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Review on antibacterial potency of combination of effective extract of *Tridax procumbens*, *Aegle marmelos*, and *Acalypha indica*

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Abstract

Chlorhexidine commonly known by its salt forms chlorhexidine gluconate or chlorhexidine acetate is a disinfectant and antiseptic which are available in many dosage forms. In recent times the emerging resistance towards the chlorhexidine and other antibiotics such as, clindamycin, gentamycin, bacitracin, polymyxin and so on. So, in this review we have assessed the methods for detection of reduced susceptibility to the following substance and the prevalence of coresistance to other antimicrobial agents. So, we selected some of the traditional way of antiseptics such as *Tridax procumbens*, *Acalypha indica*, and *Aegle marmelos*. The reason for choosing these species are found to have good antibacterial activities especially against *E. coli*, *B. subtilis*, *S. aureus*, and other streptococcus species. The work has been performed to check the antimicrobial activities of the extracted active components and then combinations of the above mentioned resistant developed antibiotic and antiseptics with the extracted active principles of the traditional antimicrobial agents and to observe the synergism and to choose the most potential extract and potential combinations among those.

Keywords: chlorhexidine gluconate, clindamycin, gentamycin, bacitracin, polymyxin *Acalypha indica*

Introduction

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. In this review, we focused on the use of antimicrobial testing methods for the in vitro investigation of extracts and pure drugs as potential antimicrobial agents.

After the revolution in the “golden era”, when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance [1]. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health.

For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today. The fact that a plant extract exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study.

Introduction to Materials

Tridax Procumbens

Tridax procumbens (Jayanthi, Coat buttons) Belongs to family – Asteraceae, The plant bears daisy like yellow-centred white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrow head- shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. Calyx is represented by scales or reduced to pappus. The plant is invasive in part because it produces so many of these achenes, up to 1500 per plant, and each achene can catch the wind in its pappus and be carried some distance. This weed can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semitropical climates.

Traditionally, *Tridax procumbens* has been in use in India for wound healing and as an anticoagulant, antifungal, and insect repellent.

The juice extracted from the leaves is directly applied on wounds. Its leaf extracts were used for infectious skin diseases in folk medicines

The chemical constituents present are alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. Mineral composition present in leaves is calcium, magnesium, potassium, sodium and selenium. Leaf mainly contains crude proteins 26%, crude fiber 17% soluble carbohydrates 39% calcium oxide 5%, Luteolin, glucoluteolin, quercetin and isoquercetin. Whereas the oleanolic acid, fumeric acid, fl-sitosterol and tannin is present in good amounts.

Acalypha indica ^[2]

Kingdom: Plantae

Class: Magnoliopsida

Order: Euphorbiales

Family: Euphorbiaceae

Subfamily: Acalyphoideae

Genus: *Acalypha*

Species: *indica* Linn.

Acalypha indica Linn. Is an annual erect herb 30-75 cm in height. Branches are numerous, long, ascending, finely pubescent. Leaves 2.5-7.5 by 2-4.5 cm, ovulate or rhombic ovulate, acute, or sub obtuse crenate serrate, glabrous thin, base cuneate somewhat 3 nerves, petiolate usually longer than the blade, slender, stipulate minute. Flower in numerous lax erect, elongated, auxiliary spikes, and cluster near the summit of the spikes, the females scattered, surrounded by a shortly pedunculate large leafy dentate cuneiform many nerves bract 6-8mm diameter. Ovary hispid, capsule small, quite concealed by the bract. Often only 1 seeded seed ovoid, smooth, pale brown, 1-2mm long.

Aegle marmelos

Kingdom: Plantae

Order: Sapindales

Family: Rutaceae

Sub family: Aurantioideae

Genus: *Aegle*

Species: *A. marmelos*

A. Marmelos is a native plant of India. *A. marmelos* belongs to Rutaceae family and commonly known as wood apple. In India, *A. marmelos* is grown as a temple garden plant and the leaves are used to pray Lord Shiva. *A. marmelos* is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. Traditionally, *A. marmelos* is used in the treatment of diarrhoea and dysentery. Leaves of this plant used to cause infertility/abortion in women ^[3]. Recently, the plant is screened for its medicinal properties by scientific techniques and reported for various medicinal properties. *A. marmelos* is extensively described in the Vedic literature for the treatment of various diseases. *A. marmelos* is traditionally used to treat jaundice, constipation, chronic diarrhoea, dysentery, stomach ache, stomachic, fever, asthma, inflammations, febrile delirium, acute bronchitis, snakebite, abdominal discomfort, acidity, burning sensation, epilepsy, indigestion, leprosy, myalgia, smallpox, spermatorrhoea, leucoderma, eye disorders, ulcers, mental illnesses, nausea, sores, swelling, thirst, thyroid disorders, tumours, ulcers and upper respiratory tract infections Different organic extracts of the leaves of *A. marmelos* have been reported to possess alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids ^[18, 19]. *Aegle marmelos* fruit pulp reported for the availability of steroids, terpenoids, flavonoids, phenolic

compounds, lignin, fat and oil, inulin, proteins, carbohydrates, alkaloids, cardiac glycosides and flavonoids.

Traditional Uses ^[4, 7]

Leaves possess laxative properties “are used as a substitute for senega”, are used in the form of powder or decoction. Mixed with garlic they are used as Anthelmintic in worms and their juice mixed with oil used to treat rheumatic arthritis.

Expressed juice of the leaves is a safe, and used as emetic for children, in smaller doses it act as expectorant, and is useful in chronic bronchitis, asthma and consumption.

The decoction is employed in ear-ache and a cataplasm of the bruised leaves is applied to syphilitic ulcers, also to relieve the pain of snakebites. Extract of fresh leaves may be employed in scabies and other skin diseases, along with lime and onion. It is a good stimulating application in rheumatism. Powder of dry leaves is used in bed sores. In congestive headache a piece of cotton saturated with the expressed juice of the plant or leaves and inserted into each nostril is said to relieve the pain. In cases of obstinate constipation of children, the leaves ground into a paste and made into a ball and introduced into the rectum, relax the sphincter and produces free motions. An infusion of the root or the root bruised in water, acts as a cathartic

The root and leaves of *Acalypha indica* Linn. Was qualitatively assayed for the presence of Anthraquinones, Alkaloids, Catechol's, Flavonoids, Phenolic compounds, Saponins, Steroids, Tannins, Triterpenoids. The result concluded the presence of Alkaloids, Catachols, Flavonoids, Phenolic compounds, Saponins, Steroids. Tannins, terpenoids and anthraquinone were not found in the root and the leaves extract

Anti-bacterial potency ^[8, 10]

Methanol ethyl acetate and hexane extract of *Acalypha indica* in concentration 50mg/ml 100mg/ml 200mg/ml against *E. coli*, *bacillus subtilis*, *s. aureus*, *s. pyogenes*, *Pseudomonas aeruginosa* and *candida auris*.

AB1 and AB2 *Aegle marmelos* oil against *E. coli*, *bacillus subtilis*, *S. aureus*, *S. pyogenes* and *candida auris* (oil is used because of high potency)

Methanol ethyl acetate and hexane extract of *tridax procumbens* in concentration 50mg/ml 100mg/ml 200mg/ml against *E. coli*, *bacillus subtilis*, *s. aureus*, *s. pyogenes* and *candida auris*.

And finally checking the combination of effective extract of above species based on polarity against the *E. coli*, *Bacillus subtilis*, *s. aureus*, *s. pyogenes* and *candida auris*.

Control for *S. aureus*, *S. pyogenes*, *Pseudomonas aeruginosa*, *E. coli* is neomycin

Control for *candida auris* is ketoconazole

Importance of Research

Emerging resistance of bacteria is increasing nowadays and we are trying to study the antibacterial potency of the natural products. Apart from the antibacterial potency *tridax* promises to show anti-inflammatory property and increased healing rates of wound so which is more desire to use as commercial product or combine with commercial product to increase the effectiveness in treating skin infection *candida auris* is most severe fungal pathogens which is resistant to all three-antifungal class of drugs so we are trying to find potential solution for that. With obtained result we will formulate the potential topical antiseptic agent with better anti inflammatory and wound healing properties.

Extraction of plant Material

The dried leaves of *Tridax procumbens* will be grounded to coarse powder. 500 mg of powder will extract with solvents like methanol, ethyl acetate and hexane extract. The extraction process carries out using Soxhlet apparatus

Acalypha indica

The fresh parts of *Acalypha indica* were collected from the local field of Perambur and Guduvancherry. The materials were washed with distilled water and air dried in the shade for two weeks.

Extraction of plant material

The dried leaves will be grounded to coarse powder. 500 mg of powder will extract with solvents like methanol, ethyl acetate and hexane extract. The extraction process carries out using Soxhlet apparatus.

Aegle marmelos

The fresh parts of *Aegle marmelos* were collected from the various places of Chennai. The materials were washed with distilled water and air dried in the shade for two weeks.

Extraction of oil from leaves

The leaf samples of *Aegle marmelos* (L.) (villa) collected were first washed with tap water followed by distilled water and then dried under shade. As 750ml of double distilled water was used with 250 gm of dried plant material for extraction process through Clevenger apparatus. The oil and hydrosol obtained were collected in different bottle's and were stored for further use.

Antimicrobial activity

Method: Agar diffusion method Species

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive round-shaped bacterium, a member of the Firmicutes, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although *S. aureus* usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. An estimated 20% to 30% of the human population are long-term carriers of *S. aureus* which can be found as part of the normal skin flora, in the nostrils, and as a normal inhabitant of the lower reproductive tract of women. *S. aureus* can cause a range of illnesses, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery.

Streptococcus pyogenes

Streptococcus is a genus of gram-positive coccus (plural cocci) or spherical bacteria that belongs to the family Streptococcaceae, within the order Lactobacillales (lactic acid bacteria), in the phylum Firmicutes. Cell division in streptococci occurs along a single axis, so as they grow, they

tend to form pairs or chains that may appear bent or twisted. This differs from staphylococci, which divide along multiple axes, thereby generating irregular, grape-like clusters of cells. Most streptococci are oxidase-negative and catalase-negative, and many are facultative anaerobes.

Escherichia coli

Escherichia coli also known as *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K₂, (which helps blood to clot) and preventing colonisation of the intestine with pathogenic bacteria, having a mutualistic relationship. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards

Bacillus subtilis

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. As a member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative anaerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, strict aerobic (although can grow anaerobically in the presence of nitrate), rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses—hospital-acquired infection such as ventilator-associated pneumonia and various sepsis syndrome.

The organism is considered as serious infection often occurs during existing diseases or conditions most notably cystic fibrosis and traumatic burns. It generally affects the immuno compromised but can also infect the immuno competent as in hot tub folliculitis. Treatment of *P. aeruginosa* infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result

Candida auris

Candida auris is a species of fungus that grows as yeast. It is one of the few species of the genus *Candida* which cause candidiasis in humans. Often, candidiasis is acquired in hospitals by patients with weakened immune systems. *C. auris* can cause invasive candidiasis (fungemia) in which the bloodstream, the central nervous system, and

internal organs are infected. It has attracted widespread attention because of its multiple drug resistance. Treatment is also complicated because it is easily misidentified as other *Candida* species.

C. auris was first described in 2009 after it was isolated from the ear canal of a 70-year-old Japanese woman at the Tokyo Metropolitan Geriatric Hospital in Japan. In 2011, South Korea saw its first cases of disease-causing *C. auris*. Reportedly, this spread across Asia and Europe, and first appeared in the U.S. in 2013. DNA analysis of four distinct but drug-resistant strains of *Candida auris* indicate an evolutionary divergence taking place at least 4,000 years ago, with a common leap among the four varieties into drug-resistance possibly linked to widespread azole-type antifungal use in agriculture.

Formulation of creams and ointments

The extraction process was formulated as creams and ointment by different concentration 1%, 5%, and 10%. Selection of oleaginous base for the formulation is based on the preliminary studies which shows the best formulation is compared to the emulsion water in oil (w/o) type, the ointment and cream bases in different concentration 1%, 5% and 10% were selected as the final base for the preparation of ointment.

Hypothesis

In traditional medicine *Acalypha indica* (Kuppameni) and *Tridax procumbens* (thatha thalavetti) applied directly in to skin for various wounds and skin infection, from this we conclude the *Acalypha indica* and *tridax procumbens* thought to have good skin permeability.

Conclusion

The methanol, ethyl acetate and hexane extract of *tridax procumbens* and *Acalypha indica* and oil of *Aegle marmelos* studied for antibacterial activity against the bacteria causing infections and increased incidence of resistance and resistant fungus *Candida auris* as alone and combination of their effective extract. Based on results extracts are formulated in to ointment with suitable base for skin infections.

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