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HERBAL DISINFECTANTS: A REVIEW

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ABSTRACT

Plants are rich in a wide variety of secondary metabolites, such as phenolic compounds, tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Search is on for phytochemicals and "leads" which could be developed into antimicrobials. The present paper is a survey in the field of herbal disinfectants, their formulations and evaluation.

Key words: phenolic compounds, tannins, terpenoids, alkaloids, and flavonoids.

INTRODUCTION

Infections are increasing alarmingly and have emerged as a critical issue in hospital care outcome. Opportunistic microorganisms primarily cause nosocomial infections; and multidrug-resistant pathogens are commonly involved in infections and are difficult to treat[1]. The transfer of bacteria from the hands to food, objects, or people plays an important role in the spread of diseases[2]. The hands of health care workers are the primary mode of transmission of these multidrug-resistant pathogens and infections to patients. Through hand hygiene a simple and least expensive means of preventing health care-associated infections specially derived from environmental surfaces can be achieved. Hence the use of safe antiseptics for hand wash purposes is necessary. Many of chemical antiseptics available in the market are alcohol based and have some short comings or adverse effects, their frequent use can lead to skin irritation and also resistance among pathogens[3]. Number of herb based products are being formulated and introduced into the market to overcome the problems. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are searching for phytochemicals and "leads" which could be developed into antimicrobials. Many plant extracts have been screened for

their antimicrobial potential as it is very likely that these phytochemicals will find their way into arsenal of antimicrobials which may be used as disinfectants, antiseptics, dentifrices and chemotherapeutic agents[4].

Organisms of concern

Skin being the most exposed part of the body requires protection from skin pathogens. Normal human skin harbours bacteria (between 10^2 and 10^6 CFU/cm). Traditionally, microorganisms residing on the hands are divided into resident and transient flora. Resident flora (e.g. *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus viridans*) colonizing deeper skin layers are more resistant to mechanical removal has lower pathogenic potential. Transient flora (e.g. *Staphylococcus aureus*, Gram-negative bacilli, Candida species) colonizes the superficial skin layers for short periods, is usually acquired by contact with a patient or contaminated environment and these microorganisms are easily removed by mechanical means such as hand washing and are responsible for most health care-associated infections and the spread of antimicrobial resistance[3].

Strains of *S. aureus* and *Streptococcus pyogenes* are the causative agents of the painful and unsightly skin condition like impetigo. The incidence of nosocomial infections is alarmingly increasing & has emerged as a critical issue in hospital care outcome, thus resulting in extended hospitalization, substantial morbidity, & mortality. Opportunistic microorganisms which cause primary nosocomial infections are Enterococcus species, *Pseudomonas species*, & *Staphylococcus aureus*. Opportunistic fungal infections have become very important especially in HIV patients and the most common infections are Candidiasis, Aspergillosis, and Cryptococcosis[1].

Antimicrobial drug resistance is a burgeoning global concern today as resistant microorganisms have emerged and spread throughout the world. Multidrug infectious diseases of bacterial and fungal origin are leading killers and account for approximately 25% of global deaths. Human pathogens like *Staphylococcus aureus* (Methicillin & Multiantibiotic resistant), *Escherichia coli*, *Pesudomonas aeruginosa*, *Candida albicans* and *Cryptococcus neoformans* are evolving themselves as multidrug resistant “Superbugs” at alarming rate. This demands for exploration of new chemical sources from biodiversity and develop therapeutic regimes to combat infectious superbugs[5].

Organisms like *Campylobacter* and *Legionella* often present in fresh water and soil and are usually transmitted in contaminated food or water. *Campylobacter* infections (or campylobacteriosis), cause diarrhea, fever, and cramps. Legionnaires disease, which most often manifests as severe pneumonia accompanied by multisystemic disease, and Pontiac fever, which is an acute, febrile, self-limited, viral-like illness. Hospitals are facing the dilemma of Legionella outbreaks as they discover the drinking water as the source of infection[6, 7].

Herbs with disinfectant properties

Plants are known to possess various secondary metabolites with profound antimicrobial properties hence they have been used extensively in traditional medicines since years. Some of the plants have proven activity on skin disinfection. Their processed extracts are generally applied to disinfect skin, hands and external wounds. The most commonly used herbs with disinfectant properties are *Azdirachta indica, Eucalyptus robusta, Aloe barbadensis, Aloe vera, Withania somniferum, Andrographis paniculata, Aegle marmelos, Berberis vulgaris, Cinnamomum verum, Piper nigrum, Rhamnus purshiana, Capsicum annuum, Syzygium aromaticum, Eucalyptus globulus, Gaultheria procumbens, Cassia angustifolia, Cassia fistula, Mentha piperita* etc. These plants with proven disinfectant activity are also referred to as plant antiseptics[8]. Some of the plant antiseptics with their active constituents are enlisted in Table No: 1.

Table no: 1. Active compounds of some herbs with disinfectant properties.

Plant	**Active compounds**
Azdirachta indica	Triterpenes, Azadirachtin.
Anethum gravveolens	Essentialoils, Phellandrene, limonene, anithofuran.
Anthemis Nobilis	Terpenoids, Flavonoids, Coumarins.
Andrographis paniculata	Andrographolides, Arabinigalactan proteins.
Aegle marmelos	Essential oil, Terpenoids.
Arctium lappa	Polyacetylene, Tannins, Terpenoids.
Allium sativum	Allicin, Ajoene, Sulfoxide sulfated Terpenoids.
Allium cepa	Allicin.
Artemisia dracunculus	Caffeic acids, Tannins, Terpenoids.
Berberis vulgaris	Berberine Alkaloid.

Cassia fistula	Anthraquinones, Fistulic acid.
Cinnamomum verum	Essential oils, Terpenoids, tannins.
Capsicum annuum	Capsaicin, Terpenoids.
Cassia angustifolia	Rhein, Anthraquinones.
Curcuma zedoaria	Curcuminoids, Demethoxycurcumin, Terpenes.
Carum carvi	Coumarins.
Centella asiatica	Terpenoids, Asiaticoside.
Camellia sinensis	Flavonoids, Catechin.
Citrus paradise	Terpenoids.
Eucalyptus globulus	Tannins, Polyphenols, Terpenoids.
Ficus religiosa	Tannins, Saponins, Flavonoids, Terpenoids.
Gaultheria procumbens	Tannins, Polyphenols.
Glycyrrhiza glabra	Glabrol, Phenolic alcohol.
Garcinia mangostana	Xanthone derivatives, Mangostins.
Galium odoratum	Coumarins.
Hibiscus sabdariffa	Flavonoids, Polyphenols.
Hydrastis Canadensis	Alkaloids, Berberine, Hydrastine.
Hypericum perforatum	Anthraquinones, Hyperecin.
Lawsonia inermis	Phenols, Gallic acid.
Matricaria chamomilla	Phenolic acid, Anthemic acid.
Matricaria recutita	Terpenoids, Flavonoids, Coumarins.
Mentha piperita	Terpenoids, Menthol.
Nelumbo nucifera	Quercetin, Myricetin, Kaempferol, Luteolin.
Ocimum basilicum	Terpenoids, Essential oils.
Olea europaea	Aldehyde Hexanal.
Panax notoginseng	Saponins.
Piper nigrum	Piperine Alkaloid.
Piper betel	Catechols, Eugnol, Essential oils.
Punica granatum	Organic acids, Phenolic compounds.
Quercus rubra	Tannins, Polyphenols.
Rhamnus purshiana	Tannins Polyphenols, Anthraquinones.

Rosmarinus officinalis	Essential oils, Terpenoids.
Salix alba	Salicin, Tannins, Phenolic glucosides.
Syzygium aromaticum	Eugenol, Terpenoids.
Thymus vulgaris	Caffeic acid, Terpenoid Thymol, Phenolic alcohol, Tannins, Polyphenols, Flavones.
Valeriana officinalis	Essential oils, Terpenoids.
Withania somniferum	Lactone, Withafarin A.

Antimicrobial properties of secondary metabolites

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives of which many have been isolated, a number estimated to be less than 10% of the total [9].

Simple phenols and phenolic acids are the simplest bioactive phytochemicals consisting of a single substituted phenolic ring. Plants containing these compounds are effective against viruses, bacteria, and fungi [10-12].

Flavones, flavonoids, and flavonols are phenolic structures containing one carbonyl group. They are known to be synthesized by plants in response to microbial infection. These compounds have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms[4].

Tannins are polymeric phenolic substances having antimicrobial potential by direct inactivation of microorganisms[13].Coumarins are phenolic substances made of fused benzene and pyrone rings. Several coumarins have been investigated for their antimicrobial properties, coumarins have been found in vitro to inhibit *Candida albicans* and several gram positive bacteria[4]. Terpenoids and Essential oils are the fragrance of plants. Various studies have shown that these compounds are effective against bacteria, fungi, viruses, and protozoa[4].

Antimicrobial screening methods

Among the vast number of methodologies, the most popular methods employed for antimicrobial screening are end point and descriptive tests. End point tests like the Disc/Well diffusion and Agar/Broth dilution methods are those in which microorganisms are challenged for arbitrary period. In descriptive tests like Rideal Walker method and Kelsey-Sykes test periodic sampling is done.

End point tests

Among the vast number of methodologies, the most popular methods employed for antimicrobial screening is the Disc/Well diffusion and Agar/Broth dilution methods[14]. Essential oils are volatile and insoluble in water and hence simple methods of antimicrobial screening are inadequate. Broth dilution method using 0.02% Tween80 to emulsify the oils has been shown to be most accurate method for testing antimicrobial activity of hydrophobic and viscous essential oils[14]. A novel fungitoxicity assay for inhibition of germination associated adhesion method for screening fungi sensitivity has been developed[15]. A Microtiter plate-based assay for screening antimicrobial activity of melanoidins against *E. coli* and *S. aureus* was reported[16].

Descriptive tests

Carrier tests: In these tests, a carrier such as a silk or catgut thread or a little stick is contaminated by submersion in a liquid culture of the test organism. The carrier is then dried and is brought in contact with the disinfectant for a specified exposure time. After the exposure, it is cultured in a nutrient broth; no growth indicates activity of the disinfectant tested whereas growth indicates no activity. By multiplying the number of test concentrations of the disinfectant and the contact times, a potentially active concentration-time relationships of the disinfectant is obtained[17].

Suspension tests

In these tests, a sample of the bacterial culture is suspended into the disinfectant solution and after exposure it is verified by subculture whether this inoculum is killed or not. Suspension tests are preferred to carrier tests as the bacteria are uniformly exposed to the activity of disinfectant. There are different kinds of suspension tests such as determination of phenol coefficient by Rideal Walker method; Chick Martin test and Disinfectant kill time tests[17]. In a study Rideal Walker method was used to test the activity of disinfectants the activity was compared with phenol and the Rideal Walker coefficient was evaluated[18].

Capacity tests

In these tests the disinfectant is challenged repeatedly by successive additions of bacterial suspension until its capacity to kill has been exhausted. Each time a soiled instrument is placed into a container with disinfectant, a certain quantity of dirt and bacteria is added to the solution. The ability to retain activity in the presence of an increasing load is the capacity of the disinfectant. The best known capacity test is the Kelsey-Sykes test. Kelsey-Sykes test is a

triple challenge test, designed to determine concentrations of disinfectant that will be effective in clean and dirty conditions[17].

Test for stability and long-term effectiveness: These tests are used to measure the activity of disinfectants upon long term storage. For this disinfectant solution is prepared for two tests. One portion is inoculated immediately and tested for growth after holding for seven days at room temperature. The other portion is kept at room temperature for seven days and then inoculated with a freshly prepared suspension of test organism. It is also tested for growth seven days after inoculation. If growth is detected, a higher concentration of disinfectant must be tested by using the same method[17].

Practical tests: The practical tests under real-life conditions are performed after measuring the time-concentration relationship of the disinfectant in a quantitative suspension test. The objective is to verify whether the proposed use dilution is still adequate in the conditions under which it would be used. The best known practical tests are the surface disinfection tests and Surface Time kill Test[19].

Herbal Disinfectants

Herbs are designed in various formulations as disinfectants and evaluated for their safety, and efficacy. Various formulations of herbs such as hand washes, hand sanitizers, gels, creams, ointments, dentifrices, herbal fumigants etc, are prepared. Most of the formulated and evaluated preparations showed good antimicrobial properties and protective effect to the skin. Following are the list of different herbs which are formulated as disinfectants and studied.

Herbal Hand washes and Sanitizers

The concept of cleansing hands with antiseptic agent emerged in the 19th century when a French pharmacist demonstrated that physicians and other persons attending patients with contagious disease would benefit and prevent the spread of infections. A lot of research has been carried out on herbal hand washes and sanitizers by using different plants with disinfectant properties.

Polyherbal handwashes were prepared and tested against different skin pathogens *Putida vulgaris, Staphylococcus auerus, Bacillus subtilus S aureus, Pseudomonas aureginosa.* The formulations are reported to possess greater activity than the commercially available hand wash[20].

Herbal handwash of *Matricaria chamomilla* flower extracts was more effective in reducing the number of organisms from hands than commercial antiseptic products [21].

The sensitivity of skin pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* to a herbal handwash prepared using extracts of leaves of *Terminalia catappa, Couroupita guanensis,* and rinds of *Garcinia indica* was more than commercial antiseptic soap[22].

Herbal Hand Sanitizers

Bacteriostatic effect of many polyherbal formulations on the bacteria present on the hands surface and on the surface of inanimate objects has been reported. Herbal Hand Sanitizer containining extracts of *Coleus vettiveroides, Coriandrum sativum, Citrus limon, Vetiveria zizanioides, and Azadirachta indica* was found to be safe, effective with significant bacteriostatic, cooling and astringent properties[23].

Effective hand sanitizers were developed using Carbomer as a gelling agent, propylene glycol as a humectant, Cucumber extract as a cooling agent, ethyl alcohol as an anti-bacterial agent, Vitamin E as a moisturizer along with fragrance[24]. Herbal hand sanitizer containing extracts of *Ocimum samctum* and *Eucalyptus globules* as anti-microbial agent was found to exhibit significant activity against *E. coli, Pseudomonas aeuroginosa, Staphylococcus aureus, Bacillus subtilis* and *Sacchromyces cerevisiae, Candida albicans* and safe on hands when compared to reference standards Ampicillin and Amphoterecin respectively[25] .

Herbal Antiseptic Gels

Development of antiseptic gels containing herbal extracts for treatment of acne, dandruff and wounds has been reported. The physical parameters and activity of these gels are compared with the market samples. Formulation and evaluation of topical anti acne formulation of coriander oil with antibacterial activity against *Propionibacterium acne (P. acne)* and *Staphylococcus epidermidis (S. epidermidis)* is reported[26]. Antidandruff hair gel containing hydro-alcoholic extract of neem leaves (*Azadirachta indica*) using gelling agent like carbopol 934 was comparable to the market formulations for physical parameters like colour, appearance, consistency, washability, pH, spreadability along with antimicrobial efficacy [27].
A comparative evaluation of antimicrobial activity of gel formulations of *Morinda citrifolia, Papain,* and *Aloe vera* against *Enterococcus faecalis* was carried out to evaluate disinfection of dentinal tubules when contaminated with E faecalis. This was compared with reference standard of Chlorhexidine gel[28].

The carbopol 934 gel formulations containing different concentrations of extracts of *Terminalia arjuna, Centella asiatica* and *Curcuma longa* showed a faster rate of wound contraction compared with controls in wound healing activity in mice[29].

Herbal creams and ointments

Antiseptic creams are water based topical creams for the skin that provide protection from contamination and infections. Development and validation of many herbal antiseptic creams have been reported. The development and validation of herbal antiseptic cream containing Oils of *J. regia, K.galanga, C. tora* and *T. arjuna* was carried out. Results showed that the formulated cream posses prominent activity compared with standard[30].

The formulation of antimicrobial polyherbal cream containing extracts of *Symplocos racemosa, Berberis aristata, Rubia cordifolia, Azadirachta indica oil* and *Cedrus deodara oil,* was evaluated against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* species. The cream showed significant anti microbial activity[31]. Antipsoriatic cream using different combinations of herbs like neem, sarsaparilla, bakuchi and daruhaldi showed good antimicrobial and anti inflammatory results[32]. The Formulation and evaluation of anti-bacterial and anti-fungal activity of an herbal ointment containing *Aloe-vera, Azadirachta indica* and *Curcuma- longa* was carried out against various organisms. Results showed a good activity against the selected organisms when compared to standard drugs [33].

The Development and evaluation of antimicrobial ointment and gel containing the methanolic extract of *Samadera indica* was carried out, it was evaluated for physical parameters and *in-vitro* antimicrobial activity. Results showed that the formulations had acceptable physical parameters and good compatibility with the skin, as well as good antimicrobial activity[34]. The ethanolic extracts of *Azadiracta indica, Elsholtzia fructicosa, Eucalyptus globules, Ocimum santrum,* and *Rhododendron setosum* were formulated into ointment and evaluated for the antimicrobial activity; the results were compared with branded marketed products[35]. The Evaluation of the Antibacterial activity of Herbal ointments formulated with Methanolic extract of *Cassia alata* against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* was done. The formulation containing *Cassia alata* extract in aqueous cream showed better activity than other formulations[36].

In a study a polyherbal ointment was prepared using methanolic extracts of *Azadirachta indica, Chromolaena odorata, Mimosa pudica, Samadera indica* and evaluated for its

physicochemical antibacterial and antioxidant properties. The results showed better physicochemical and antibacterial properties[37].

Herbal Dentifrices

Oral hygiene is an essential part of health of a person. Oral health when neglected, results in different types of oral diseases like dental caries and periodontal diseases. The main causes of oral diseases are infections, food habits and life style. Dental caries and periodontal diseases are the two common threats to oral health. The diversity of plant secondary metabolites as antimicrobials is utilized by the traditional practice in various forms of medicine. Medicinal plants have been used extensively in folk medicine for maintaining oral hygiene. It is also observed that the microorganisms found in inflamed gums are resistant to antibiotics but not to antibacterial plant extracts. And unlike antibiotics, antibacterial plant extracts produced no allergy in the gingiva[38]. Medicinal plants used in traditional system to treat a variety of dental ailments are enlisted in **Table No: 2.**

Gel formulations of *Morinda citrifolia, Papain,* and *Aloe vera* were formulated and evaluated for antimicrobial properties against *Enterococcus faecalis.* Results showed that the overall percentage inhibition of bacterial growth was significant when compared to standard[28].

An herbal toothpowder was formulated using extracts of *Cinnamomum zeylanicum, Thea sinensis, Moringa oleifera, Glycyrhhiza glabra, Terminalia chebula* and clove oil. It was evaluated for its effect on microbial flora of oral cavity. It was found that the toothpowder was as effective as branded products Colgate and Pepsodent and showed 47 % reduction in oral salivary microbial count[39].

An *in-vitro* study was conducted to evaluate the efficacy of commercial dentifrices and over the counter herbal dentifrices on *Streptococcus sangui,* by measuring the Maximum Zone of Inhibition at 24 hours. Colgate Total toothpaste was used as the positive control and sterile normal saline was used as negative control. Results showed that all dentifrices selected for the study were effective against the test organism but to varying degree[40].

The antimicrobial efficacies of two toothpaste formulations containing natural antimicrobials (herbal extracts and chitosan) against oral biofilms of *Actinomyces naeslundii, Streptococcus oralis* was carried out. Chlorhexidine mouthrinse was used as positive control and a buffer were used as negative control. Results indicated that the herbal and chitosan based

supernatants showed immediate killing of oral biofilm bacteria which was comparable with chlorhexidine[41].

Table No: 2. List of herbs used in traditional systems as Dentifrices[38].

Plant	Part used	Traditional use
Acorus calamus	Rhizomes	Painful teeth and gums
Allium sativum	Bulb	Infected gums and cavities
Bombax ceiba	Gum	Tooth ache
Cinamomum tamala	Stem bark	Tooth decay, Tooth ache
Citrus medica	Fruit	Bleeding gums
Datura stramonium	Seeds	Oral ailments
Juglens regia	Oil & Fruits	Tooth ache, Pyorrhoea
Justicia adhatoda	Twigs	Pyorrhoea
Myrica esculenta	Bark	Tooth ache
Ocimum sanctum	Leaves	Painful teeth
Phyllanthus emblica	Fruit	Bleeding gums
Punica granatum	Fruit	Bleeding gums
Ricinus communis	Twigs & Leaves	Dental caries, Pyorrhoea
Urtica dioica	Root	Tooth ache
Vitex negundo	Leaves	Tooth ache
Zanthoxylum armatum	Fruit & Twig	Tooth ache
Zingiber officinale	Rhizome	Tooth ache, Tooth decay

Disinfection of water by herbs

The disinfection of water was carried out using the leaves and fruits of *Hardwickia binata, Cyperus rotundus, Andropogon muricatus* and *Luffa cyllindrica.* A kinetic model was formulated to predict the disinfection of water. Results showed that all herbs showed effective removal of *E.coli* [42]. The evaluation of disinfectant activity for water purification was carried out for the plants *Ocimum Sanctum, Azadirachta indica, Triticum aestivum, Phyllanthus emblica* and *Strychnos potatorum.* Results showed that in all herbs maximum removal of *E.coli* was found at 30 minutes contact time onwards[43].

The evaluation of disinfection efficiency between sodium hypochlorite and chlorine dioxide on spa water was reported. Results indicated that Disinfection efficiencies for chlorine

dioxide were 99.4% and sodium hypochlorite was 89.5%. Chlorine dioxide was found to be more effective than sodium hypochlorite[44].

Herbal Fumigants

In a study the preparation and evaluation of antimicrobial herbal based incense sticks for fumigation against infectious bacteria was carried out. The herbal based incense stick was formulated using authentic samples of herbal powders of various woods , barks , gums along with volatile oils using mould method, the herbal incense sticks were evaluated for its antimicrobial activity and appearance. The formulated herbal based incense stick not only showed good antimicrobial activity against airborne microbes but also these incense sticks were easy to prepare , user friendly, economical and did not lead to health hazards[45].

It is reported that in China the herbs usually used for herbal fumigants are Chinese atractylodes, argy wormwood leaf, Mosla chinensis oil, fire wood, pine wood etc. The fumigants are, for convenient application, usually made in the form of incense or smoking agents. Coil and bar incenses are mostly used for air disinfection to prevent respiratory infectious diseases. Smoke guns or tanks containing large amount of smoke are also used for terminal disinfection in epidemic foci and big public places. Results revealed that these herbal fumigants dramatically reduced the natural bacteria [46].

Plant oils as disinfectants

In a study the antimicrobial activity of topical formulation containing the essential oils of *Eugenia caryophyllata* and *Myritus communis* were evaluated on selected skin disease causing pathogens, Results indicated that the formulations have shown significantly high antimicrobial activity against selected pathogens[47].

The antimicrobial and antifungal activity of volatile oil based gel formulation of *Allium sativum* against skin pathogens *Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeroginosa, Candida albicans, Aspergillus niger* and *Cryptococcus neoformans* was tested. The gel showed significant activity against skin pathogens when compared to the standard drugs Ciprofloxacin and Amphotericin-B [48]. A Comparative evaluation in the efficacy of peppermint (*Mentha piperita*) oil with standards antibiotics against bacterial pathogens *Bacillus cereus, Bacillus subtilis, E coli, S aureus, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* was carried out, results indicated that peppermint oil was found to be effective against all organisms[49].

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