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Antimicrobial Activity of *Thymus leucotrichus* and *Origanum laevigatum*

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The *in vitro* antimicrobial activity of *Thymus leucotrichus* and *Origanum laevigatum* leaf and extracts were studied against selected fungi and bacteria, following disc diffusion method. Leaves were extracted using ethanol and lyophilized. Remaining solid were dissolved in water or carbon tetrachloride. From these solutions, 3 different concentrations were applied to disk (100, 150, 200 µg/disc). The water solutions of extracts of both plants were found to be more effective against bacteria. The carbon tetrachloride solutions of extracts were ineffective in inhibiting the bacterial growth or showed poor inhibition. The inhibition zone diameter was seen to increase with the concentration. These results were compared with results obtained using standard antibiotics, imipenem and ampicilin which served as a reference for inhibition zone diameter.

Key Words: *Thymus leucotrichus, Origanum laevigatum*, Antimicrobial assay.

INTRODUCTION

The use of plants as a source of medicine has been inherited and is an important component of the health care system. Recently, extracts and bioactive compounds which isolated from medicinal plants are used for antibacterial, antifungal and antiviral therapy[1-3].

Turkey has a very rich flora in terms of existing plant diversity. It is located in a region where 3 fitogeographical regions intersect and constitutes a bridge between Southern Europe and Southwestern Asia and Anatolia. As a consequence, endemism of species is very high[4-6]. Some medicinal plants were being used traditionally as medicines in Turkey like other countries in the world. Today, the chemical compounds obtained from medicinal plants are used by people. However, most of these have not been evaluated scientifically and their effects have not yet been explained experimentally. About 25 % of the medicines used for public health have been obtained from these plants so far[7]. Origanum and thymus species is used as medicinal plant for several works. The advantage of use of these plants is no side effects like antibiotics and developed any resistance to pathogen microorganisms[8-10]. The flora

of Turkey has 36 species of thymus and 23 species of origanum[11]. The essential oils of *O. laevigatum* were previously reported[12,13]. Unlike other thymus species for *T. leucotrichus* taxonomic studies was reported[14]. According to the literature, no study has been done about antimicrobial activity of *O. laevigatum* and *T. leucotrichus*.

The principle aim of the present work is to study the antimicrobial activity of *Thymus leucotrichus* and *Origanum laevigatum* leaf extracts in different solvents like, water and carbon tetrachloride against gram positive bacteria including *Bacillus subtilis, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa* and gram negative bacteria *Proteus vulgaris, Escherichia coli* and fungi *Candida albicans*.

EXPERIMENTAL

The fresh matured leaves of the *T. leucotrichus* and *O. laevigatum* were collected randomly during the month of July, from the outskirts of Ahir Mountains from Kahramanmaras, Turkey. The plants were identified by systemic botanist Dr. H. Guray Kutbay Department of Botany, Sciences-Art Faculty, University of Ondokuz Mayis.

Preparation of extracts: Fresh leaves were dried in the dark within 1 week at room temperature. The leaves were extracted with ethanol-water (90 % v/v) for 24 h in soxhlet apparatus. The extracts were lyophilized and then both of them were dissolved in carbon tetrachloride or water and stored in 5 ºC refrigerators.

Test microorganisms: Selected pathogenic bacteria; *Bacillus subtilis* KUEN 16 II D-75, *Candida albicans* KUEN 1475, *E. coli* W 3110, *Proteus vulgaris* KUEN 1329, *Pseudomonas aureginosa* ATCC 28753, *Staphylococcus aureus* ATCC 46300, *Streptococcus pyogenes* KUEN 719 were obtained from culture collection of American Type Culture Collection (ATCC), Microorganisms Collection Center, Faculty of Medicine, University of Istanbul, Istanbul, Turkey (KUEN), Faculty of Pharmaceutical Sciences, Chiba University (W 3110). All the test bacterial species were maintained on media. The bacterial cultures were maintained in Mueller Hinton Agar (MHA), Candida albicans was maintained on Sabroud Dextrose Agar (SDA).

Antimicrobial assay: Antimicrobial activity of water and carbon tetrachloride solutions of extracts of the plants were determined by the disc diffusion method[15]. Suspension of the antibacterial strains with an optical density of McFarland 0.5 were made in distilled water. Sterile petri dishes (∅ 14 cm) with 60 mL of sterile MHA and SDA were seeded with appropriate bacterial suspension. Sterile paper discs (Whatmann, 5 mm diameter) were impregnated with the solutions 100, 150, 200 µg/disc and discs were allowed to dry and then discs were spaced on the agar surface of each sterile petri dishes negative control of discs water and carbon tetrachloride were included. The diameter of the inhibition zone were measured after 24 h at 38 ºC. The reference antibiotics imipenem (10 µg/disc) and ampiciline (10 µg/disc) were used for comparative and control purpose.

RESULTS AND DISCUSSION

The ethno botanical screening tests of ethanol extracts of *T. leucotrichus* and *O. laevigatum* leaves were prepared. The solution of lyophilized extract in different solvents against both bacteria and fungi using disc diffusion technique are depicted in Table-1. The water solutions of the extracts are found to be more effective against bacteria rather than fungi. Carbon tetrachloride extract was found to be ineffective or showed poor inhibition of bacterial and fungal growth.

TABLE-1
INHIBITION ZONE DIAMETER (mm) OF ANTIBACTERIAL ACTIVITY

Bacterial	Conc. (μg)	*Origanum laevigatum*		*Thymus leucotrichus*		Ampicilin (10 μg)	Imipenem (10 μg)
		CCl_4	Water	CCl_4	Water		
*Staphylococcus aureus**	100	-	10	-	8	15	41
	150	-	14	-	10		
	200	-	16	8	17		
*Streptococcus pyogenes**	100	7	6	8	-	35	32
	150	7	7	9	7		
	200	7	10	11	8		
*Proteus vulgaris***	100	6	8	-	-	19	26
	150	7	9	-	-		
	200	8	9	-	-		

*Gram positive; **Gram negative.

Extract of *O. laevigatum* showed the antibacterial activity on 3 bacteria (*P. vulgaris*, *S. aureus* and *S. pyogenes*). The antibacterial activity of *T. leucotrichus* was observed on two bacteria (*S. aureus* and *S. pyogenes*). Inhibition zone diameters obtained (mm) was given in Table-1. *O. laevigatum* showed antimicrobial activity against both gram positive and gram negative bacteria, *T. leucotrichus* only showed inhibition against Gram positive bacteria. Extracts of plants would generally be active Gram positive bacteria than Gram negative bacteria[3,16]. The solutions of leave extracts showed no antimicrobial activity against *E. coli*, *B. subtilis*, *P. aureginosa* and *C. albicans*.

The solution of extracts in polar and non-polar solvents showed different activities. The water solution of *O. laevigatum* extract showed similar or higher activity than the non-polar CCl_4 solution. Since the essential oils of *O. laevigatum* consist of Thymol and carvacol *etc.*, (Fig. 1) which are polar compounds they are readily soluble in polar solvents such as water. On the other hand the ethanol extract of leaves were not soluble completely in the CCl_4 due to non-polar character of CCl_4 because of this problem some of the active compounds possibly not dissolved and the effect of antibacterial activity of CCl_4 solution is not high.

The inhibition zone diameters of all the extracts were seen to increase with the concentration increased. This is generally observed in water solution of the extract.

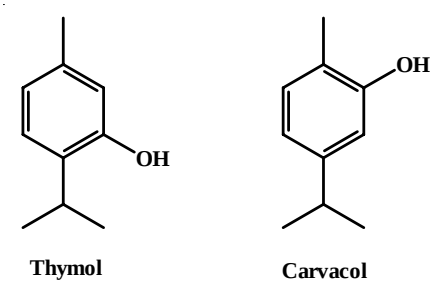

Fig. 1. Molecule structures of thymol and carvacol

For example when the water solution of *T. leucotrichus* extract applied 100, 150 and 200 µg against *S. aureus* measured inhibition zone diameter in were 8, 10 and 17 mm, respectively. Similarly increasing inhibition zone diameters were also measured water solution of *O. laevigatum* extract which are 10, 14 and 16 mm, respectively. This inhibition zone diameter against *S. aureus* is comparable with inhibition zone diameter ampicilin used. Similar inhibition zone diameter CCl_4 and water solution of *O. laevigatum* extract were observed against *S. pyogenes*. On the other hand *T. leucotrichus* extract in CCl_4 was more effective than water solution against *S. pyogenes*. The effect of the water solution of *O. laevigatum* and *T. leucotrichus* extract were more active against *S. aureus* than *S. pyogenes*. Antimicrobial activity of *O. laevigatum* on *P. vulgaris* in the water and CCl_4 showed similar inhibition zones which are between 6-9 mm, on the other hand under same conditions *T. leucotrichus* showed no inhibition. We also studied activity of extract against *B. subtilis*, *P. aeruginosa* and gram negative bacteria *E. coli* and fungi *C. albicans* and no inhibition were observed in all studied conditions.

Thymol and carvacol are known for having active ingredients of *O. laevigatum* and *T. leucotrichus*[14,17] which are reported as effective antimicrobial compounds in plants[18]. Due to phenolic OH these compounds are slightly polar and therefore they are soluble in water. When ethanol extracts of the plants were dissolved in non-polar solvents such as CCl_4 possibly little amount of active compounds were taken into solvent. Because of this CCl_4 solution of ethanol extract has low concentration of carvacol and thymol and thus, showed little or no inhibition on bacteria studied.

Conclusion

Antimicrobial activity of ethanol extract of *O. laevigatum* was observed on *S. pyogenes, S. aureus* and *P. vulgaris*. Similarly *T. leucotrichus* showed antimicrobial activity against *S. pyogenes* and *S. aureus*. The water solution of ethanol extracts of *T. leucotrichus* and *O. laevigatum* showed strong inhibition against *S. aureus* where higher inhibition zone diameters measured than ampicilin.

Further work is needed to isolate the active principle compounds from the plant extracts and carry out pharmaceutical studies.

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