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PHYTOCHEMICAL ANALYSIS AND ANTI -FUNGAL ACTIVITY OF *CINNAMOMUMZEYLANICUM* (CZ)

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ABSTRACT

Plants have a property to synthesize many sustainable aromatic substances. Mostly the phytochemical process undergoes to change simple compounds to complex secondary metabolite derivatives. Those secondary metabolites have medicinal properties. Present work reports the antifungal and phytochemical analysis of *Cinnamomumzeylanicum* which havebiofractions extracted from bark and leaves. Analysis of *Cinnamomumzeylanicum* leaf, stem, bark and root bark oils indicated 72 essential oils. Extract from leaf, stem, and bark oils are investigated for phytochemical constituents and anti-fungal activites. Extract with ethyl alcohol gives alkaloids, glycosides, terpenoids, saponins, flavonoids, anthraquinones, phlobatanins, steroids, phenolic, amino acids, proteins, quinones, tanins, reducing sugars. Acetone, Methanol, Ethyl alcohol, and aqueous extracts of *Cinnamomum zeylanicum* were tested against plant pathogen fungi, *Fusariumoxysporum* MTCC 7392 and *Alternariasp* MTCC 9692. Flucanazole (fungicide) standard was used as a positive control. After incubation, minimum inhibitory concentration (MIC) is measured. The zone indicates the antifungal activity. This study reveals the phytochemical characterization and antifungal effect of bark and leaf extracts of indian spice plant, *Cinnamomumzeyalnicum(CZ).*

INTRODUCTION

A Spice is defined as natural compound that is extracted from the seeds, fruits, flowers or trunks (skin, roots, leaves) of several plants are added to food in order to provide taste, smell or flavor. Spices are a diverse group of a wide variety of staple dietary additives consumed all over the world, significantly more tropical, oriental, Hispanic and Mediterranean cuisines. The Spice is a culinary term not a botanical category it does not refer to a specific kind of plant or plant part (*B. Shineyramya 2012*). Each spice has a unique aroma and flavour, which derive from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (*Walker, 1994)*. Cinnamon is a common spice used by different cultures around the world for several centuries. It is obtained from the inner bark of trees from the genus Cinnamomum, a tropical evergreen plant that has two main varieties*; Cinnamomumzeylanicum* (CZ) and Cinnamon cassia (CC) (also known as *Cinnamomumaromaticum/Chinese cinnamon)*. In addition to its culinary uses, in native ayurvedic medicine, Cinnamon is considered a remedy for respiratory, digestive and gynaecological ailments. Almost every part of the cinnamon tree including the bark, leaves, flowers, fruits and roots, has some medicinal or culinary use. The volatile oils obtained from the bark, leaf, and root barks vary significantly in chemical composition, which suggests that they might vary in their pharmacological effects as well. (*Ranasinghe et al 2013*).

The genus *Cinnamomum* comprises of about 300 species of which four species are used to obtain the spice 'cinnamon'.Ceylon/True cinnamon(*Cinnamomumzeylanicum*) and Chinese cassia cinnamon(*Cinnamomumaromaticum*) are the most widely available varieties (*JayaprakashaG,2011*). Studies have demonstrated many beneficial health effects of cinnamon,such as anti-inflammatory properties, anti-microbial activity, blood glucose control, reducing cardiovascular disease, boosting cognitive function and reducing cardiovascular disease, boosting cognitive function and reducing risk of colonic cancer *(Ouattara B,2010,Gruenwald J,2010)*. *Cinanamomumzeylanicum* also known as 'Ceylon cinnamon or true cinnamon' is indigenous to srilanka (*Jayaprakasha G,2011*).

Antimicrobial properties of herbs and spices have been used since time immemorial for food preservation and medicinal purposes. Fungi cause major damage to the food commodities. The major drawback related with the use of synthetic fungicides is that they enter into the food chain and causes several ill effects. The bark and leaves of *Cinnamomumspp* are

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commonly used as spices in the home kitchen and their distilled essential oils or synthetic analogs are used as flavouring agents in the food and beverage industry *(Jham, 2005*). It can be used in treatment of diarrhea, stomach upset, respiratory ailments, skin antiseptic and rubefacient. (*Mishra AK, 2008, InouyeS, 2001, Juglal, 2002*). Antifungal and anti-bacterial principles present in essential oil are effective in preventing food spoilage (*Fabio A,2007, Ranasinghe L, 2002, Valero M, 2003*). Phytochemical moieties in *Cinnamomumspp* possess antioxidant action that may prove beneficial against free radical damage to cell membranes (*Jayaprakasha GK, 2008*).

The objectives of this study were to elucidate phytochemical analysis and their antifungal activites of *Cinnamomumzeylanicum*.

MATERIALS AND METHODS

The botanical material was identified by Prof NirmalaBabuRao and a voucher specimen was deposited in the Herbarium of the Botanical Garden of Department of Botany (Osmania University).

Preparation of Solutions

1. ***Fehling's Solution:*** **-** A mixture of equal volume of copper sulphate, sodium potassium tartarate and sodium hydroxide was prepared in a beaker.
2. ***Wagner's Reagent:*** **-** Mixing 2gm of Iodine, 6gm of potassium iodide in 100ml of water.

Collection of Sample: Barks of *Cinnamomumzeylanicum* were taken and washed under running water to remove the dust and other external pollutants. The barks were air dried for few days (normally 10 to 15 days). *Cinnamon* barks were ground into powder and stored in clean polythene bags.

Preparation of Plant Extract with Distilled Water: 10 grams of bark powder and added 50ml of distilled water stirred it constantly for 30 minutes and the solution was kept at room temperature for 24 hours and then filtered. The filtered solution is again filtered with whatman filter paper No.3 and stored at 4ºC until further use.

Tests

A. ***Phytochemical Screening:*** Chemical test is carried out on the distilled water extract of *Cinnamomumzeylanicum* using standard procedures to identify the constituents.
B. ***Procedure for Alkaloids:*** 2ml of extract is taken and added 2ml of wagner's reagent a brownish precipitate indicate the presence of alkaloids.
C. ***Cardiac Glycosides:*** 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. Deep reddish brown colour at the inter face of steroid ring indicates the presence of cardiac glycosides.
D. ***Flavonoids:*** 2ml of extract is treated with 2 ml of 10%lead acetate. Brownish green colour indicates the presence of flavonoids.
E. ***Saponins:*** 2ml of extract is dissolved with 2ml of Benedict's reagent. Blue black ppt indicates the presence of saponins.
F. ***Tanins:*** 2ml of extract is treated with 0.1% of ferric chloride. There was no brownish green ppt found hence tannins were absent.
G. ***Terpenoides:*** (Salkowski test) 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour indicates the presence of terpenoids.
H. ***Anthraquinones:*** 1ml of extract is boiled with 10% HCL for few minutes in a water bath. It is filtered and allowed to cool. Equal volume of CHCl3 is added to the filtrate few drops of 10% Ammonia is added to the mixture and heat. Formation of rose pink colour indicates the presence of anthraquinones.
I. ***Glycosides:*** The extract is hydrolysed with HCL solution and neutralised with NaoH solution. A few drops of Fehlings solution A&B are added red precipitate indicates the presence of glycosides.

Screening for antifungal assay

The Acetone, Methanol, Ethyl alcohol, and aqueous extracts of *Cinnamomumzeylanicum* were tested against plant pathogen fungi, *Fusariumoxysporum* MTCC 7392 and *Alternariasp* MTCC 9692. Suspensions of different fungi were prepared by using 24hours old fungal cultures. About 0.3 ml of the each fungal suspension was mixed in separate 15 ml aliquots of sterilized molten state potato dextrose agar medium and poured into oven sterilized petridishes. Then wells (6mm) were made in the medium using sterile cork borer. 100ul of each extracts were transferred in to the separate wells. Flucanazole (fungicide) standard was used as a positive control. The plates were incubated at 27˚c for 48-72 hours. After the incubation the plates were observed for formation of clear zone around the well indicated the presence of antifungal activity. The zone of inhibition was measured.

RESULTS AND DISCUSSION

Table 1 Showing Results of Phytochemical analysis of *Cinnamomumzeylanicum*

Sl. No	Phytochemicals	Distilled Water	Methanol	Acetone	Ethanol
1	Tanins	Negative	Positive	Positive	Positive
2	Anthraquinones	Positive	Positive	Negative	Positive
3	Flavanoides	Negative	Negative	Negative	Negative
4	Alkaloides	Positive	Positive	Positive	Positive
5	Terpenoids	Positive	Positive	Positive	Positive
6	Saponins	Positive	Positive	Positive	Positive
7	Cardiac glycosides	Positive	Positive	Positive	Positive
8	Glycosides	Positive	Positive	Positive	Positive
9	Reducing Sugars	Positive	Positive	Positive	Positive
10	Phlobatanins	Positive	Positive	Positive	Positive
11	Steroids	Positive	Positive	Positive	Positive
12	Phenolic	Positive	Positive	Positive	Positive
13	Aminoacids	Positive	Positive	Positive	Positive
14	Proteins	Positive	Positive	Positive	Positive
15	Quinones	Positive	Positive	Positive	Positive

Antimicrobial susceptibility remains an area of interest and provides development of new drug discovery. The present work demonstrates the fungicidal efficacy of C.*zeylanicum* bark and leaf extracts and seems antifungal principles are mostly concentrated in polar fractions as evident from experimental findings.MIC(minimum inhibitory concentration)

of polar extract fractions shows against test fungi showing appreciable inhibitory potential in some of the extracts. The inhibitory effects of spices are mostly due to the volatile oils present in their composition.

The results of the Antifungal screening are given in table-2. Anti-fungal activity was screened by agar well diffusion method. Among all the different solvent extract, the Acetone extract showed significant antifungal activity on two fungal species compared with other solvent extracts and positive control (Flucanazole). Acetone, Methanol and Ethyl alcohol extracts of *Cinnamonzeylanicum* bark extract exhibited larger zone of inhibition than positive control against *Alternarissps*. The positive control (Flucanazole) does not show zone of inhibition on *Fusariumoxysporum*. This activity of the results against the fungi provides confirmation biological activity of *Cinnamon zeylanicum* bark extracts.

Table.2 showing minimum inhibitory concentration (MFC)

Test organism	Zone of inhibition (mm)				
	Acetone	Methanol	Ethanol	Aqueous	Positive control
Fusariumoxysporum MTCC 7392	17	14	16.5	12	--
Alternariasp MTCC 9692	25.6	21.5	19.8	16	17.5

CONCLUSION

The preliminary phytochemical analysis of ethanolic extract of cardamomum showed the presence of flavonoids, tannins and phenolic compounds, terpenoids, alkaloids, saponins and phytosterols, carbohydrates and proteins, fixed oils and fatty acid. The available reports revealed that C.zeylanicum possess at least three to four secondary metabolites. Therefore, thepresence of phytochemicals comes to justify the observed antifungal activity in the present study. The extract demonstrated potential of antifungal activity by inhibiting spore germination in *Alternaria* and *Fusariam*. This explains the medicinal properties shown by Cardamomun in various studies.

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