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ANTIBACTERIAL ACTIVITY OF VARIOUS INDIAN SPICES TARGETING *ENTEROCOCCI*

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ABSTRACT

Spices are widely used for flavoring and preserving foods since the ancient times. They are also used as natural and household medicines against variety of infections. Most of the Asian countries have rich heritage of traditional medicinal system and a diverse range of spices are used for treatments. *Enterococci* are common bacteria that inhabit the gastrointestinal tract, oral cavity & vagina of humans & animals. *Enterococcus spps* in particular *Enterococcus faecalis* have been found to be associated with chronic periodontitis and failed root canal treatments involving chronic apical periodontitis. Antimicrobial activity of some spices such as Clove, Turmeric, Black pepper against *Klebsiella pneumonia* & *Staphylococcus aureus* has been studied (Bhawana Pandey *et al*, 2014). Antibacterial activity of some important naturally grown spices like (alcoholic extract) Cardamom, Lemon grass, Clove showed activity against Gram positive as well as Gram negative bacteria (Ranganathan Kapilam, 2015). Antimicrobial activity of some of the south Indian spices against serotypes of *Escherichia coli, Salmonella, Listeria monocytogenes* & *Aeromonas hydrophila* was determined. Extracts of spices such as Garlic, Nutmeg, Ginger, Onion, Pepper was studied (M.N.Indu *et al* 2006).

KEYWORDS: Indian spices, Antibacterial activity, *Enterococcus faecalis*, Nagkesar, Kalonji, Rockflower, Nutmeg, Mace, Poppy seeds, Periodontitis.

INTRODUCTION

Enterococci have evolved over eons as highly adapted members of the gastrointestinal consortia of a wide variety of hosts—humans and other mammals, birds, reptiles and insects—but for reasons that are not entirely clear, they emerged in the 1970s as some of the

leading causes of multidrug-resistant, hospital-acquired infections. The taxonomy of *Enterococci* has changed considerably over the past ten years, and the genus now includes over forty distinct species with various habitats, tropisms, and metabolic and phenotypic characteristics. These habitats include animal hosts, as well as plants, soil and water, and man-made products, including fermented foods and dairy products. Antibiotic-resistant strains of enterococci have emerged in many of these habitats, and strains with novel resistance mechanisms are isolated with alarming regularity. As a result, the relationship between the non-therapeutic use of antibiotics and the occurrence of *Enterococci* in various non-human habitats is of substantial interest.

Phylogenetically, the genus *Enterococcus* belongs to the low GC branch of Gram-positive bacteria (Paulsen, et al., 2003). Enterococci were classified as group D streptococci (Sherman J. M., The streptococci, 1937; Sherman J. M., The enterococci and related streptococci, 1938) until 1984, when *Streptococcus faecalis* and *Streptococcus faecium* were reclassified as *Enterococcus faecalis* and *Enterococcus faecium*, respectively (Schleifer & Kilpper-Balz, 1984).

The *Enterococcus* genus is placed in the Enterococcaceae family (Ludwig, Schleifer, & Whitman, 2009), along with genus Bavariicoccus, Catellicoccus, Melissococcus, Pilibacter, Tetragenococcus and Vagococcus, and consists of species that occur in human and animal gastro-intestinal (GI) tracts (Mundt, 1963; Mundt, 1986), as well as in the guts of insects (Martin & Mundt, 1972), traditional fermented food and dairy products (Mundt, 1986), and in various environments including plants ((Mundt, 1963), soil (Mundt, 1961) and water (Ator&Starzyk, 1976).

Enterococci are ubiquitous in GI tracts even though they constitute a small proportion of the gut consortium, typically comprising less than 1% of the adult microflora (Finegold, Sutter, &Mathisen, 1983; Sghir, Gramet, Suau, Violaine, Pochart, & Dore, 2000). Little is known about the main mechanisms used by enterococci to colonize GI tracts of either healthy individuals or hospitalized patients. Nevertheless, exposure of hospitalized patients to antibiotics results in major modifications of the gut microbiota, which facilitate colonization of the GI tract by drug-resistant enterococci (Donskey, et al., 2000; Ubeda, et al., 2010).

Enterococci are important human pathogens that are increasingly resistant to antimicrobial agents. These organisms were previously considered part of the genus *Streptococcus* but have

recently been reclassified into their own genus, called *Enterococcus*. To date, 12 species pathogenic for humans have been described, including the most common human isolates, *Enterococcus faecalis* and *E. faecium*. *Enterococci* cause between 5 and 15% of cases of endocarditis, which is best treated by the combination of a cell wall-active agent (such as penicillin or vancomycin, neither of which alone is usually bactericidal) and an aminoglycoside to which the organism is not highly resistant; this characteristically results in a synergistic bactericidal effect. High-level resistance (MIC, greater than or equal to 2,000 micrograms/ml) to the aminoglycoside eliminates the expected bactericidal effect, and such resistance has now been described for all aminoglycosides. *Enterococci* can also cause urinary tract infections; intraabdominal, pelvic, and wound infections; superinfections (particularly in patients receiving expanded-spectrum cephalosporins); and bacteremias (often together with other organisms). They are now the third most common organism seen in nosocomial infections. For most of these infections, single-drug therapy, most often with penicillin, ampicillin, or vancomycin, is adequate. Enterococci have a large number of both inherent and acquired resistance traits, including resistance to cephalosporins, clindamycin, tetracycline, and penicillinase-resistant penicillins such as oxacillin, among others. The most recent resistance traits reported are penicillinase resistance (apparently acquired from staphylococci) and vancomycin resistance, both of which can be transferred to other enterococci. It appears likely that we will soon be faced with increasing numbers of enterococci for which there is no adequate therapy.

Enterococci have been extraordinarily successful at rapidly acquiring resistance to virtually any antimicrobial agent put into clinical use. Introduction of chloramphenicol, erythromycin and tetracyclines was quickly followed by the emergence of resistance, in some cases reaching a prevalence that precluded their empirical use. While the occurrence of ampicillin resistance in *E. faecalis* has been quite rare, there is now widespread, high-level resistance to ampicillin among clinical *E. faecium* isolates.

In response to the growing problem of vancomycin resistance in *Enterococci*, the pharmaceutical industry has developed a number of newer agents that have activity against vancomycin-resistant enterococci (VRE). However, none of these newly licensed agents (quinupristin-dalfopristin, linezolid, daptomycin, tigecycline) has been entirely free of resistance. Thus, the widespread resistance of enterococci has had a substantial impact on our

use of both empirical and definitive antibiotics for the treatment of enterococcal infections, a situation that is likely to persist for the foreseeable future.

Modern medicine is moving towards researching herbal remedies for medical treatment to prevent unnecessary bacterial resistance. Therefore, new antimicrobial agents that could overcome this resistance need to be discovered. Many spices can exhibit antimicrobial activity against *Enterococcus faecalis*. Therefore, spices have a great potential to be developed as new and safe antimicrobial agents.

Why Spices

- Spices are widely used for flavoring and preserving foods since the ancient times.
- They are also used as natural and household medicines against variety of infections.
- Most of the Asian countries have rich heritage of traditional medicinal system and a diverse range of spices used for treatments.
- Human body naturally develops resistance to some antibiotics in the long run, but the herbs or spices that are in practice have no such resistance.
- The antibacterial activity is due to the phytochemicals present in spices.

PHYTOCHEMICAL ANALYSIS:

Spices	Phytochemicals Present
Myristica fragrans (Mace)	Alk Fla Gly Ste Tan Ter
Papaver somniferum (Poppy seeds)	Gly Ste Ter
Lichens (Rockflower)	Alk Gly Ste Tan Ter

- Alk- alkaloids, Fla- flavonoids, Gly-glycosides Phe-phenols, sap-saponnins, ste- steroids, Tan- tannins, Ter-terpenes.

A. *Ceylon ironwood* (Nagkesar)

The name, literally, translates to Cobra's saffron. But it is also known as "**Indian Rose Chestnut**" or "**Sri Lankan Ironwood**" in different parts of the world. "Ironwood of Assam" or "Ceylon ironwood" areother names for this tree in English. Nagkesar is a very famous Ayurvedic herb used in treating fever, vomiting, urinary tract disorders, migraine etc. It is one among Chaturjata group of herbs. It is used as in powder form along with other spices, and put into many herbal jams including Chyawanprash. There are no known side effects with this herb. It is safe to use this during lactation and in children.

Oil is used to treat skin diseases and its local application is also recommended in rheumatism. The essential oil has antimicrobial and anthelmintic activity. Others have shown anti-inflammatory and antipyretic activity. Recently it has been shown that calophyllolide is effective in reducing the increased capillary permeability induced in mice by iiistamine, 5-HT and bradykinin. Main use of stamen has been described for controlling bleeding in menorrhagia and piles.

Oil from the seeds has been used in the treatment of sores, scabies, wounds, and rheumatism. It has been attributed to have beneficial effects as a digestant, and to have anti-poisonous, anti-microbial, anti-inflammatory, anti-pyretic and anti-helmintic activity.It has also been used in the treatment of fevers, itching, nausea, leprosy, skin disorders, erysipelas, bleeding piles, metrorrhagia, menorrhagia, excessive thirst, and sweating.

Nagakeshar is slightly hot in potency, has pungent and bitter tastes. It helps to relieve Kapha. It is useful in the treatment of fever, pruritis (itching), excessive thirst, sweating, vomiting, diseases pertaining to Basti – Urinary bladder, throat diseases and headache.It enhances the complexion. It leads to fragility transparency to the skin.

The flowers are acrid, anodyne, digestive, constipating, stomachache. They are useful in conditions like asthma, leprosy, cough, fever, vomiting and impotency.The seed oil is considered to be very useful in conditions like vata and skin diseases.

Dried flowers are used for bleeding hemorrhoids and dysentery with mucus. Fresh flowers are useful remedy for itching, nausea, erysipelas, bleeding piles, metrorrhagea, menorrhagea, excessive thirst, and sweating. Oil from the seeds is used for sores, scabies, wounds, and rheumatism.The leaves are applied to the head in the form of a poultice for severe colds.

The resin produced by the tree is slightly poisonous yet is very useful. It helps in aggravates the fairness of skin.The flowers are fragrant, acrid, anodyne, digestive and useful in stomach-ache, and constipation. In some countries the extract of flowers are used in perfumes.The flowers are also used to treat asthma, leprosy, cough, fever, vomiting and impotency.

Desiccative nature of Nagkesar helps to quench the thirst.Nagkesar is a help in the parasitic intestinal worms.It is a tonic to the heart.It helps to expel wind (air) from the body.It is also useful in the urinary system.

B. *Nigella sativa* (Kalonji)

Nigella sativa (of the family *ranunculaceae*) is a plant that is synonymous with *nigella cretica* and is commonly called black cumin. The seeds are the main medicinal component, although a seed oil pressed from the seeds (Black Cumin Oil or Black Seed Oil) also shares the same bioactives.Medicinal usage of these seeds mostly centers around diarrhoea and abdominal pain, dyslipidaemia, asthma and coughs, headache, dysentery, renal calculi, infections, obesity, back pain, hypertension, and dermatological problems. *Nigella sativa* is a simple spice used in food that appears to be a very well renowned seed, and is used for a large variety of medicinal purposes as well as general wellbeing and longevity.*Nigella sativa* is known to have antibacterial properties *in vitro*and have shown efficacy against both gram positive and gram negative bacteria as well as yeast which has been noted to influence anti-biotic resistant bacteria.

Due to all the aforementioned and how an *in vitro* study noted complete eradication of *Heliobacter pylori* within an hour it has been investigated for reducing this particular bacteria in humans where supplementation of 2g of the seed extract of *nigella sativa* (in addition to 40mg omeprazole) was able to eradicate two thirds (66.7%) of a *Heliobacter pylori* infection, whereas lower (1g) and higher (3g) doses were less effective at 47.6-47.8%. The reference therapy (clarithromycin, amoxicillin, omeprazole) reached 82.6% eradication but was statistically comparable to 2g *Nigella sativa*. The combination of an antibiotic or two with a proton pump inhibitor (omeprazole) tends to be standard therapy for the eradication of *Heliobacter pylori* and *Nigella sativa* has not yet been tested in isolation.

Nigella sativa appears to have anti-ulcer properties against the *Heliobacter pylori* bacteria, with a potency somewhat comparable to a clarithromycin and amoxicillin combination

therapy. It has not yet been tested in isolation, but alongside a proton pump inhibitor (omeprazole).

C. *Papaver somniferum* (Poppy seeds)

Also known as 'khuskhus' in Hindi, poppy seeds are oilseeds obtained from poppy (which is a flowering plant).

There are different varieties of poppy seeds. Some of the popular types are.

Blue poppy seeds – Also called European poppy seeds as they are mostly seen on Western breads and in confectionery.

White poppy seeds – Also called Indian or Asian poppy seeds, they are featured in the respective cuisines.

Oriental poppy seeds – Also called opium poppy, this one yields opium and is grown for commercial purposes.

Uses

Poppy seeds are sources of insoluble fiber, which can aid digestion. It also increases the bulk of the stool and helps treat constipation. Though there is limited research on this, anecdotal evidence suggests that poppy seeds can help cure mouth ulcers. Poppy seeds are known to have a cooling effect on the body, which plays a role in mouth ulcer treatment.Simply mix some crushed dry coconut, powdered sugar candy, and ground poppy seeds. Shape it into a pellet and suck on this for instant relief from mouth ulcers. Poppy seeds are loaded with dietary fiber, which helps lower cholesterol levels and ultimately improves heart health. As per a study, the cholesterol content in a food item decreased with a higher amount of poppy seed oil. This simply proves that adding the oil to your diet can make it more heart-healthy.

D. *Myristica fragrans* (Nutmeg and Mace)


Nutmeg and mace are plant products. Nutmeg is the shelled, dried seed of the plant Myristica fragrans, and mace is the dried net-like covering of the shell of the seed. Nutmeg and mace are used to make medicine.

Nutmeg and mace are used for diarrhea, nausea, stomach spasms and pain, and intestinal gas. They are also used for treating cancer, kidney disease, and trouble sleeping (insomnia); increasing menstrual flow; causing a miscarriage; as a hallucinogen; and as a general tonic. Nutmeg and mace are applied to the skin to kill pain, especially pain caused by achy joints (rheumatism), mouth sores, and toothache.In foods, nutmeg and mace are used as spices and flavorings.

In manufacturing, nutmeg oil is used as a fragrance in soaps and cosmetics. Nutmeg oil is distilled from worm-eaten nutmeg seeds. The worms remove much of the starch and fat, leaving the portions of the seed that are rich in oil.

Uses

- Producing hallucinations. Eating 5-20 grams of nutmeg powder (1-3 whole seeds) might cause psychoactive effects. Because nutmeg and mace are so similar, high doses of mace might also have psychoactive effects but, as yet, this has not been proven.
- Can be used in treatment of Diarrhea, stomach problems, intestinal gas, cancer, kidney disease and pain.

E. *Lichens* (Rockflower)

Parmotrema perlatum, commonly known as black stone flower or kalpasi, is a species of lichen used as spice in India.Kalpasi or black stone flower is a species of lichen used as spice in India. Alternatively known as daagar ka phool or patthar ka phool.

Uses

1. A good pain reliever, Kalpasi helps heal wounds.
2. It helps treat skin problems and reduces inflammation.
3. It has antibacterial activity and is effective against protozoans.
4. It improves digestion and helps suppress respiratory disorders.
5. It tones up urinary tract and helps maintain body temperature.

DISCUSSION

Most effective 15 spices discussed and reported were *Allium cepa*, *Brassicajuncea*, *Cinnamomumtamala*, *Cinnamomumzeylanicum*, *Coriandrum sativum*, *Cuminum cyminum*, *Curcumalonga*, *Mentha spicata*, *Murrayakoenigii*, *Nigella sativa*, *Papaver somniferum*, *Piper nigrum*, *S.aromaticum*, *Trachyspermumammi*, and *Trigonellafoenum*, *Zingiber officinale* showed the maximum antibacterial activity against the Enterococcal isolates. Cinnamaldehyde present in *Cinnamomum cassia* (Blume) bark reveled pointed inhibition against *Clostridium perfringens* and *Bacteroides fragilis* which are human intestinal bacteria. Black pepper (*Pippalinigrum*) showed antibacterial activity on *E.coli*.

Curcuma longa, *Zingiber officinale*, *Piper nigrum*, *Eletteria cardamom*, *Cinnamomum vernum*, *Syzygium aromaticum*, *Trigonella foenumgraecum*, *Myristica fragrans* have action against oral microorganisms like, *S.mutans*, *C. albicans* and various periodontal pathogens.Cinnamon and Ginger were found to have activity against e*nterococcal* isolates. *Eugenia caryophyllus*, *Cinnamomum zeylanicum* and *Myristica fragrans* have the maximum

antibacterial activity against all the five urinary pathogens *Escherichia coli, Klebsiella pneumoniae, Citrobacter diverses, Pseudomonas aeruginosa* and *Proteus vulgaris.*

Thus, spices not only have application in culinary art to impart flavor to food, but also have medicinal properties and in treatment of various diseases. Research done on the phytochemicals present in these spices show that they have action against oral, gastrointestinal tract and urinary tract microorganismshence further research is necessary to incorporate these chemicals in oral, gastrointestinal tract and urinary tract health care products.

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