To study the Antifungal Activity of Indian Spices against Aspergillus Niger and Trichoderma Reesei

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Abstract: ***There has been constant increase for the search of efficient and alternative for food conservation with the aim of replacing antimicrobial chemical additives. Spices provide a capable alternative for food safety issues. In the present attempt, fifteen Indian spices were investigated using different solvents such as ethanol, methanol and acetone for determining antifungal potential of some common Indian Spices against two fungal species - Aspergillus niger and Trichoderma reesei using agar well diffusion method . Ethanolic extracts of Glycyrrhiza glabra were found to be highly effective against Aspergillus niger . Also the ethanolic, methanolic and acetone extracts of Glycyrrhiza glabra were found to be very effective against trichoderma reesei showing maximum zone of inhibition.***

Keywords: Aspergillus niger, Trichoderma reesei, Glycyrrhiza glabra, Spices

1. Introduction

Spices and herbs have been added to food since ancient times, not only as flavoring agents, but also as ancient medicine and food preservatives [1]. Microorganisms and increasing consicious food consumers have to be using new and natural antimicrobials [2]. A new area of research is the advance of new and improved methods of food preservation. Additives such as monosodyum glutamate, aspartame, saccharin, sodium cyclamate, sulfites, nitrates, nitrites and antibiotics are harmful for human health. It causes headache, nausea, weakness, mental retardation, seizures, cancer and anorexia [3].This resulted in consumer's interest in natural products, especially plant extracts, including their essential oils and essences. It is well established that these extracts possesses antimicrobial properties against A.niger and T.reesei. Antimicrobial properties of certain spices have been reported in meat and meat products, e.g. poultry meat, turkey breast and beef, broth and foods and turkey frankfurter sluries [4]. The aim of this study was to compare the inhibitory effects of spice extracts on the growth of two commonly found fungal species i.e., A.niger and T.reseei.

Conventionally the people of India have an ancient practice of using wide diversity of herbal products in cure of diseases or as preservatives in foods. Spices are essential components of Indian cuisines since ancient times. Spices are rich source of bio-active antimicrobial compounds.

Traditionally the people of India have ancient practice of using wide variety of plant products in treatment of diseases. Spices are indispensable components of Indian cuisines since ancient times. These are used in minute amounts to impart flavour, taste and aroma in food preparation to improve their palatability [5]. Spices are also used for stabilizing several food items from deterioration [6]. Spices are considered as rich source of bio-active antimicrobial compounds [7]. The typical Indian spices like cumin, mustard, fenugreek, ajowain, turmeric, cardamom are usually used in curries, pickles, sauces etc. These spices are also known to have some ethno-medicinal or anti-microbial properties [8].

Uncontrolled use of chemical antimicrobial preservatives has been inducing feature for manifestation of microbial strains more and more resistant to classic antimicrobial agents. Fifty years of increasing use of chemicals antimicrobials have created a situation leading to an ecological imbalance and enrichment of multiples multiresistant pathogenic microorganisms [9]. The successful story of microbial chemocontrol lies in the continuous search for new antimicrobial substances to control the challenge posed by resistant strains [10]. Antimicrobial activity of spices depend on several factors, which includes: i) kind of spice, ii) composition and concentration of spice, iii) microbial specie and its occurrence level, iv) substrate composition and v) processing conditions and storage [11]. Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods [12]. Although, spices have been well known for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf-life [13] [14] has emphasized the possible use of spices and derivatives like alternatives for inclusion in a new perspective of food conservation called "natural antimicrobial system", which could use the synergistic effect of antimicrobial compounds from animal, plant and/or microbial origin, more physical procedures in order to create an inhospitable environment for microbial survival in foods.

2. Materials and Method

Sample collection: The spices- Black cardamom (Amomum subulatum), Mustard seed (Brassica nigra) , Red Chilli (Capsicum annuum), Bay leaf (Cinnamomum tamala), Cinnamon (Cinnamomum verum), Coriander seed (Coriandrum sativum), Cumin seed (Cuminum cyminum), Green cardamom (Elettaria cardamomum), Liquorice (Glycyrrhiza glabra), carom seed/Thyme (Trachyspermum ammi)) , Anise (Pimpinella anisum) , Black pepper (Piper nigrum), Fenugreek (Trigonella foenum-graecum), Turmeric (Curcuma longa), Dry ginger (Zingiber officinale) were

collected from the local area market of Lucknow . The spices were grind well to make powder form by using hand homogenizer and mixer grinder and then fine powder form of spices was used for extraction procedure and other evaluation.

Test organism: Two commonly found fungal species were taken for the purpose of evaluation of antifungal property of the fifteen spices taken. Pure culture of these fungal species (A.niger and T.reesei) were provided by CytoGene Research & Development, Lucknow.

Preparation of culture broth: Potato Dextrose Broth was prepared for subculturing of each species. The colonies were picked from the culture plates and inoculated into the broth. The broth was kept at 37^0 C for 24 hours for obtaining growth.

Antibacterial study (Agar well diffusion method): The agar well diffusion assay was used to determine the growth inhibition on A.niger and T.reesei. Fungal strains were maintained at 4˚C on PotatoDextrose Agar media plates before use. PDA media was sterilized in autoclave at 121˚C than poured in to petri dishes. The PDA broth was inoculated with the two different fungal species taken and incubated at 37˚C overnight. Each broth was inoculated with 200µl of different fungal species, mixed well, transferred into sterile petri dishes and allowed to set. Using a sterile cork-borer of 5mm diameter, four holes per plate were made into the set agar.40 µl of broth culture containing the fungal species were poured and spread uniformly with the help of a spreader onto the agar. A total of 20µl of spice extracts were poured into the wells and one containing 100ppm solution of antifungal drug (Terbinafine), the plates were kept in incubator overnight and the zone diameter was then recorded if greater than 5mm.

3. Result and Discussion

The spices taken showed different zone of inhibition in different solvents against the two test organisms. While few spices showed maximum antimicrobial activity, others did not produce any significant result.

Table 1: Diameter of inhibition zone shown by Trichoderma reesei on different spice extracts

Spices	*Methanol*	*Ethanol*	*Acetone*
Red chilli	0	0	0
Turmeric	10	9	0
Coriander	0	0	0
Liquorice	10	15	0
Carom	7	0	0
Fenugreek	6	13	8
Black pepper	7	6	6
Black Cardamom	0	0	0
Bay leaf	7	15	10
Green Cardamom	10	10	8
Anise	0	7	10
Dry ginger	15	15	8
Cumin	0	12	6
Cinnamon	9	7	6
Mustard seed	0	0	0

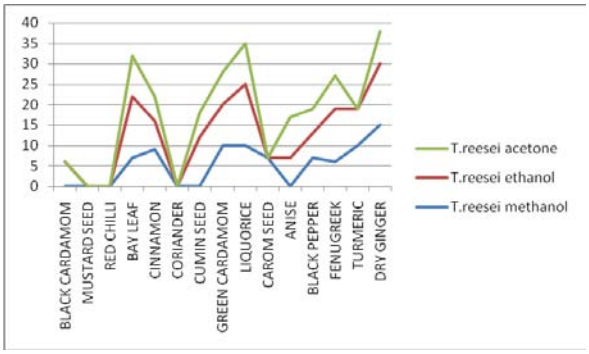

Figure 1: Graphical representation of inhibition zone shown by different spices on T.reesei

Table 2: Diameter of inhibition zone shown by Aspergillus niger on different spice extracts

Spices	*Methanol*	*Ethanol*	*Acetone*
Red Chilli	0	0	0
Turmeric	11	10	10
Coriander	13	15	7
Liquorice	13	25	0
Carom	8	15	0
Fenugreek	0	0	0
Black Pepper	0	10	15
Black Cardamom	0	0	0
Bay leaf	7	17	10
Green Cardamom	0	0	10
Anise	0	6	0
Dry ginger	0	7	0
Cumin	6	8	9
Cinnamon	10	15	9
Mustard seed	8	7	12

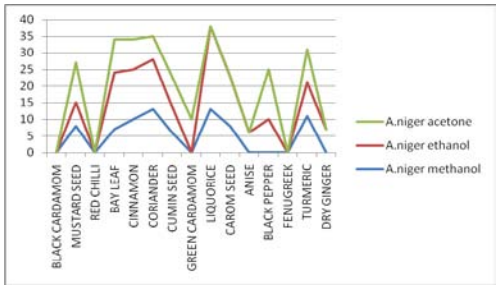

Figure 2: Graphical representation of inhibition zone shown by different spices on A. Niger

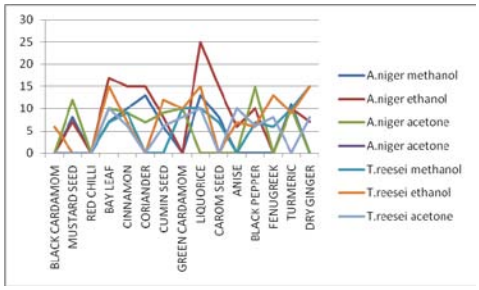

Figure 3: Graphical representation of comparison of the diameter of inhibition zones shown by different spices on A.niger and T.reesei

Terbinafine , an antifungal drug has been taken as a positive control against the two fungal species .in case of Cinnamomum tamala shows inhibition zone of 15 mm in case of ethanol and 10 mm in case of acetone against Trichoderma reesei. While Trigonella foenum graecum showed 13 mm of inhibition zone in case of ethanol, minimum of 6 mm in case of methanol and 8mm in case of acetone. Glycyrrhiza glabra showed 15 mm of inhibition zone in ethanol, 10 mm in methanol and 10 mm in acetone in case of Trichoderma reesei..

On the other hand, Piper nigrum showed inhibition zone of 10 mm in ethanol, 15 mm in acetone against aspergillus niger. Piper nigrum have shown to possess the following medicinal effect:-Anthelmintic, Carminative, Alterant, Antiperiodic, Diuretic, Digestive, Emmenagogue, Rubefacient, Stimulant, Stomachic, used in fever, Asthma, Cough, Dyspepsia, Flatulence, Arthritis. Trachyspermum ammi showed no zone of inhibition in ethanol and acetone against aspergillus niger. Liquorice in ethanol showed maximum zone of inhibition (25 mm) in case of Aspergillus niger .

4. Conclusion

It is well-known that spices and their derivatives could be appropriate alternatives for addition in food conservation systems and could act sometimes as main or adjuvant antimicrobial compounds. Before including spices and/or their derivatives in food conservation systems, some evaluations about their microbiological value, financial viability, and antimicrobial result for a long time and toxicity should be carried out. Use of spices as microbial growth inhibitor in foods is often limited because of flavor considerations as effective antimicrobial dose may exceed the organoleptically accepted level [15]-[16]. Due to this and due to the fact that spices are as GRAS, the antimicrobial properties of spices continue to be of interest [15].

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