Petroleum jelly)

All the ingredients are mixed well in the form a thick paste using UZDN-A ultrasonic dispersor in the following mode, 0.5 A, 44 Khz and 3 cycles for 25-30 seconds with 1 min breaks for cooling. The mixer is again stirred with ER-10 (MLW) mixer making sure that no air bubbles accumulated in the resulting ointment.

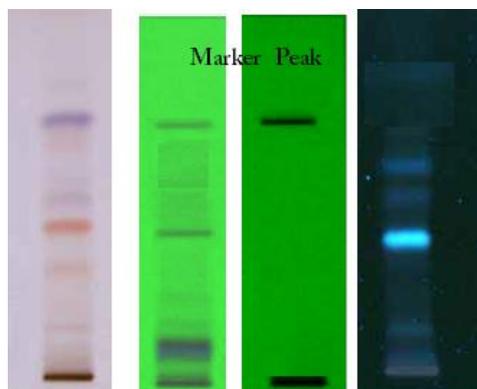
III. RESULTS AND DISCUSSION

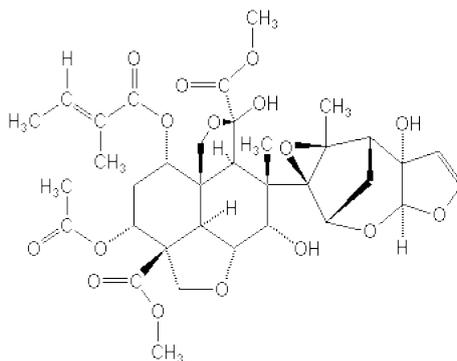
3.1 Physicochemical study

The Ayurvedic formulation is prepared as explained above and subjected for its various physical and chemical studies and the obtained results are tabulated as follows.

Colour	-	Pale brown
LOD	-	NMT 0.5 %w/w
Fixed oil content	-	NLT – 65 %w/w
Unsaponifiablematter	-	NMT – 35 %w/w
Melting Point	-	35-40 deg. C
Congeaing point	-	28 – 25 deg. C
Boric acid	-	0.5-0.8%w/w
Assay of Azadirachtin A	-	NLT 0.08 %w/w

High Performance Thin Layer Chromotography:





The chemical structure of the marker Azadirachtin A

3.2 Antibacterial Study

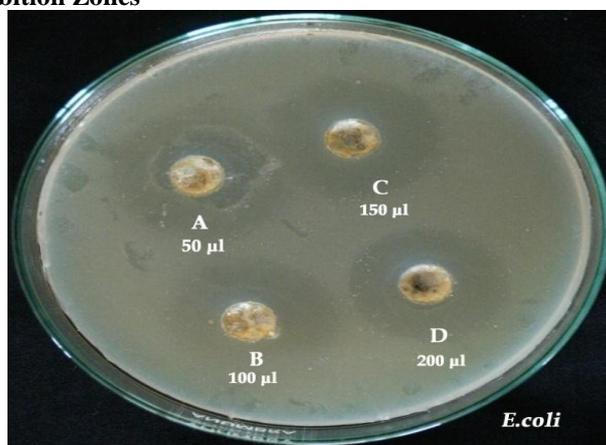
The antibacterial activity of the Ayurvedic preparation is studied by Agar well diffusion method. The minimum inhibitory concentration (MIC) was determined by broth micro dilution method. The MIC was recorded as 1 mg/mL. Bactericidal activity against both Gram positive and Gram negative strains were investigated. Standard referal bacterial strains of *Eshcherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853 (Both Gram negative) and *Staphylococcus aureus* ATCC 25923 (Gram positive) were obtained from IMTECH, Chandigarh, India.

About 25mL of Mueller Hinton Agar (MHA) were poured in to sterile petri dish and allowed to solidify. The bacteria in Mueller-Hinton broth at 37°C for 18 hours and then stocked at 4 °C in Agar. Sub cultures were prepared from the stock for bioassay. The bacterial culture was inoculated in to sterile nutrient broth and incubated at 37°C for 3h untill the required turbidity is achieved. The final inoculum size was standardized to 10⁶ CFU/mL with the help of SPC and Nephlo-tubidometer. Inoculum was spread over the nutrient agar plate using sterile cotton swab in order to obtain uniform microbial growth. In each plate four wells were punched and 50µL, 100µL, 150µL, 200µL of prepared Ayurvedic formulation was separately added and allowed to diffuse at room temperature. The plates were incubated for 24 hours at 37°C and the average diameter of clear zone of inhibition is recorded. This results were compared with that of standard Antibiotic Cefixime hydrochloride. All the experiments were done in duplicate and the average diameter of inhibition zones were recorded.

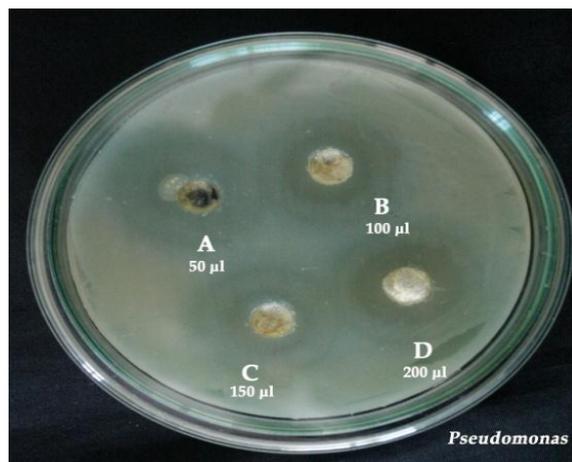
Media details

For *E. coli* - 3.2% peptone, 2% yeast extract, and 0.5% NaCl
For *Pseudomonas aeruginosa* - Enzymatic Digest of Gelatin 20 g, Magnesium Chloride. 1.4 g, Potassium Sulphate 10 g, Irgasan 0.025 g, Agar 13.6 g, Glycerol 20 mL.

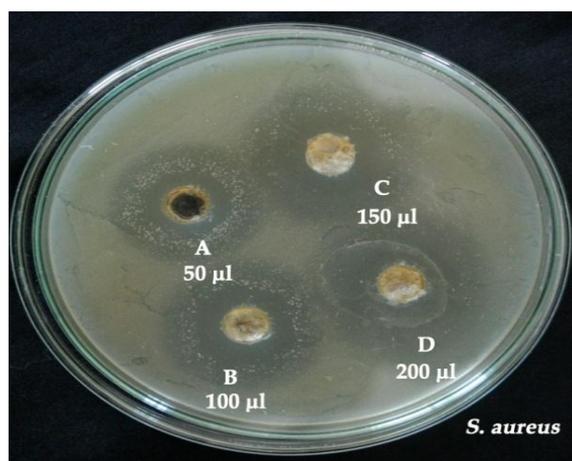
Invitro Antibacterial Inhibition Zones



Antibacterial study against *E. coli* bacteria.



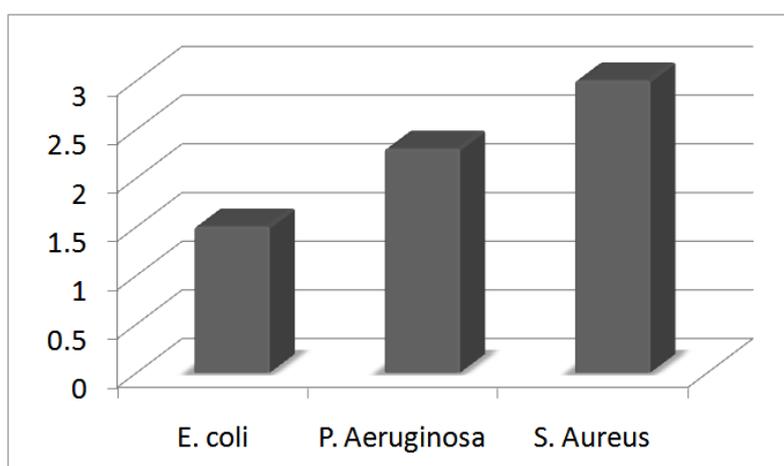
Antibacterial study against *Pseudomonas aeruginosa* bacteria



Antibacterial study against *Staphylococcus aureus* bacteria

The results of inhibition zone diameter study can be summarised and tabulated as shown below

Compound	Max. Zone of inhibition in mm		
	Gram positive	Gram negative	
	S.aureus	E.coli	P. aeruginosa
Ayurvedic formulation	3.0	1.5	2.3
Cefixime Hydrochloride	3.2	5	3.2



IV. SUMMARY

The prepared cream showing antibactericidal property against pathogenic bacteria like *S.aureus*, *E.coli*, *P.aeruginosa* is suggested for use in the following diseased conditions.

- For reddened skin due irritation and insect bites
- For cuts and burns
- For exposed epithelial tissue damages
- For inflammatory Gangrene
- For ring worm infections.

The final conclusion is that Ayurvedic herbal preparations containing extracts of *Azadirachta indica*, *Terminalia chebula*, and *Hemigraphis colorata* have great potentials as antimicrobial agents against bacterial pathogens like *E.coli*, *S.aureus*, and *P.aeruginosa*. The antibacterial effect may be attributed to the phytoconstituents present in the cream. This may be either due to individual effect of the ingredients or can also be due to the additive effect of the herbal extracts. Also the histogram clearly shows that anti microbial action of prepared Ayurvedic formulation is more effective against Gram positive bacteria than that against Gram negative bacteria. The results justify the use of these herbal extracts being used in many Ayurvedic formulations against microbial related diseases since Vedic period. Further studies will be focussed on the effect of controlled addition of nanocarbon material (prepared by the pyrolysis of edible oils) on the antimicrobial activity of Ayurvedic formulation.

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