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Antifungal Evaluation of Cumin Oil for development of natural anti fungal agent against fungal nails infection

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Abstract

The purpose of this study was to determine the antifungal evolution against fungal nail infection in spices (celery, coriander, fennel and cumin) extracts. In this study antifungal activity of cumin, celery, fennel and coriander extracts were evaluated against isolated fungal species from soil and infected nails from the person who suffered oncomycosis disease. *A. niger*, *A. flavus*, *A.fumigatus* and *Trichophyton rubrum* were isolated form soil and infected nail sample respectively. Antifungal detection of spices was done against fungal species by agar well diffusion method and determined their MIC values .For identification of the bioactive components of spices extracts chromatography technique was done. Due to this antifungal activity we will cure from oral drugs causes disorders, inflammation in liver or gastrointestinal disorders and so on. Some small children are targeted by Oncomycosis are not capable of eating tablets or oral syrups are cured by this type antifungal agent.

Keywords: Nail infection, Fungal Species, Zone of inhibition, MIC, HPLC.

Introduction

In India Spices have been used since ancient times although they have been employed mainly as flavoring and coloring agents, their role in food safety and preservation. Spices have exhibited numerous health benefits in preventing and treating a wide variety of diseases such as cancer, aging, metabolic, neurological, cardiovascular, and inflammatory diseases. Many spices such as clove, oregano, celery, fennel, cinnamon, and cumin have been applied to treat

infectious diseases or protect food because they were experimentally proved to possess antimicrobial activities against pathogenic and spoilage fungi and bacteria (Lai and Rai 2004; De *et al.*, 1999; Arora and Kaur 1999). Moreover, the secondary metabolites of these spices are known as antimicrobial agents, the majority of which are generally recognized as safe materials for food with insignificant adverse effects (Nabavi *et al.*, 2015). There are many species that have been reported to be human pathogens. Some fungus causes many diseases such as, Ringworm, Aspergillosis, Mycosis, etc. which is fatal and analyzed the medicinal effects of these species, which are used and can minimize the activity of *fungus* species (Jardan and Chakraborty 1995). Cumin is an aromatic plant belonging to the Apiaceae family. The Cumin seed oil have strong potential to control the production of Aflatoxins which are highly toxic and carcinogenic metabolites produced by fungus *Aspergillus parasiticus* on food and agricultural products (Khosravi *et al.*, 2011)**.** The Celery is native to moist habitats in temperature regions of Eurasia. The celery seed oil is as a fixative, as an important ingredient in perfumes, in medicine and in flavoring of different types of foods, such as meats, sausages, canned soups, sauces and Fennel belonging to family Umbellifarae (Singh *et al.*,2006), is widely planted in temperate zones and the tropical belt for its aromatic fruits, and is used as an ingredient in the cooking (Diao *et al.*, 2014). Fennel is used for various digestive problems including heartburn, intestinal gas, bloating, loss of appetite, and colic in infants. Fennel seeds are primary flavor component in Italian sausage. An herbal tea or tissue can be made from fennel (Shan 2016).Coriander is being an annual herb is most commonly used for seasoning purpose it is also well known for its antioxidant, anti-diabetic, anti-mutagenic, and antimicrobial activity along with analgesic and hormone balancing effect that promotes its use in foods due to numerous health benefits and its protective effect to preserve the food for longer period.

Methodology

1) Isolation and identification of fungal Species

Fungal species was isolated from soil and fungal infected nail samples, using PDA (potato dextrose agar) media. Lacto phenol cotton blue staining, sugar fermentation, catalase, Urease, Starch hydrolysis and casein hydrolysis test were done in order to identify the isolated fungal species.

2) Preparation of Spices Extract

The spices (cumin, celery, fennel & coriander) seeds were dried at 60°C in hot air oven till constant weight was attained. Then seeds were grinded and maked it powder formed. The powdered seeds were extracted with six solvents methanol, ethanol, Isopropanol, benzene, toluene and acetone for 48 hours. Collected extracts was filtered through double layer muslin cloth and stored in air tight bottles at -20°C for further use.

3) Phytochemical analysis of Spices Extract

Phytochemical tests were done to find the presence of the active chemical constituents such as Alkaloids, amino acid, Flavanoids, Glucosidase, Phenol, Protein, Saponins, Steroid and Tannin in spices extracts. According to Harborne **(**1973) phytochemical analysis were qualitative and quantitative method to to detect the chemical compounds in spices. Amino acid (ninhydrin) test was done by using 0.25% w/v ninhydrin reagent added the blue colour indicates the presence of amino acids in the extracts. Alkalonids test also known as Mayer test the aliquot sample of extract was added with ammonium solution a cream colour precipitation was indicates the presence of alkalonoids in the species extracts. Tannins (gelatin) test was done using 1% gelatin solution containing and formation of white precipitates indicates its presence in the species extracts In Protein (xanthoprotic) test, extracts were treated with few drops of conc. nitric acid and its presence gives yellow color. For saponins (froth) test extracts were diluted with distilled water formation of foam indicates its presence while in Phenols (ferric chloride) test extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols. The steroid test was done by using chloroform and sulphuric acid the yellow with green florescence color indicates its presence while glycosides test was done by using glacial acetic and ferric chloride the blue green color indicates the presence of glycosides.

4) Determination of Antifungal Activity by Well- Diffusion Method

Antifungal activities of the spices were done against isolated fungus on PDA plate. The antifungal activity was done by agar well diffusion method. 20µl of spices extract suspension was poured onto each of wells on the plates. After overnight incubation at 37°C, the different levels of zone of inhibition were measured.

5) Determination of MIC by broth dilution method

The Minimum inhibitory concentration of fungi was done on broth dilution method .The cultures were added into the broth of MIC and add antifungal agent, continue to started serial dilution at last of tube and incubated for 48 hours.

6) Identification of different Spices components using Chromatography technique: HPLC

Dissolved Aliquot 1ml of sample in HPLC grade water. If there was some undissolved material, the sample was filtered through a filter paper and then prepared solvent which was filtered before used, This removes particulates that could block solvent lines or the column and also serves to de gases the solvent Once a stable base line is obtained, inject 20µL of the sample (either manually or via an automatic injector) and use a linear gradient from 0 to 100% HPLC grade water over 10 minute to elute and analyzed the sample.

Result and Discussion

1) Isolation and identification of fungi

Fungal species was isolated from soil and fungal infected nail sample was to be found *A. niger, A. fumigates* and *A. flavus* and *Trichophyton rubrum.* For observed the morphology of fungi Lacto phenol cotton blue staining was done and the result showed on table no.1. The biochemical activity of fungi was also done and the result was summarized on table no.2

2) Phytochemical Screening result of Spices extracts

In our study the Phytochemical extracts were done by different solvents. Saponins was absent in all six different solvents and alkolides were present only in acetone, benzene, Toluene and Isopropanol. Steroids, Tannin and Glucosidase was present on Isopropanol, Toluene extracts of cumin celery and fennel expect Coriander. Flavanoids test was absent in Benzene and Isopropanol solvent extracts of cumin fennel and coriander and present in Celery .Protein test was present in all solvents of cumin, celery, fennel & coriander expect in benzene and toluene. In coriander extracts protein test was present in all solvents expect methanol, Isopropanol. A study done by Kamble *et al.*,1987 in Phytochemical analysis in cumin their result showed that Flavanoids, protein, amino acid, phenol was present on Isopropanol, ethanol, methanol extracts expect benzene. Another study done by Vaitcho *et al.*, 1950 determined Phytochemical analysis in celery and their result showed that protein, alkaloids,

amino acid, and protein was found in toluene and benzene extracts and phenol was present on Isopropanol, methanol and ethanol spice extract.

3) Antifungal of spices extracts by Agar well diffusion method

Antifungal activity of different type of spices extract was checked again isolated fungi species such as *A.fumigatus*, *A. Flavus*, *A. niger* and *Trichophyton rubrum* and their inhibiting effect was seen in PDA media. Cumin, Celery, Fennel and coriander showed maximum zone of inhibition in methanol acetone and Ethanol extracts against Aspergillus and Trichophyton Species. According to the study Hossain. Mohammad pour *et al., 2012* observed maximum zone inhibition 13,9,12 mm respectively in Cumin extract against Aspergillus species. According to the Omr Ertutk 2006 study the Cumin showed antifungal activity against *A. niger* and *C. albicans* with inhibition zone diameter range of 10–16 mm.

4) Determination of MIC (minimum inhibitory concentration) in Spices extracts

The MIC value of spices extract of this study was showed at 10^{-2} and 10^{-3} in methanol and acetone extract against Aspergillus and Trichophyton species. According Hong Zeng *et al.,* 2015 their MIC Value showed 10^{-4} and 10^{-5} for spices extract oils against Trichophyton . According to Qing Liu *et al.,* 2017 MIC value of Cumin seed extract showed10^{-4} against fungal species.

5) Characterization and identification using HPLC

The different extracts of spices were used for characterization and identification of bioactive components by HPLC. The Chromatogram of different spices extract was done for the identification of their bioactive components in the extracts. The result of HPLC showed the highest peak in cumin species in benzene solvent at 1453 approx and then fennel showed highest solvent in acetone at 1252 (approximate). According to Esther LH tang *et al.,* 2013 their HPLC chromatogram of coriander seed extract showed highest peak in ethyl acetate extract Ascorbic acid and *p*-coumaric acid were detected in the extract of their study.

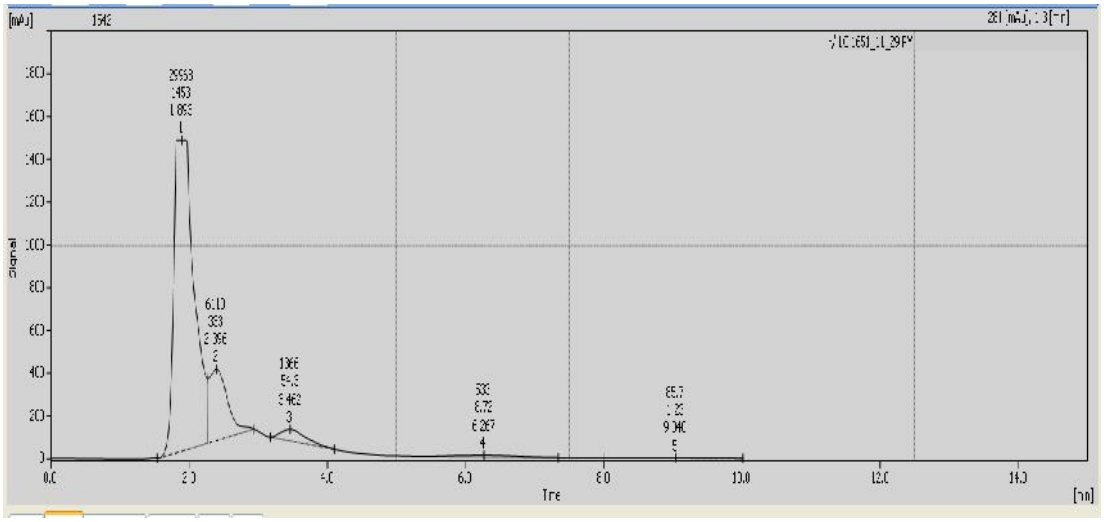
Table No. 1: Observation of Isolated Fungal species on Lacto phenol Blue Staining

LCB staining	***A. niger***	***A. flavus***	***A. fumigates***	**T. rubrum**
Observation	Blue Hyphae	Blue Hyphae	Blue hyphae	Pale Blue
Morphology	Conidia, Conidiophores	Conidia, hyphae	Conidiophores	Conidia

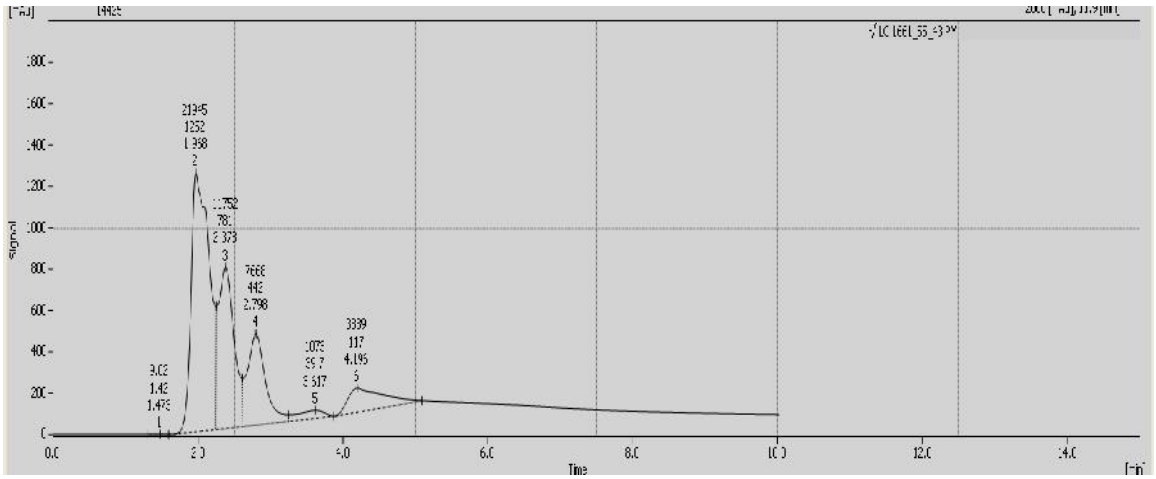
Table no.2: Result of biochemical test of isolated fungal species

S. No	Biochemical Test		*A. flavus*	*A. fumigates*	*A. niger*	*T. ruburum*
1.	Sugar fermentation Test	Glucose	+	+	+	+
		Sucrose	+	+	+	-
		Lactose	-	-	+	-
2.	Starch Hydrolysis Test		+	+	+	+
3.	Casein hydrolysis test		+	+	+	_
4.	Urease test		+	+	+	_
5.	Catalase test		+	+	+	+

Positive(+),Negative(-)



Graph no.1: Shows HPLC Chromatograph of cumin in benzene solvent



Graph 2: Shows HPLC Chromatograph of Fennel in acetone solvent.

Conclusion

The aim of our study to detect antifungal activity of cumin, fennel, celery and coriander against antifungal nail infection called as Onchomycosis.and developed a oil or oilments for fungal nail infection for childrens and old age groups people who are unable to take medicine orally against fungal nail infection. In this study we take four Indian spices and determined their antifungal activity against fungal species *A. niger*, *A. flavus*, *A. fumigates* and *Trichophyton ruburum* and extraction of these four spices was done by using six solvents methanol, ethanol, Isopropanol, toluene, benzene, and acetone. The Phytochemical analysis of these four spices showed the presence of protein, steroid, alkaloid, phenols etc. Antifungal activity of spices was found more against to Aspergillus and Trichophyton species. The MIC was showed at 10^{-2} and 10^{-3} for all extraction of spices cumin, celery, fennel & coriander. The identification of bioactive components in spices was done by HPLC technique the highest peak was found in cumin than fennel spices.

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