March 2009 Volume 5 Issue 1

Trade Science Inc.

Natural Products

An Indian Journal

Short Communication

NPAIJ, 5(1), 2009 [26-28]

Preliminary studies on *in vitro* sntifungal activity of citrus peel distillates

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Received: 18th February, 2009 ; Accepted: 22rd February, 2009

ABSTRACT

The citrus peel essential oils are the most versatile essential oils. The present paper describes the antifungal activity of steam distillates of peels of different citrus fruits namely *Citrus limetta, C.sinensis* and *C.limon*. A simple laboratory apparatus has been employed to get steam distillate and the distillate thus obtained was subjected to antifungal activity against *Aspergillus* species by poison food technique. The results revealed that the steam distillates of *C.sinensis* and *C.limetta* were potent enough to inhibit the growth of test fungi while lemon distillate was ineffective against test fungi. Lack of efficacy of *C.limon* distillate could be due to low concentration of compound in the distillate. Among distillates tested, *C.limetta* distillate was found to be superior to other distillates. The results are in justification with the antimicrobial activity of essential oils of citrus peel being considered as a waste in citrus industries.

KEYWORDS

Citrus peel;
Antifungal activity;
Steam distillate;
Poison food technique;
Essential oils.

INTRODUCTION

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition[1]. Essential oils are products, generally, of rather complex composition comprising the volatile principles contained in the plants, and more or less modified during the preparation process[2]. The citrus peel essential oils are the most versatile essential oils. These oils are mainly used for flavouring fruit beverages, confectioneries, soft drinks, perfuming eau de cologne, soaps, cosmetics and household products. They are also used in medical treatments as immune stimulating as well as being anti-inflammatory agents. The citrus peel essential oils are known to exhibit antimicrobial properties such as antifungal, antibacterial, antiviral and antiparasite[3]. Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil normally float on top of the Hydrosol (the distilled water component) and may be separated off[4].

In current citrus industry, citrus fruits are marketed fresh or as processed juice and canned segments, while fruit peel is produced in great quantities and normally regarded mainly as waste. Exploring essential oil seemed to be an alternative way to evaluate the underlying economical values of citrus due to the special roles it played in food, flavor and cosmetics industries[5]. The present paper describes the extraction and antifungal activity of steam distillates of peels of three citrus fruits namely *Citrus limetta* (Sweet lime), *C.sinensis* (Sweet orange) and *C.limon* (Lemon) against *Aspergillus* spe-

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cies, most of which are known to be the causative agents of opportunistic mycotic infections.

MATERIALS AND METHODS

Collection of steam distillates of citrus peels

Fresh fruits of *Citrus limetta*, *C.sinensis* and *C.limon* were purchased from the market and the peel was separated. The peel of the fruits was made into paste by grinding separately.

A simple laboratory quick-fit apparatus with a 1000ml distilling flask (to boil the mixture of plant material and water), a condenser (to condense the steam to obtain the steam distillate), and a receiving vessel, was used for the steam distillation. A known weight of (100 grams) paste was subjected to steam distillation in the assembly. When heated up, the plant cells release their components and some of them are volatilized and carried by the steam. The volatile components were collected into the receiving flask during 3 hours of steam distillation[6,7]. The distillates were transferred into clean containers and stored in refrigerator until use.

Screening for antifungal activity

The antifungal activity of steam distillates of citrus peels was assessed against three species of the genus *Aspergillus* namely *A.niger*, *A.oryzae* and *A.nidulans*. The suspension of spores of the test fungi (for inoculation on poisoned plates) was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[8].

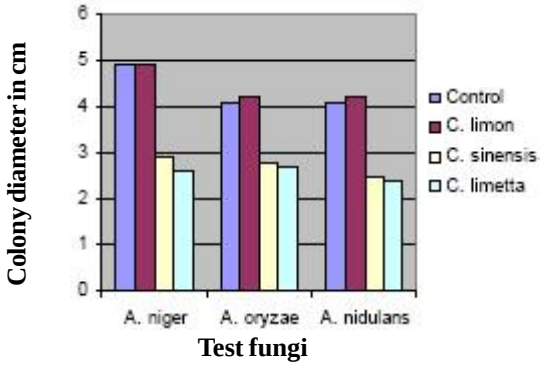
The antifungal activity was assessed using Poison food technique[9]. The test fungi was allowed to grow in Sabouraud's dextrose agar plates poisoned with steam distillates (10% concentration). The test fungi were inoculated by Point inoculation method. The effect of extract on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control (plates not poisoned with extract). The experiment was carried in triplicate to arrive concordant readings.

RESULTS AND DISCUSSION

TABLE 1 and the graph show antifungal activity of steam distillates of peels of different citrus fruits. From the table, it becomes clear that *C.limetta* distillate was more potent in inhibiting test fungi followed by *C.sinensis* and *C.limon*. In case of *C.limon*, the growth of *A.oryzae* and *A.nidulans* has been enhanced and was found to be more than the growth in control. The growth of *A.niger* was found to be not affected by lemon distillate. The stimulation of fungal growth in case of *C.limon* distillate could be due to low concentration of the compound in distillate.

TABLE 1: Antifungal activity of steam distillates of citrus peel

Steam distillate	Average colony diameter of test fungi in cm		
	A.niger	*A.oryzae*	*A.nidulans*
Control	4.9	4.1	4.1
C.limon	4.9	4.2	4.2
C.sinensis	2.9	2.8	2.5
C.limetta	2.6	2.7	2.4



Graph: Reduction in colony diameter of test fungi by citrus peel distillates

Essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. Therefore, the intimate knowledge of essential oil composition allows for a better and specially directed application. They are essentially obtained by hydrodistillation as opposed to steam distillation, hydrodiffusion or CO_2 extraction[10]; in addition to expression of the pericarp (or cold pressing) which is a special method for *Citrus* (Rutaceae) peel oils extraction[11,12] from fresh or dried material.

A number of compounds and substances have been found to inhibit fungal growth, while others have stimulatory properties[13]. In many instances low concentrations of test compounds stimulated fungal growth, while higher concentrations completely inhibited them. Clove oil at 50 and 10µg/ml and cinnamon oil at 50µg/ml stimu-

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lated the growth of *A.flavus* in liquid media whereas higher concentrations reduced the mycelial growth[14]. Citrus peel essential oils extracted by cold expression from malta (*Citrus sinensis*) and mossumbi (*Citrus sinensis*) were applied in different forms (treatments) separately on breads. The essential oils inhibited and delayed the microbial growth in the bread. Maximum inhibitory effect was achieved against molds and bacteria by spraying the malta peel essential oil on bread[3]. There has been speculation on the contribution of the terpene fraction of the oils to their antimicrobial activity[15]. The antimicrobial activity varies widely, depending on the type of spice or herb, test medium and microorganism[16]. A content of essential oils in different species is influenced by genetic material, culture conditions, environment and by crop and post-crop processing[17,18].

CONCLUSION

In citrus industry, fruit peel is produced in great quantities and normally regarded mainly as waste. Exploring essential oil seemed to be an alternative way to evaluate the underlying economical values of citrus. The present paper highlighted antifungal activity of steam distillates of three citrus fruit peels against *Aspergillus* species. Thus, the steam distillates of citrus peel can be recommended against fungal infections. Regular consumption of distillate could make the body resistant against fungal infections. Further experiments in animal models could possibly reveal the real potential of the distillates.

ACKNOWLEDGMENTS

The authors express their sincere thanks to Head of the Dept. of Microbiology, Principal, S.R.N.M.N College of Applied Sciences and N.E.S, Shivamogga for providing all facilities and moral support to conduct the work.

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