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Influence of extraction time on antifungal activity of *Hemidesmus indicus* and *Swertia chirata*

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ABSTRACT

Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents. The present investigation highlights the effect of extraction time on antifungal activity of Hemidesmus indicus and Swertia chirata. The aqueous extraction was carried out for about 2 hours and microwave extraction was carried for about 10-20 minutes. The aqueous and microwave extracts were subjected for antifungal activity by poison food technique. The results revealed the potential of both the extracts against test fungi. Microwave assisted extraction was found to be more superior to aqueous extraction in terms of reduction in fungal growth when compared to control. The results of the study are in justification that microwave extraction is of superior choice as it takes considerably lesser time to extract the components from cells than other extracts.

KEYWORDS

Microwave assisted extraction;
Antifungal activity;
Poison food technique;
Hemidesmus indicus;
Swertia chirata.

INTRODUCTION

The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents[1]. The fact that one single plant can contain up to several thousand secondary metabolites, makes the need for the development of high performance and rapid extraction methods an absolute necessity[2]. Keeping in pace with such requirements recent times has witnessed the use and growth of new extraction techniques with shortened extraction time, reduced solvent consumption, increased pollution prevention concern and with special care for thermolabile constituents. Novel extraction methods including microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized solvent extraction (PSE) have drawn significant research attention in the last decade. Although many reports have been published on application of microwave heating for extraction of organic compounds and pesticide residue from environmental samples[3,4], microwave has only recently been applied to extraction of plant materials. Very few publications in scientific journals do exist related to this area. So extraction of phytoconstituents by microwave provides a vast scope of research exploration.

Hemidesmus indicus commonly known as Indian Sarsaparilla, belonging to the family Asclepiadaceae.

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TABLE 1: Antifungal activity of aqueous and microwave extracts of selected plants

Treatment	Average colony diameter in cm		
	A.niger	*A.oryzae*	*A.nidulans*
Control	4.3	4.1	3.8
Hemidesmus Aq	2.8	2.9	2.3
Hemidesmus Mw	3.1	3.0	2.4
Swertia Aq	3.2	3.4	3.4
Swertia Mw	3.4	3.5	3.5

Aq- Aqueous, Mw- Microwave, Results are average of triplicates

The roots are used as antipyretic, anti-diarrhoeal, astringent, blood purifier, diaphoretic, diuretic, refrigerant and tonic. Roots are useful in biliousness, blood diseases, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism. Root bark is used to cure dyspepsia, loss of appetite, nutritional disorders, fever, skin diseases, ulcer, syphilis, rheumatism and leucorrhoea. Stem of *H.indicus* is used as diaphoretic, diuretic, laxative and in treating brain, liver and kidney diseases, syphilis, gleet, urinary discharges, uterine complaints, leucoderma, cough and asthma[5]. *S.chirayita* belongs to family Gentianaceae, which records the occurrence of taxonomically informative molecules, namely iridoids, xanthones, mangiferin and C-glucoflavones. The biological activities attributed to *Swertia chirayita* are Alternative, Antihelmintic, Antileishmanial, Anticholinergic, Anticonvulsant, Antiedemic, Antiinflammatory, Antimalarial, Antipyretic, Antitubercular, Astringent, Bitter, Cardio stimulant, Cholagogue, Choleretic, CNS depressant, Emollient, Hepatoprotective, Hypnotic, Hypoglycemic/antidiabetic, Laxative, Secretagogue, Stomachic, Tonic, Undersedative, Vermifuge[6].

The present study employs extraction of *Hemidesmus indicus* and *Swertia chirata* using aqueous extraction and Microwave assisted extraction and describes the influence of extraction time on Antifungal activity of both extracts.

MATERIALS AND METHODS

Plant materials selected for study

Hemidesmus indicus and *Swertia chirata* were procured from local markets of Udupi city and were authenticated to their identity by Dept. of Botany, S.R.N.M.N College of Applied Sciences, Shimoga. The voucher specimens were kept in the department for future references. The dried plant materials were mechanically powdered and used for extraction.

Aqueous extraction

Extraction of plant materials with distilled water (aqueous extraction) was carried[7] with minor modifications. About 25g of powdered plant material was taken in a flask containing distilled water and extracted for about 2 hours in slow heat. After 3 hours, the contents were filtered through 8 layers of muslin cloth and the filtrate was concentrated to make the final volume $1/5^{th}$ of the original volume. The condensed plant extract was used for antimicrobial assay.

Microwave-assisted extraction

Microwave-assisted extraction was carried out by mixing the powdered material and water which was used as solvent and the container was placed in a microwave oven and irradiated at 2450Mhz for 10 or 20 min. After cooling to room temperature, the extract was filtered and the filtrate was kept in refrigerator until analysis[8].

Screening for antifungal activity

In the study, species of the genus *Aspergillus* were selected as target fungi which are known to cause opportunistic mycotic infections in susceptible individuals. The pure cultures of test fungi on SDA slants and were maintained in refrigerator. Periodic subcultures were done aseptically. 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[9]. The antifungal activity was assessed using Poison food technique[10]. The test fungi was allowed to grow in Sabouraud's dextrose agar plates poisoned with extracts (Aqueous and Microwave extracts separately). The test fungi were inoculated by Point inoculation method where the spore suspension of test fungi were taken with the help of a sterile inoculation needle and touched the centre of the medium. 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 done in triplicate and average reading was recorded.

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RESULTS AND DISCUSSION

Antifungal activity of aqueous and microwave extracts of *Hemidesmus* and *Swertia* is depicted in the table. It has been observed that the microwave extract was found to exert more antifungal activity against test fungi than aqueous extract. The average colony diameter in poisoned plates was found to be more reduced in case of microwave extract when compared to aqueous extract. More inhibition of test fungi was observed in microwave extract in relatively shorted period of time. From the results, it can be concluded that the efficacy of microwave extracts against test fungi is quite similar (slightly higher) when compared to aqueous extraction. The microwave extraction was carried for only about 20 minutes while the extraction time is about two hours in aqueous extraction process.

Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz and positioned between the X- ray and infrared rays in the electromagnetic spectrum[11]. The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenons': ionic conduction and dipole rotation, which in most cases occurs simultaneously[3,11]. Even though dried plant material is used for extraction in most cases, but still plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect, evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell. The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptures cells to the surrounding solvent thus improving the yield of phytoconstituents. This phenomenon can even be more intensified if the plant matrix is impregnated with solvents with higher heating efficiency under microwave. Higher temperature attained by microwave radiation can hydrolyze ether linkages of cellulose, which is the main constituent of plant cell wall, and can convert into soluble fractions within 1 to 2 min. The higher temperature attained by the cell wall, during MAE, enhances the dehydration of cellulose and reduces its mechanical strength and this in turn helps solvent to access easily to compounds inside the cell[12].

CONCLUSION

MAE has been considered as a potential alternative to traditional solid-liquid extraction. Some of its potential advantages are reduced extraction time, reduced solvent usage, improved extraction yield, better accuracy and precision in automation, suitable for thermolabile constituents, extraction of even minute traces of constituents including heavy metals and pesticide residue from a few milligram of plant sample, agitation during extraction, which improves the mass transfer phenomenon etc. The results of the study are in justification that the microwave technology is very useful in Medicinal plant research as it is the efficient extraction method as it consumes less time and has several other advantages. The microwave technique is thus found to be superior extraction technique when compared to traditional extraction method.

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