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Comparison of antimicrobial activity of crude ethanolic extracts and essential oils of spices against five strains of diarrhoea causing *Escherichia coli*

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Abstract

Crude ethanolic extracts and essential oils of 5 spices including coriander (*Coriandrum sativum L.)*, Ginger (*Zingier officinale)*, Turmeric (*Curcuma longa*,), Cloves (*Syzygium aromaticum*), and garlic *(Allium sativum)*, were examined for their antibacterial activity against 5 strains of diarrhoea causing *Escherichia coli* using disk diffusion methods. Of these cloves showed the highest inhibitory effect in the both case while coriander, ginger and turmeric showed no inhibitory effect in the case of crude ethanolic extracts. The constitute of these spices mainly Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons are the antimicrobial activity whose was shown by present work.

Key-Words: Antimicrobial activity, crude ethanolic extracts, essential oil, *E.coli*

Introduction

Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavour, colour and aroma of food. In addition to boosting flavour, herbs and spices are also known for their preservative[1] and medicinal value, which forms one of the oldest sciences[2]. Yet it is only in recent years that modern science has started paying attention to the properties of spices. Spices can be defined as Òany dried fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms that contribute flavour, whose primary function in food is seasoning rather than nutrition and that, may contribute relish or piquancy of foods and beverages[3]. Although as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because a substance being derived from a plant does not mean it is always harmless.

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One drug used for one ailment could actually be detrimental to the treatment of another. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, mutagens and abortifacient.

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants .such as Coriander (*Coriandrum sativum L.)* belongs to the family, Umbelliferae) is considered both an herb and a spice since both its leaves and seeds are used as a seasoning condiment. It is native to the Mediterranean and Middle Eastern regions and has been known in Asian countries for thousands of years. Coriander seeds have a health-supporting reputation that is high on the list of the healing spices. It has traditionally been referred to as an anti-diabetic, anti-inflammatory and recently been studied for its cholesterol-lowering effects. In addition, it is also used as carminative, diuretic, tonic, and stimulant, stomachic, refrigerant, aphrodisiac and analgesic. The coriander seeds contain 0.5-1% essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol, and linalool. Coriander's flavonoids include quercitin, kaempferol, rhamnetin, and epigenin. Coriander also contains active phenolic acid compounds including caffeic and chlorogenic acid.

Zingiber officinale Rosc. (Ginger), belonging to the family, Zingiberaceae is widely used around the world as a spice or food additive and medicine. This plant is a perennial herb consisting of an underground stem or rhizome, bearing erect leafy shoots. The pungent element of ginger is the oleoresin-gingeroles, shogaols and zingerone, which are credited with anti-nausea or antiemetic, abortifacient, antimicrobial, anti-inflammatory[4] antioxidant[5] anticoagulant, antihypercholesterolemic, antihypertensive, antihyperglycaemic, and anti-spasmodic, aperient[6] alexeteric, circulatory stimulant, counter irritant, sialagogue and vasodilator effects.

Turmeric is a spice which is obtained from rhizomes of plant *Curcuma longa*, a member of the family Zingaberaceae. Components of tumeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin[7]. The significance of turmeric in medicine has changed considerably since the discovery of the antioxidant[8] and antimicrobial properties of its naturally occurring phenolic compounds. They also have antiinflammatory[9], anticancer, hepatoprotective, antiallergic, wound healing, anti-tumour, antispasmodic and anti-HIV[10] properties.

Allium sativum, commonly known as garlic, is a species in the onion genus, Garlic is also claimed to help prevent heart disease (including atherosclerosis, high cholesterol, and high blood pressure) and cancer[11]. Garlic is used to prevent certain types of cancer, including stomach and colon cancers.

Clove is the dried (flower bud of *Eugenia caryophyllus*, family Mytraceae. It has a wide range of medicinal properties; even it is now commonly used in Western medicine. Recently antibacterial property of clove has been reported.

Keeping in view the important role of spices in inhibition of different cultures of bacteria and its role as antibacterial, the present study was conducted to compare the antibacterial activity of the essential oils and extracts of coriander, cloves ,ginger and turmeric against 5 strains of diarrhoea causing E.Coli. such as Enteroinvasive *Escherichia coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC) and . Enteroaggregative *E. coli* (EAEC).

Material and methods

Bacterial strains

Five different strains of E.Coli were used for this study. Enteroinvasive *Escherichia coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC) and . Enteroaggregative *E. coli* (EAEC) were obtained from the collection of the Department of microbiology, Sanjay Gandhi post graduate institute of medical science, Lucknow They were subculture monthly and subsequently stored at 4^{o}C.

Culture preparation

A loopful of 24 h surface growth on a NA slope of each bacterial strain was transferred individually to 5 ml of Brain Heart Infusion (BHI) broth (pH 7.6, Difco). After incubation at 37°C for 24 h, bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match that of a 5 McFarland standard (10^{8} CFU/ml). Then, a 1:10 dilution of the cell suspension was performed to give inoculums concentration of 10^{7} CFU/ml.

Preparation of crude ethanolic extracts

The spice materials were cut into small pieces; 20g of each were soaked in 100 ml of 95% ethanol, and shake at 150rpm for 4 days at ambient temperature. The mixtures were then filtrated, evaporated a frozen at -80 C. Stock solutions of crude ethanolic extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 400 mg/ml.

Preparation of essential oils

The small pieces of spice materials (300 g) were placed in a flask (2 L) together with distilled water (1 L). After steam distillation, the 100% pure essential oils were collected, dispensed into dark bottles, and stored at 4°C until used. The stock solutions of crude ethanolic extracts and essential oils were ready to use for disk diffusion test.

Screening of spice extracts using disk diffusion technique

The disk diffusion test was performed using the standard procedure as described by Jorgensen *et al.* [12]. The inoculums suspension of each bacterial strain was swabbed on the entire surface of Mueller-Hinton agar (MHA, pH 7.3 ± 0.1, Difco). Sterile 6-mm filter paper discs (Schleicher & Schuell) were aseptically placed on MHA surfaces, and crude ethanolic extracts or essential oils were immediately added to discs in volumes of 20 µl or 15 µl, respectively. A 20-µl aliquot of 10% DMSO was also added to a sterile paper disc as a negative control, whereas a disc containing 10 µg amoxycillin was placed in the plate as a positive control. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation at 37 °C for 24 h.

Results and Discussion

Five strains of *E.Coli* were used which is commonly found in clinical diagnosis of stool. The essential oils of coriander, cloves, ginger, and garlic showed the broadest antibacterial activity by inhibiting growth of all *E.Coli* strains tested (the diameter of inhibition zone, 8-16) which is shown in the table 1. Cloves showed highest antimicrobial activity against all strains of *E.Coli*. The crude ethanolic extracts showed narrower antibacterial activity only cloves showed the degree of inhibition in table 2. Ethanolic extract of coriander, ginger and turmeric were inactive against strains of *E*.Coli. In general the inhibitory activity of essential oils was greater than that of ethanolic extracts. Oils of coriander include quercitin, kaempferol, rhamnetin, and epigenin. Coriander also contains active phenolic acid compounds including caffeic and chlorogenic acid. The major antimicrobial compound in garlic is allicin [13]. The major pungent components of ginger are gingerone and gingerol which have strong inhibitory activity against pathogenic bacteria[14]. Turmeric consists of 3-5% curcuminoids. Curcumin is the most important fraction which is responsible for the biological activities of turmeric.

In the present study, most of the spice oils exhibited stronger antibacterial activity than their own ethanolic extracts. The antimicrobial property of spices has been shown to be attributable to the essential oil fraction [15]. This is because of the fact that some essential oils contain active components which influence certain metabolic functions of microbial cells. As most components of spice oils belong to the terpenoid family, there has been much speculation on the contribution of the terpene fraction of the oils to their antimicrobial activity[16]. Cyclic terpene compounds have been reported to cause loss of membrane integrity and dissipation of proton motive force[17]. Wilkins and Board[18] suggested that the antimicrobial action of spices is due to the impairment of a variety of enzyme systems involving in the production of energy or synthesis of structural components in microbial cells.

It is concluded from the present study that both the extracts of cloves can be used as the natural potential against diarrhoea causing E.coli when if applied to food products. And other can be used in the combination with the other preservatives such as acid, sugar, and other chemical preservative systems [19].

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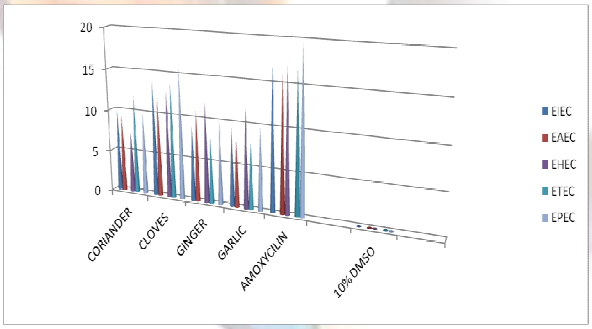
Table 1: Antimicrobial activity of essential oils against five strains of *E.Coli* Essential oils Diameter of inhibition (mm)

	EIEC	EAEC	EHEC	ETEC	EPEC
Coriander	10	9.5	8	12	10
Cloves	14	12	13	14	16
Ginger	9	11	12	8	10
Garlic	10	9	12	8	10
Amoxycilin	17	16	17	17	20
10% DMSO	-	-	-	-	-

Table 2: Antimicrobial activity of crude ethanolic extracts against five strains of *E.Coli* Crude ethanolic extracts Diameter of inhibition (mm)

	EIEC	EAEC	EHEC	ETEC	EPEC
Coriander	-	-	-	-	-
Cloves	10	12	9	9.5	10
Ginger	-	-	-	-	-
Turmeric	-	-	-	-	-
Amoxycilin	17	16	17	17	20
10% DMSO	-	-	-	-	-



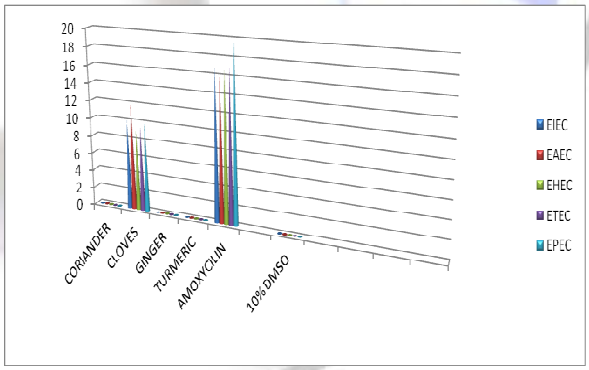


Fig 1: Comparison of antimicrobial activity of essential oils of spices against five strains of *E. coli*
Fig 2: Comparison of antimicrobial activity of essential oils of spices against five strains of *E. coli*