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Effect of Sage Herb (*Salvia officinalis*) on *Candida albicans* and *F. hpatitca*

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ABSTRACT

Because of resistance and side effects of common anti-parasite and antifungal drugs, there have been many studies on the use of the herbal antifungal Herbal Extract. In this study, the anti-Candida and anti-Fasiola activities of Sage Herb (Salvia Officinalis) extract in comparison with anti-fungal and anti -parasite drugs were examined. The research disc diffusion method was used to study the inhibitory effects of the extract of Sage Herb (Salvia officinalis) and Miconazole, Triclabendazole and fluconazole on species of Candida Albicans and Fasciola Hpatitca, which are isolated from patients who suffered from vulvovaginal candidiasis and Fasciolasis at 25°C and 37°C. The transparent area around the manuscript discs showed that thyme extract has the most effect against Candida Albicans. The inhibitory effects of Sage Herb (Salvia Officinalis) extract on the growth of Candida and Fasciolaat 37°C were better than 25°C. The inhibitory effect of Miconazole, Triclabendazole was better than fluconazole. The results of the present study showed that Triclabendazole and Sage Herb (Salvia Officinalis) extract had inhibitory effects on the growth of Candida Albicans and Fasciola Hpatitca.

Keywords: Sage Herb (*Salvia Officinalis*), *Fasciola Hpatitca*, *Candida albicans.*

INTRODUCTION

Sage has one of the longest histories of use of any culinary or medicinal herb. Ancient Egyptians used it as a fertility drug [1]. In the first century C.E. Greek physician Dioscorides reported that the aqueous decoction of sage stopped the bleeding of wounds and cleaned ulcers and sores. He also recommended sage juice in warm water for hoarseness and coughs. It was used by herbalists externally to treat sprains, swelling, ulcers, and bleeding [2]. Internally, a tea made from sage leaves has had a long history of use to treat sore throats and coughs; often by gargling. It was also used by herbalists for rheumatism, excessive menstrual bleeding, and to dry up a mother's milk when nursing was stopped [3]. It was particularly noted for strengthening the nervous system, improving memory, and sharpening the senses. Sage was officially listed in the United States Pharmacopoeia from 1840 to 1900 [4].

Figure 1.Sage Herb (Salvia Officinalis)

Sage Tea or infusion of Sage is a valuable agent in the delirium of fevers and in the nervous excitement frequently accompanying brain and nervous diseases. It has a considerable reputation as a remedy, given in small and often-repeated doses [5]. It is highly serviceable as a stimulant tonic in debility of the stomach and nervous system and weakness of digestion generally [6]. It was for this reason that the Chinese valued it, giving it the preference to their own tea. It is considered a useful medicine in typhoid fever and beneficial in biliousness and liver complaints, kidney troubles, hemorrhage from the lungs or stomach, for colds in the head as well as sore throat, quinsy, measles, for pains in the joints, lethargy and palsy [7]. It has been used to check excessive perspiration in phthisis cases, and is useful as an emmenagogue. A cup of the strong infusion will be found good to relieve nervous headache [8].

The German Commission E approved the internal use for mild gastrointestinal upset and excessive sweating as well as for external use in conditions of inflamed mucous membranes of the mouth and throat. An unpublished, preliminary German study with people suffering from excessive perspiration found that either a dry leaf extract or an infusion of the leaf reduced sweating by as much as 50% [9].

In Germany, sage tea is also applied topically as a rinse or gargled for inflammations. Sage extract, tincture, and essential oil are all used in prepared medicines for mouth and throat and as gastrointestinal remedies in fluid (e.g., juice) and solid dosage forms (Leung and Foster, 1996; Wichtl and Bisset, 1994. Sage has been used effectively for throat infections, dental abscesses, infected gums and mouth ulcers. The phenolic acids in Sage are particularly potent against Staphylococcus aureus [10]. In vitro, sage oil has been shown to be effective against both Escherichia coli and Salmonella species, and against filamentous fungi and yeasts such as Candida albicans and F. Hpatitca. Sage also has an astringent action due to its relatively high tannin content and can be used in the treatment of infantile diarrhea. Its antiseptic action is of value where there is intestinal infection. Rosmarinic acid contributes to the herb's anti-inflammatory activity [11].

Sage has an anti-spasmodic action which reduces tension in smooth muscle, and it can be used in a steam inhalation for asthma attacks. It is an excellent remedy for helping to remove mucous congestion in the airways and for checking or preventing secondary infection. It may be taken as a carminative to reduce griping and other symptoms of indigestion, and is also of value in the treatment of Dysmenorrhoea. Its bitter component stimulates upper digestive secretions, intestinal mobility, bile flow, and pancreatic function, while the volatile oil has a carminative and stimulating effect on the digestion. It has a vermifuge action. There also seems to be a more general relaxant effect, so that the plant is suitable in the treatment of nervousness, excitability and dizziness. It helps to fortify a generally debilitated nervous system [12].

In 1997, the National Institute of Medical Herbalists in the United Kingdom sent out a questionnaire to its member practitioners on the clinical use and experience of the sage. Of 49 respondents, 47 used sage in their practice and 45 used it particularly in prescriptions for menopause [13]. Almost all references were to sage's application for hot flashes, night sweats, and its estrogenic effect [13]. The age range of the menopause patients was 40 to 64, with an average of 49.76. Three-quarters were aged 47 to 52. Forty-three practitioners also noted its use in infections, mainly of the upper respiratory tract, 29 reported its use in sore throat, and 15 reported its use in mouth and gum disease, taken in the form of gargles and mouthwashes. Another main area emphasized by the respondents was its use as a general tonic, for fatigue, nervous exhaustion, immune system depletion, and poor memory and concentration, at any age. Dosage form preference was also reported. Sage was prescribed as tea (aqueous infusion) by 37 practitioners, alcoholic tincture by 30, fresh tincture by 14, alcoholic fluidextract by 2, fresh juice of 2, and fresh leaf by 1 [14].

It is well documented that Sage leaf helps to reduce menopausal sweats. In one study, excessive sweating was induced by Pilocarpine. The sweating was reduced when participants were given an aqueous extract of fresh Sage

leaf. In a further study 40 patients were given dried aqueous extract of fresh sage (440mg) and 40 were given an infusion of sage (4.5g) herb daily. Both groups of patients experienced a reduction in sweating [15].

Sage has a strong anti-hydrotic action, and was a traditional treatment for night sweats in tuberculosis sufferers. Its oestrogenic effects may be used to treat some cases of Dysmenorrhoea and menstrual irregularity or amenorrhea and can reduce breast-milk production [16].

Research has suggested that the presence of volatile oil in Sage is largely responsible for most of its therapeutic properties, especially its anti-septic, astringent and relaxing actions. Sage is also used internally in the treatment of night sweats, excessive salivation (as in Parkinson's disease), profuse perspiration (as in TB), anxiety and depression. Externally, it is used to treat insect bites, skin, throat, mouth and gum infections and vaginal discharge.

It is thought that Sage is similar to Rosemary in its ability to improve brain function and memory. In a study involving 20 healthy volunteers Sage oil caused indicated improvements in word recall and speed of attention. Meanwhile the activity of Sage and its constituents have been investigated in the search for new drugs for the treatment of Alzheimer's disease with promising results. ESCOP (European Scientific Cooperative on Phytotherapy) indicate its use for inflammations such as stomatitis, gingivitis and pharyngitis, and hyperhidrosis (ESCOP, 1997).

The aim of the present study was to evaluate the anti-Candida and anti-Fasciola activities of Sage Herb (Salvia Officinalis) extract against some clinical isolates of Candida Albicans and Fasciola Hepatitca by using research disc diffusion to find alternative materials to synthetic antifungal and anti-parasite drugs [16].

MATERIALS AND METHODS

Two clinical isolates of Candida albicans and Fasciola Hepatitca isolated from women and Man who suffered vulvovaginal candidiasis, Fasciolasis and also C.Albicans and F.Hepatitca (GENETIC RESOURCES IN IRAN) were used for sensitivity test. These species were approved by common distinction method for yeasts. Then the prepared suspensions were kept in 50% glycerin liquid at -70°C. The fungus suspension was cultured on Sabouraud dextrose agar, SDA, (Merck, Germany) and rabbit for Inoculation of Fasciola supplemented with chloramphenicol (SC) and incubated at 30°Cfor 48h.

Extraction

One pure herbal extract of Sage Herb (Salvia Officinalis) was purchased from the Barij extract pharmaceutical company. These extract Were maintained at 4°C before use and during the process. Instructions, the extract was emulsified in hexane.

Miconazole, TRICLABENDAZOLE (Sigma Co., USA) and fluconazole (Fuji Co., Japan) were used for positive controls. Miconazole, TRICLABENDAZOLE and fluconazole were dissolved in 100% Dimethyl Sulfoxid, DMSO (Sigma Co,. USA) and distilled water, respectively, to obtain stock solutions at a concentration of 128mg/ml. The Small size of the stock solutions was stored at -70°C to be used.

Drugs were diluted in distilled water for final concentration, 8-512mg/ml for TRICLABENDAZOLE and 64-2048mg/ml for fluconazole.

Culture of F.Hepatitca in In Vitro

All culture procedures were carried out in a laminar-flow hood (Microflow Ltd) using aseptic techniques. Newly existed metaeercariae were freed from contaminating micro-organisms by allowing them to sediment at 1 g through five 10 ml volumes of sterile HBSS containing 30% v/v human serum. The highest concentration of serum was essential to prevent sticking of metacercariae in the Pasteur pipettes used for transfer. Washed metacercariae (15~0) were transferred to each plastic Leighton-type culture tube (Nuclon, Gibco-Biocult Ltd.) and 2 ml of culture medium added [17].Culture media contained human type A serum prepared from donated Nood. The serum was separated by centrifugation for 30 min at 10,000 g and filtered through a Sartorius positive pressure filter using a glass fiber prefilter and cellulose nitrate membrane (0.22 lam pore-size). Enough serum was obtained from a single donor to complete a single cultural experiment. In two experiments rabbit serum obtained by ear-Needing New Zealand white rabbits was used. Sera were diluted with one of the following: Earte's balanced saline with 0.65% iactalbumin hydrotysate (ELac,(RPMI 1640, or NCTC 135, all obtained from Gibco-Biocult Ltd. Culture media contained washed human type A red blood cells (RBC) at 2% v/v. Twenty percent v/v washed RBC were stored at 4~ in RPMI 1640 at pH 7.4. With culture media containing rabbit serum, washed rabbit RBC were used. Typing of rabbit sera and cells to prevent agglutination is unnecessary because levels of agglutinins are very low in normal rabbits. All media contained 50 units ml- ~ penicillin and 50 gg ml- 1 streptomycin (Gibco-Biocult Ltd) [17]. Cultures were

gassed individually for 30 s with %8CO2 in air passed through sterile cotton-plugged Pasteur pipettes and the screw tops of the culture tubes sealed with paraffin. Cultures were incubated at 37~ and medium replaced at 4-3day intervals. Growth of metacercariae was assessed by observation through the optical base of the plastic culture tube using a Leitz inverted microscope and measurements were made with a calibrated eyepiece micrometer. The growth rate was expressed on the basis of the mean length+_ standard deviation of the six most rapidly growing parasites in each culture. Duplicate cultures were set up for each medium in every experiment. At the termination of culture organisms were fixed in 70% ethanol and stained with Gowers carmine. Aceto-orcein squashes were prepared as described by Smyth (1956) [17].

Statistical analysis

The statistical method of ANOVA was used for comparison of the effectiveness of the anti-Candida and anti-parasite activities of extracts and drugs.

RESULTS

The anti-Candida and anti-parasite activity of the herbal extract showed that thyme extract has the most inhibitory effect on Candida Albicans and Fasciola Hepatitca, followed by Sage Herb (Salvia Officinalis). The difference between them was statistically significant (P<0.001) Although F. Hepatitca was most sensitive to treatment with an extract, there weren't any significant differences between them. It should be noted that the anti-Candida and the anti-Fasciola effect of extract at 37°C was better than 25°C (Table 1). In comparison with the control group, 100% and 87.5% of Candida Albicans and Fasciola Hepatitca were inhibited by the Sage Herb (Salvia Officinalis) extract (in dilutions of 6.25-50%), respectively (Fig. 1). The Sensitivity of these species to the lemon was about 12.5% (Table 2).

Table 1.*The anti-Candida and anti-Fasciola activity of extract by research disc diffusion*

			Sage Herb (Salvia Officinalis)	
Spp	**No (%)**	**Extract**	**25oC**	37oC
C.Albicans	34 (80/36)	30	32±3.2	51±0/1
		21/4	34±0/2	12.4±0/6
		62	24±0/5	23±0/4
		21/3	19±0/4	21.4±0/9
		54	10.9±0/3	10.4±1
		25/32	6.3±0/3	20.5±1.5
		4/12	25.14±1.3	15.7±0/4
		0/36	32±0/4	23.4±0/2
F.Hepatitca	2(3.4)	30	52±1.4	20±0/4
		21/4	33±2.1	23.5±0/7
		62	42±0/1	62±0/2
		21/3	21.3±0/3	12.1±0/1
		54	15.7±0/4	14.7±0/4
		25/35	9.3±0/5	23.4±0/1
		4/12	7.2±0/1	56±1
		0/36	6.4±0/1	1.4±0/6

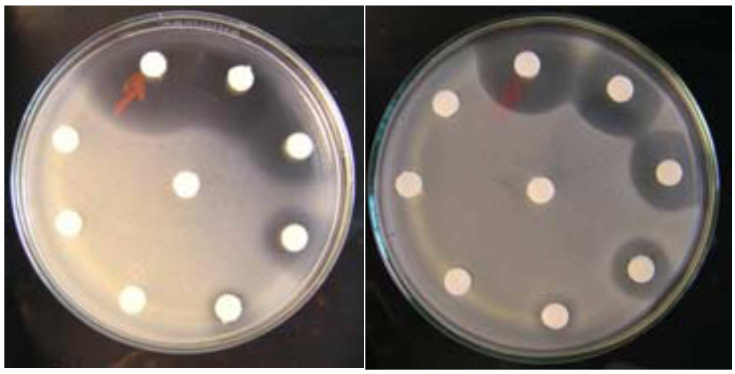

Figure 2.Anti-Candida and anti-Fasciola activity of extract and antifungal drugs at 25°C

Table 2.The anti-Candida and anti-Fasciola activity of antifungal drugs by Article disc diffusion

Drug	Concentration (µg/ml)	*C.Albicans*	*F.Hepatitca*
miconazole	512	14±1.2	18.5±1.2
	234	16±0/4	45±0/1
	123	21±1.7	25.4±2.1
	63	11.4±0/4	23.4±1.2
	34	21±0/4	23.1±0/1
	25	95±1.5	20.1±0/2
	12	8.5±0/2	23±0/3
	16	7.2±0/1	1.4±0/1
triclabendazole	56	45.5±0/1	57.1±0/4
	62	38.5±1.5	46±0/3
	12	34±0/2	42±0/3
	23	30.1±0/3	40.2±0/1
	41	26±0/4	39±0/2
	23	24.2±0/7	32±0/1
	20	20±0/1	23±0/1
	12	8.5±0/2	20.2±0/4
fluconazole	58	43±0/7	59±0/4
	42	23±0/4	44.3±0/1
	34	21±0/1	42±0/3
	22	18.2±0/4	41±0/4
	12	13±0/1	32.4±0/4
	10	10.1±1.5	21.5±1.2
	32	9.5±1.2	18.7±0/1
	10	2.4±0/1	8.7±0/2

DISCUSSION

The results of the present study show that Sage Herb (Salvia Officinalis) extract had the most inhibitory effect on Candida growth. The effects at37 C were better than 25°C. Fluconazole had a bigger inhibitory zone, but Miconazole had a better inhibitory effect on Candida and Fasciola than fluconazole in the same concentration. The anti-Candida and anti-Fasciola activities of Sage Herb (Salvia Officinalis)extract were significantly different, although they were almost similar when compared to Sage Herb (Salvia Officinalis) [18].

Miconazole and Triclabendazole had better inhibitory effects at lower concentrations than fluconazole. They indicated that Triclabendazole had a more inhibitory effect against to see. albicans and F.Hepatitca than fluconazole. Fluconazole had zone inhibition bigger than Triclabendazole with the same concentration.This matter may be due to type of solvent.Because the primary solvent for fluconazole was water, so these preparations can induce diffuse of this drug better than Triclabendazole [19].

Triclabendazole is the drug of choice for treatment of fascioliasis. It is the medication recommended by the World Health Organization. It is not yet widely available to treat people. In the United States, it is not approved by the Food and Drug Administration (FDA); the drug is not yet commercially available in the United States. However, it is available to U.S.-licensed physicians through the CDC Drug Service, under a special protocol, which requires both CDC and FDA to agree that the drug is indicated for treatment of a particular patient [19].

As with all medications, use of triclabendazole should be individualized. It is a benzimidazole compound that is active against immature and adult Fasciola parasites. The therapy usually is effective and safe. Triclabendazole is given orally, with food, to improve absorption.

The medication comes in scored tablets. The dosage is calculated on the basis of the patient's weight. The typical regimen is a single oral dose of 10 mg of triclabendazole per kilogram of body weight (10 mg/kg).

Two-dose (double-dose) triclabendazole therapy can be given to patients who have severe or heavy Fasciola infections (many parasites) or who did not respond to single-dose therapy. Of note, some experts routinely use 2-dose therapy, which might have a higher response rate, on the basis of limited data [20].

Two-dose therapy means that the patient is given 2 individual doses of 10 mg/kg, separated in time by 12 to 24 hours. In other words, the patient receives a total dose of 20 mg/kg, given in 2 divided doses, 12 to 24 hours apart.

Triclabendazole, an antihelmintic agent long used in veterinary medicine, has recently been proven to provide substantial human benefit at much lower doses than bithionol. In 1990, the WHO and pharmaceutical company,

Ciba-Geigy, partnered to conduct clinical trials of triclabendazole and found that 1-2 oral doses of 10 mg/kg body weight administered in a single 24 hour period result in virtually no side effects and has a success rate approaching 100%. Following this research, the Ministry of Health of the Arab Republic of Egypt registered triclabendazole for human use and the WHO Expert Committee on the Use of Essential Drugs has recommended that it be added to the WHO's list of essential drugs (Chitsulo, Montresor, and Savioli). Triclabendazole remains unavailable in most countries, but its future, both in terms of individual treatment and broad-scale public health measures appears very promising [21].

Although use of micro-dilution in determining anti-Candida and anti-Fasciola activity is the best way other methods such as agar diffusion and Article disc diffusion could be utilized as a screening method or in combination with a micro-dilution assay In this study the anti-Candida and anti-Fasciola activities of extract were assessed by measuring the diameter of the zone around the Article discs (6.4 mm in diameter); in other studies discs with different diameters were used The smaller or bigger zone around the colony is related to the sensitivity or resistance of the fungi to the tested materials. Because there are differences in measurement of the transparent zone and also because different amounts and dilutions of the tested materials were used, comparisons are difficult [22].

CONCLUSION

There are limitations in the use of antifungal and anti-parasite drugs, such as resistance, toxic effects, and allergic reactions. Further, in some countries plants are used in traditional medicine. For these reasons we need to compare antifungal and anti-parasite drugs with plant-derived ones, which are cheaper, safer and more nature-friendly. Although the anti-Candida and anti-Fasciola activities of essential oils are different due to factors such as environmental conditions, extraction methods and non-standardized processing,we will hope for the futures of these products. Essential oils could be used as herbal medicine for some diseases, after, of course, further experiments with more strains of Candida and Fasciola in animal models and human volunteers.

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