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ORIGINAL ARTICLE

Study on Antifungal activity of *Artemisia L.* extract in Compared with Tryptophan against trichophytonmentagrophytes

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ABSTRACT
Dermatophytosis or ringworm is a clinical condition caused by fungal infection of the skin in humans, pets such as cats, and domesticated animals such as sheep and cattle. The genus Artemisia (Asteraceae) comprises over 400 species, many of which have an aromatic, bitter taste. These herbs have been used worldwide in folk medicine since ancient times. They have been used as tonics, antimalarials, antihelmintics, and antidiabetics, and in treating wounds, bronchitis, ulcers, and tuberculosis in traditional Anatolian medicine. 40 male Wistar rats (200±20 g and 9-weeks aged) were selected for the study. Rats were randomly divided into four equal groups: 1- normal controls; 2- infected rats received no treatment; 3- infected rats treated with tryptophan and 4- infected rats treated with Artemisia extract. After 12 weeks, blood samples were taken from retro-orbital plexus for cultivate in the mycobiotic agar medium. Data obtained from measurement of colonies diameter showed that there is significant difference in groups which have received different doses of amino acid and herbal extract (P<0.05). Also, it has been shown that the efficacy of high doses of amino acid is more than low doses so can state that it act as dose dependently. But in compared with group 4, herbal extract showed better antifungal activity against trichophytonmentagrophytes.The strong effects of the essential oils of Artemisia are probably due to the high amount of terpenoids and flavonoids especially α-thujone content.
Keywords: *antifungal activity, Artemisia L. extract, tryptophan, trichophytonmentagrophytes, Rats.*

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INTRODUCTION

Dermatophytosis or ringworm is a clinical condition caused by fungal infection of the skin in humans, pets such as cats, and domesticated animals such as sheep and cattle. The term "ringworm" is a misnomer, since the condition is caused by fungi of several different species and not by parasitic worms. The fungi that cause parasitic infection (dermatophytes) feed on keratin, the material found in the outer layer of skin, hair, and nails. These fungi thrive on skin that is warm and moist, but may also survive directly on the outsides of hair shafts or in their interiors. In pets, the fungus responsible for the disease survives in skin and on the outer surface of hairs[1].

It has been estimated that currently up to twenty percent of the population may be infected by ringworm or one of the other dermatophytoses. It is especially common among people who play sports, wrestling in particular. Wrestlers with ringworm may be withheld from competition until their skin condition is deemed non-infectious by the proper authorities [2].

Misdiagnosis and treatment of ringworm with a topical steroid, a standard treatment of the superficially similar pityriasisrosea, can result in tinea incognito, a condition where ringworm fungus will grow without typical features like a distinctive raised border thus, we aimed to use herbal medicine against fungal agents which is safer than synthetic drugs[3].

The genus Artemisia (Asteraceae) comprises over 400 species, many of which have an aromatic, bitter taste. Some say that it is named after the Greek Artemis, who was goddess of the hunt, of forests, and of childbirth [4,5]. Plants of this genus, as for instance A. absinthium, were used to control pain in childbirth and to induce abortions. Most importantly, however, the species Artemisia annua L is now known worldwide for its antimalarial properties. Other Artemisia species have also been used for the treatment of fevers and malaria. A. absinthium and A. abrotanum were used to treat malaria in Europe, and A. afra in Africa [6,7,8].

These herbs have been used worldwide in folk medicine since ancient times [9,10]. They have been used as tonics, antimalarials, antihelmintics, and antidiabetics, and in treating wounds, bronchitis, ulcers, and tuberculosis in traditional Anatolian medicine [11]. There are also several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different Artemisia species [12,13]. The chemical studies on Artemisia species indicate that all classes of compounds are present in the genus with particular reference to terpenoids and flavonoids.

MATERIALS AND METHODS

Extract preparation:

Dried aerial parts (20 g) of the plant cultured on the MS [14]medium were powdered with mortar and pestle. They were extracted with n-hexane (AR grade) with the aid of ultrasonication. The collected supernatants were evaporated into dry extract using rotary evaporator. The crude extracts were dissolved in a combination of acetonitrile (Sigma) and n-hexane (Sigma) solvents and partitioned using a separation funnel. The partitioned parts of solvents were tested for artemisinin using thin layer chromatography (TLC). The fraction with artemisinin was dried using rotary evaporator. Then, the dried fraction was weighed and purified via column chromatography based on the method by El-Feraly[15]. Fractions of 1 ml were tested for presence of artemisinin and fractions that contained artemisinin and a precursor located very near to artemisinin (tested via TLC) were then pooled together and dried with rotary evaporator. It was then purified again by eluting in column chromatography as mentioned above. Fractions with artemisinin and a precursor were pooled into a flask respectively and weighed.

In-vivo procedure

40 male Wistar rats (200±20 g and 9-weeks aged) were selected for the study. The animals were housed under standard environmental conditions (23 ± 1 °C, with 55 ± 5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Rats were randomly divided into four equal groups: 1- normal controls; 2- infected rats received no treatment; 3- infected rats treated with tryptophan and 4- infected rats treated with Artemisia extract.After 12 weeks, blood samples were taken from retro-orbital plexus for cultivate in the mycobiotic agar medium. Rats were killed by dislocation in the cervical vertebrates.

In-vitro procedure

First, the saboroud glucose broth culture media was provided. Thereby 30 gram of ready powder scaled and added to 1 litter distilled water. Erlenmeyer contain culture media and distilled water was occupied on the magnetic heater and during the boiling mixed. Environment was shaded into 10 centimeter head screwd tubes and was autoclaved.0.5CC of tween 80 was shaded into other sterile and head screwed tubes. By spike beak fieldoplatin some of dermatophyte colony were achieved and were resolved in tween 80. Contents of each saboroud glucose broth tubes were empties on one of the dermatophytes resolved in tween 80. The samples after closing the curved (the curved should not be quite sealed) for 21 days were kept in laboratory temperature and after 21 days, tubes were centrifuged and upper portion were discarded and from their sediments used to culturing in solid media. 36 gram of mycobiotic agar powder were scaled and added into 2 litter erlenmeyer that 1 litter of this was distilled water. Afteroccupation of magnet into Erlenmeyer were located on magnetic heater while during the boiling assimilated quietly(from this media were provided in more amounts). Into ten of 250cc erlenmeyer that each of them contains 200ccculture media by turn were provided 5 different concentrations of tryptophan (1, 0.75, 0.5, 0.25, and 0.1 percent). As concentration of 1%, 2gram, for concentration of 75%, 1.5 kilogram, for concentration of 0.5%, 1gram, for concentration of 0.25%, 0.5 gram and for concentration of 0.1%, 2 gram of tryptophan was scaled and added. In control erlenmeyer no added any amino acid. Erlenmeyer after autoclaving in temperature at 121°C and pressure of 15 atmospheres, were divided into 8 centimeter plates and on plates the name of amino acid and their concentration were wrote. Each of trichophytonmentagrophytes counterfoils were cultured in plates contains amino acid and also in plates without amino acid. Cultured plates were located into incubator at temperature of 25°C and after 14 days the diameter of grown colonies were measured. Fungus culturing were done near the gas flame and under sterile conditions.

Statistical analysis

Data were presented as Mean±SEM. The data obtained were tested by ANOVA followed by Tukey'sposthoc multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Data obtained from measurement of colonies diameter showed that there is significant difference in groups which have received different doses of amino acid and herbal extract ($P<0.05$). Also, it has been shown that the efficacy of high doses of amino acid is more than low doses so can state that it act as dose

dependently. But in compared with group 4, herbal extract showed better antifungal activity against trichophytonmentagrophytes (table 1).

Table 1: Comparison of colonies diameter in different concentration of tryptophan and extract in normal and treated groups

Groups		Mean±SEM (diameter)
Control group		28.33±1.02
infected rats received no treatment		32.25±1.48
infected rats treated with tryptophan	1	15.06±1.10
	0.75	11.57±1.03
	0.5	7.04±0.84
	0.25	5.23±0.63
	0.1	2.36±0.42
infected rats treated with Artemisia extract		1.14±0.34

DISCUSSION AND CONCLUSION

Terpenoids are the most commonly studied class of metabolites of the genus Artemisia. The essential oil of A. absinthium is found in several pharmacopoeias and there have been numerous studies performed on it. Mainly 4 major components, β-thujone, cis-epoxyocimene, trans-sabinylacetate, and chrysantenyl acetate, have been described from A. absinthium, primarily depending on the origin of the plant [16].

It is known that sabinene is the first bicyclic intermediate to arise in the biosynthetic pathways to the epimericthujones, so the majority of this compound might be due to the stage of the collection. Kordali et al. [17] described chamazulene as the main compound from the A. absinthium of eastern Anatolia.

It might be produced from the unstable sesquiterpene lactone artabsin during the hydrodistillation process [11]. Both A. campestris and A. scoparia contain 1,2-dehydro acenaphthylene, which is a polyaromatic hydrocarbon (PAH), a ubiquitous class of environmental contaminants [18]. It was first wondered whether there had been environmental pollution of the plants or wax contamination during the processing. However, identification of the same compound in 2 different species and a literature survey of this compound confirmed that this compound is indeed synthesized by the plant. It was also found in Italian A. variabilis essential oil and in the essential oil of residues of A. scoparia from India [19,20]. Monoterpenethujone is one of the most characteristic compounds of Artemisia species which have antifungal activity.

Akrout et al. (2010) reported the antimicrobial and antiradical activities of the essential oil of A. campestris originating from Tunisia[21]. Methanolic extracts of A. campestris were also evaluated for antibacterial properties [22]. The extract was reported to have a strong effect on S. aureus and Bacillus subtilis strains.We showed the antifungal activity of Artemisia L. extract which is compatible with several previous studies [23,24,25].

Dermatophytosis is caused by pathogenic, keratin-digesting fungi in the genera Microsporum, Trichophyton and Epidermophyton. Members of Microsporum and Trichophyton cause illness in both humans and animals. E. floccosum is the only species of Epidermophyton known to cause disease, and it usually affects only people. Some authors use the term “dermatophytoids” for soil-dwelling members of Microsporum, Trichophyton and Epidermophyton that are never or rarely associated with disease (e.g., T. terrestre). Dermatophytes, like many fungi, may have two different species names. One name belongs to the asexual form (the anamorph state), which is the form that occurs in vertebrate hosts. The other name is given to the sexual state of the organism [1,3]. The latter form, called the teleomorph form or the “perfect state,” is produced by mating between anamorphs. For example, the dermatophyteMicrosporumcanis infects animals; however, when this organism mates with a compatible environmental organism, the resulting sexual form is called Arthrodermaotae. The teleomorph (perfect) states of both Microsporum and Trichophyton belong to the genus Arthroderma, and dermatophytes known to have sexual states are placed in the phylum Ascomycota, family Arthrodermataceae. Dermatophytes that currently have no known sexual state, like other medically important fungi with this characteristic, are classified as Deuteromycota (Fungi Imperfecti).

Although dermatophytes originated from soil-dwelling keratinophilic organisms, only a few pathogenic species still reside primarily in this niche. These organisms, known as geophilicdermatophytes, are associated with decomposing keratin sources in the environment. M. gypseum and M. nanum are the only two geophilicdermatophytes that are important pathogens in animals. M. gypseum is also seen in people, but M. nanum occurs infrequently [26].

Most species that cause dermatophytosis have become adapted to people or animals, and are now maintained in these reservoirs. Although they can infect other hosts, each dermatophyte tends to be

associated with a particular host or group of hosts, and it is not maintained in other species long term. Zoophilic dermatophytes are adapted to various animal species, while anthropophilicdermatophytes occur in humans [26].

With considering to current study this appears that increase in tryptophan level in serum probably causes hypersensitivity in people against dermatophytosis and were stimulated the dermatophytes growth. Also in Sarasgani and Firozrai study revealed that none of them were inhibited growth of dermatophytes with exception the L-lusin that were elicited to growth inhibition of microsporiumgypseum. Argenine also inconcentration of 1 and 0.1 have inhibitory effects but were not causes complete growth inhibition even inconcentration of 1 gr/dl. Methionine also has no effect on trichophytonverrucosum and was shown mildly effect on microsporiumgypseum. In one other study that was done by Garachorlou et al., [27] reveled that aspargin and methionine amino acids cause decrease in the trichophytonrubrum and trichophytonverrucosum growth . Acidic amino acids also either was shown inhibitory effect on two dermatophytes that the acid aspartic inhibitory effects on microsporiumgypseum growth were determined in pandy study. In one other study by Garachorlou et al., [27] reveled that histidine has inhibitory effect on Trichophyton Mentagrophytes Growth. In current study the inhibitory effect of tryptophan on trichophytonverrucosum were assessed and shown that concentration of 1% tryptophan causes maximum decrease in trichophytonverrucosum growth. The colony diameter in different concentrations of tryptophan in experimental fungi than control group was decreased. This appears that tryptophan causes growth decreasing in epidermophytonfloccosum. A comparison of our results with those of previous studies shows that the locality of the plant material and the extraction procedure cause differences in the antifungal activity of the plants. The strong effects of the essential oils of Artemisia are probably due to the high amount of terpenoids and flavonoids especially α-thujone content.

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