ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *OLEAEUROPAEA* AND *CUMINUM CYMINUM* EXTRACT

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خلاصہ

اویلا ایوپیوا اور Cuminum cyminum کے antibacterial سرگرمیوں بیکٹیریا Staphylococcus aureus، strains E. coli اور *aeruginosa* P اتحقیقات میں. Olea یوروپیوا اور Cuminum cyminum کی اینٹیفیلل سرگرمیوں میں فنگل strains میں *A. niger* s، Fusarium oxysporum اور Candida albicans کی تحقیقات کی گئی. اویلا ایوپیوا اور Cuminum cyminum آکسوں کو مسابقتی مقدار اور تیل میں تیار کیا گیا تھا. سالوینٹس میتانول (50٪ v / v) اور DMSO (50٪(v / v تھے. اویلا یورپیا کے تیل کے مختلف مقدار (V / V٪) پر مشتمل آٹھ مختلف حجم (3.1µl، 6.25µl، 12.5µl، 25µl، 50µl، 100µl، 500µl) اینٹی ویکٹیریل سرگرمی کے لئے استعمال کیا جاتا تھا. Zera [3.1(1.55mg) µl، 6.25 µl (3.1 ملی گرام)، 12.5 µl (6.25 ملی گرام)، 25 µl (12.5 ملیگرام)، 50(25mg) µl، 100(50mg) µl، 200(100mg) µl] استعمال کیا گیا تھا.

اے یوروپیا اور سی کیمینسم آکسوں کی اینٹی بیکٹیریل خصوصیات کو چیک کرنے کے لئے، ایک اینٹی بیکٹیریل عہد قائم کیا گیا تھا.

ترقی کی جانچ پڑتال کی گئی تھی جس میں آپریشنل کثافت (اوڈی) کی پیائش کی جاسکتی ہے جس میں 600 ملی میٹر پر سپیکٹروفیٹو میٹر کا استعمال ہوتا ہے. پلانٹ کے نکات کی اینٹیفنگل سرگرمیوں کا تجزیہ کرنے کے لئے (اے یوروپیا: 150 µl، 300 µl، 600 µl اور C. سیمنز: 75 ملیگرام، 150 ملیگرام، 300 ملی گرام)، اینٹیفیلل ہمت ڈیزائن کیا گیا تھا. اینٹیفنگل سرگرمی کے لئے، الگموم ڈیبیمیو اور میتھول الگ الگ [50٪(v / v) میں Cuminum cyminum بیج کے نکات [604.80 µl (300 75 ملی گرام)، 302.40 µl (150 ملی گرام) اور 151.20 µl (75 ملی گرام)] تیار کئے گئے تھے.]. زیتون کا تیل (50 µl) اور زیرہ (25 12.5 / µl ملی میٹر) کے میتھولک نکات کے ساتھ، پی. ایر گینوسا میں زیادہ سے زیادہ اینٹی بیکٹیریل سرگرمی (اوڈی: 0.006) دیکھی گئی. زیادہ سے زیادہ اینٹی بیکٹیریل سرگرمی (اوڈی: 0.004) ایس جی کی بیکٹیریل کلچر میں بھی دیکھی جاسکتی ہے جس میں میتھولول (25 12.5 / µl ملی گرام) کی میتانول نکالنا ہے. اس کے علاوہ، زیادہ سے زیادہ antibacterial سرگرمی E. coli (OD: 0.007) کے بیکٹریل ثقافت میں دیکھا گیا تھا جنات کے ساتھ میتانول نکالنے کے ساتھ (3.1 1.55 / µl ملی گرام).

سی سیمینم میبتانول کے 151.20 µl کی ایک کم از کم حراست میں، اے اینگر کے خلاف زیادہ سے زیادہ فنگل نمائش (آر جیبی: 1.6 سینٹی میٹر) ظاہر ہوا. سی سیمینوم میبتانول نکالنے کے اسی کم از کم حراستی کے ساتھ، سی البیسیوں کے خلاف دوسرا زیادہ سے زیادہ فنگل انشورنس (RGIB: 1.8 سینٹی میٹر) کا مشاہدہ کیا گیا تھا. اے یوروپیا میبتانول کے 150 µl کی کم از کم حراست میں سے ایک نکالنے سے سی ایلیسیوں کے خلاف زیادہ سے زیادہ فنگل نمائش (آر جی بی: 1.3 سینٹی میٹر) ظاہر ہوا. اے یوروپیا میبتانول نکالنے کا ایک ہی کم از کم حراستی کے ساتھ، ایک نجی آر جیبی: 1.9 سینٹی میٹر) اے اینگر کے خلاف منعقد کیا گیا تھا. اے یوروپیا کے DMSO نکالنے (150 µL) نے ایک جی جی بی: 1.9 سینٹی میٹر ایف. آکسسیفورم کے خلاف بھی دکھایا ہے. مجموعی طور پر، Cuminum cyminum (میبتانول نکات) ایک اینٹی آلودگی ایجنٹ کے طور پر O. یوروپا کے مقابلے میں زیادہ مؤثر پایا گیا تھا. مجموعی طور پر، سیمینوم اور اے یوروپیا دونوں کے میتھولیولک نکات ڈیبیمیائی آکسوں کے مقابلے میں زیادہ سے زیادہ نمائش کے لحاظ سے antifungal سرگرمیوں میں زیادہ مؤثر تھے. فنگل ہمسایہ میں، سی. یوروپیا کی نکالنے سی سیومینم کے مقابلے میں آر جیبی: 1.3 سینٹی میٹر (150 µL) کے ساتھ سی کے اضافہ پر سلنٹ میبتانول میں زیادہ موثر تھا. ان دونوں پودوں کو اینٹیفنگل اور اینٹی بیکٹیریل انفیکشنوں میں دواؤں کے طور پر استعمال کیا جاسکتا ہے.

Abstract

Antibacterial activities of *Oleaeuropaea* and *Cuminum cyminum* in bacterial strains *Staphylococcus aureus*, *Escherichia coli*&*Pseudomonas aeruginosa*were investigated. Antifungal activities of *Oleaeuropaea* and *Cuminum cyminum* in fungal strains *Aspergillus niger*, *Fusarium oxysporum*&*Candida albicans*were investigated. Eight different volumes (3.1µl, 6.25µl, 12.5µl, 25µl, 50µl, 100µl, 250µl, 500µl) containing different amounts (v/v%) of *Olea europaea* oil were used for the antibacterial activity. The different concentrations of *Cuminum cyminum* [3.1µl (1.55mg), 6.25µl (3.1mg), 12.5µl (6.25mg), 25µl (12.5mg), 50µl

(25mg), 100µl (50mg), 200µl (100mg)] were used. The bacterial growth was checked by measuring optical density (OD) using a spectrophotometer at 600 nm. To analyze the antifungal activity of plant extracts (*O. europaea:* 150 µl, 300 µl, 600 µland *C. cyminum:* 75 mg, 150mg, 300 mg), antifungal assay was designed. For antifungal activity, three different concentrations [604.80µl (300 mg), 302.40µl (150 mg) and 151.20µl (75 mg)] of *Cuminum cyminum* seeds extracts were prepared in solvents DMSO and methanol separately [50% (v/v)]. With methanol extracts of Olive oil (50 µl) and cumin (25 µl/12.5 mg), the maximum antibacterial activity (OD: 0.006) was seen in *P. aeruginosa*. Maximum antibacterial activity (OD: 0.004) was also seen in the bacterial culture of *S. aureus* with methanol extract of cumin (25 µl/12.5 mg). A minimum concentration of 150 µl of *O. europaea* methanol extract showed greater fungal inhibition (RGID: 1.3 cm), against *C. albicans*. Overall, *Cuminum cyminum* (methanol extracts) was found more effective as an antibacterial agent as compared to *O. europaea*. Overall, methanolic extracts of both *C. cyminum* and *O. europaea* were more effective in antifungal activities in terms of maximum inhibition as compared to DMSO extracts. In fungal assay, the extract of *O. europaea* was more effective in solvent methanol on the growth of *C. albicans* with RGID: 1.3 cm (150 µl) as compared to *C. cyminum.* Both these plants can be used as pharmaceutical in antifungal and antibacterial infections.

Keywords: *Cuminum cyminum*; *Olea europaea*; Methanol extract; Dimethylsulfoxide extract; Antibacterial; Antifungal;*Pseudomonas aeruginosa; Staphylococcus aureus; Candida albicans; A. niger;* Radial growth inhibition diameter (RGID)

Introduction

In this research, the antibacterial and antifungal effects of whiteCumin *(Cuminum cyminum)*seedsand *Olea europaea* (Olives) were determined. Plants, herbsand their extracts withtheir specific chemical compositions have proved less toxic as per traditional medicine. It is evident that *O. europaea* and *C. cyminum* show antibacterial and antifungal effects against bacterial and fungal pathogens. Plants play a critical role in the control and treatment of numerous diseases. The occurrence of microbial resistance is because of an excessive use or misuse of antimicrobial drugs (Bibitha *et al*., 2002; Bax *et al*., 2000; Alade and Lrobi,1993). Therefore, there is a strong need to find natural, broad spectrum and less toxic antimicrobial alternatives. Antimicrobial activities of plant extracts have now been employing in different medicines (Cowan, 1999). Natural medicinal flora extracts have shown inhibitory results towards phytopathogenic fungi *in vitro* (Senhaji *et al*., 2005; Pak *et al*., 2006; Oyedeji *et al*., 2011). Plants contain numerous phytochemicals which are essential in defense against microorganisms, fungi, herbivores, insects and viruses (Duke and Bogenschutz-Godwi, 1998). Outcomes of various spices, crucial oils and natural extracts on the growth inhibition of mycotoxin generating *Aspergillus spp, Fusarium spp* and *Penicillium spp* have been studies. Due to phenolic properties of plants, the antimicrobial abilities of many such molecules derived from herbs, spices or primary oils (Droby *et al.,* 2000). Research has been done to evaluate the feasibility of natural drug treatments to control bacterial infections (Bhuvaneswari *et al*., 2006; Abutbul *et al*., 2005; Bhattacharjee *et al*., 2010 and Chatterjee *et al*., 2011). Aflatoxins, fumonisins, trichothecenes, ochratoxin A (OA), cyclopiazonic acid, zearalenone, deoxynivalenol, citrinin, gliotoxin and sterigmatocystin are the essential mycotoxins and are extremely poisonous and cancerous (Reddy *et al*., 2010). Exposure to such fungus can cause genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive problems and immune suppression (Lacey, 1988). The bactericidal activity of virgin olive oil for numerous microorganisms has been studied *in vitro*. Olive fruit is considered to certainly possess anti-bacterial actions against microbial strains. The antimicrobial natural components of the plant leaves had been proved effective in conventional medicinal drugs to treat fever and infections. Olive's phenolic chemicals have been tested in the inhibition of the growth of *E. coli, K. pneumoniae* and *S. aureus* (Aziz *et al*., 1998; Paster *et al*., 1988). Many gram-positive and gram-negative bacteria had been found sensitive to olive oil polyphenols (Medina *et al.,* 2007). Olive leaves extracts contain phenolic compounds and they show antimicrobial actions against various bacteria, yeasts, molds, fungi, parasites and viruses. It is know that phenolic compounds of olive fruit obstruct growth of *K. pneumoniae*, *S. aureus* and *E. coli* (Korukluoglu *et al.* 2006). Candidiasis is a common invasive fungal infection, which commonly exists in non-neutropenic patients (Eggimann *et al*., 2003). Resistance in anti-fungal diseases has also been increased (Rapp, 2004). Crucial oils from many vegetation include antifungal activity (Kalemba and Kunicka, 2003). A human consumption of mycotoxins contaminated grains especially from the species of *Aspergillus, Fusarium* and *Penicillium* can develop fungal diseases. The primary toxic consequences of these fungal metabolites are reported in the form of carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity and reproductive disorders (Rocha *et al*., 2005). White cumin (*Cuminum cyminum*) is known for its antioxidant activity (Burits and Bucar, 2000), antimicrobial activity (Hanafy and Hatem, 1991), the hypoglycemia effect, antitumor impacts (Salomi *et al.,* 1992), and the anti-nociceptive impacts (Abdel-Fattah *et al.,* 2000; Ani and Okorie, 2006). Cumin aldehyde, cymene, and terpenoids are the principal elements of volatile oils of *cumin*. The cumin oil exhibited excessive antioxidant activity due to its monoterpene alcohols, flavonoids and different polyphenolic compounds (Najda

et al., 2008). Active compounds to cumin have been proved beneficial for lymphocytes, adrenal glands and spleen. The aim of the current research was to determine antibacterial and antifungal potential of *Oleaeuropaea* and *Cuminum cyminum* extracts prepared in methanol and DMSO. The antibacterial effect was determined against *Staphylococcus aureus*, *Escherichia coli*&*Pseudomonas aeruginosa* while antifungal effect was determined against *Aspergillus niger*, *Fusarium oxysporum*&*Candida albicans*.

Materials and Methods

Antibacterial activities of *Oleaeuropaea* and *Cuminum cyminum* against bacterial strains *Staphylococcus aureus* (grampositive), *Escherichia coli* (gramnegative) &*Pseudomonas aeruginosa* (gram negative)and antifungalactivities of these two species *Oleaeuropaea*&*Cuminum cyminum*were determined againstfungal strains *Aspergillus niger* (mycotoxin and spore producing Fungi), *Fusarium oxysporum* (mycotoxin generating Fungi) &*Candida albicans* (Yeast) were investigated. Dimethylsulfoxide (DMSO) and methanol were used as solvents for this research. *Olea europaea*oil extracts were prepared in equal amount of solvent (10 ml oil and 10 ml of each solvent). So the final amount of methanol and DMSO was 50% (v/v). The extracts of *Cuminum cyminum* were also prepared by using same solvents. Eight different volumes (3.1µl, 6.25µl, 12.5µl, 25µl, 50µl, 100µl, 250µl, 500µl) containing different amounts (v/v%) of *Olea europaea* oil were used for the antibacterial activity. The different concentrations of *Cuminum cyminum* [3.1µl (1.55mg), 6.25µl (3.1mg), 12.5µl (6.25mg), 25µl (12.5mg), 50µl (25mg), 100µl (50mg), 200µl (100mg)] were used. The prepared extracts were microfiltered by using 0.2µm pore's size syringe micro-filter. All glass wares, tips and media used in the current research were autoclaved before use. Bacterial and fungal strains used in current research were obtained from the Fungal Culture Bank, Institute of Agriculture Sciences, University of the Punjab, Lahore.

Extracts Preparation

The *Cuminum cyminum* seed samples were washed with sterile distilled water and dried in laminar flow hood. The plant substances had been weighed by using a weighing balance and 10g seeds of *Cuminum cyminum* were crushed in pestle and mortar with the addition of 10 ml of each solvent. The seeds were crushed separately for each of the solvent. Two different solvents used were methanol and DMSO. The extracts in both solvents were centrifuged at 8000 rpm for 5 minutes. The supernatant containing plant extract was filtered through a 0.2 µm syringe micro-filter into 1.5 ml Eppendorf tube. The extract was stored at 4^0C in refrigerator until further used. The extraction process was carried out in laminar flow to ensure sterility.

*Oleaeuropaea*oil was mixed with equal amounts of both of the solvents in separate test tubes. A 5 ml olive oil was mixed in 5 ml Dimethylsulfoxide solvent (DMSO) in test tube and with 5 ml of methanol in separate test tube. The two prepared oil extracts were left overnight in a shaking incubator at 37^0C to allow for proper mixing. Following overnight incubation, the two types of extracts were subjected to microfiltration with a 0.2 µm syringe micro-filter and transferred into 1.5 ml Eppendorf tube. The extracts were stored at 4^0C in refrigerator until further use.

Antibacterial bioassay

To check the antibacterial properties of *O. europaea* and *C. cyminum* extracts, an antibacterial assay was set up using Eppendorf tubes and labeled accordingly. Each Eppendorf tube contained the following components, LB broth medium (100µl), bacterial culture (10µl), *Cuminum cyminum* seed extracts in methanol and DMSO (variable volumes) as mentioned in Table 1and *Olea europaea* oil extracts in methanol and DMSO (variable volumes) as mentioned in Table 1. All Eppendorf tubes were incubated at 37^0C for 72 hours and checked for the growth after incubation period. The growth was checked by measuring optical density (OD). Spectrophotometerwas used to measure the optical densities of experimental samples to calculate antimicrobial effects of plants extracts by measuring OD at 600 nm for bacteria as described by Wahab *et al*. (2016). The medium only (without any bacterial strain and plant extract) was used as blank while medium with bacterial strain but devoid of any plant extract was used as negative control. The medium used was LB broth and 1L medium contained tryptone 10g, yeast extract 5g and sodium chloride 10g.Comparative results were organized into tables and analyzed for bacterial growth inhibition effect.

Antifungal assay

For antifungal activity, three different concentrations [604.80µl (300 mg), 302.40µl (150 mg) and 151.20µl (75 mg)] of *Cuminum cyminum* seeds extracts were prepared in solvents DMSO and methanol separately. Three different volumes (150µl, 300µl, 600µl) of *Olea europaea* oil were prepared in solvents DMSO and methanol separately by maintaining total concentration of stock as 50% (v/v).The antifungal assay method adopted with some modifications and this assay was described earlier by Awad *et al*., (2018). Potato Dextrose Agar (PDA) prepared in a flask and autoclaved. The composition of 1 Liter PDA was Potato Infusion 200g, Dextrose 20g, Agar 20g and final volume of the PDA medium was adjusted to 1 Liter with distilled water before

autoclaving.After autoclaving the PDA medium was cooled at 55 ^{0}C and *C. cyminum* extract with final concentration (75 mg, 150mg, 300mg) and *O. europaea* oil were added in separate flasks containing PDA media and the medium was poured into petri plates under aseptic conditions. The media plates were allowed to solidify at room temperature after 24 hours incubation. The central area of the petri plates was cut with a disk and placed a fungal disk in the center of the plates under strict aseptic conditions. The fungal disk containing media plates were incubated at 37 ^{0}C for 5 days and the results were recorded in terms of fungal growth inhibition diameter in cm. In order to avoid bacterial growth in media, Ampicillin drug (100 µg/ml) was added.

Table 1.Experimental design showing concentrations of sample plant extracts.

Exp. No.	Bacterial strain	Olive Oil				White Cumin seeds				Control 2
		DMSO		Methanol		DMSO		Methanol		
		3.1 µl	**50 µl**	**3.1 µl**	**50 µl**	**3.1 µl** (1.55mg)	**25 µl** (12.5mg)	**3.1 µl** (1.55mg)	**25µl** (12.5mg)	**100 µl**
Exp. 1	*P.aeruginosa*(P)	3.1 µl	50 µl	3.1 µl	50 µl	3.1 µl	25 µl	3.1 µl	25 µl	100 µl
	S.aureus(S)	3.1 µl	50 µl	3.1 µl	50 µl	3.1 µl	25 µl	3.1 µl	25 µl	100 µl
	E.coli(E)	3.1 µl	50 µl	3.1 µl	50 µl	3.1 µl	25 µl	3.1 µl	25 µl	100 µl
		6.25 µl	**100 µl**	**6.25 µl**	**100 µl**	**6.25 µl** (3.1mg)	**50 µl** (25mg)	**6.25 µl** (3.1mg)	**50 µl** (25mg)	**100 µl**
Exp. 2	*P.aeruginosa*(P)	6.25 µl	100 µl	6.25 µl	100 µl	6.25 µl	50 µl	6.25 µl	50 µl	100 µl
	S.aureus(S)	6.25 µl	100 µl	6.25 µl	100 µl	6.25 µl	50 µl	6.25 µl	50 µl	100 µl
	E.coli(E)	6.25 µl	100 µl	6.25 µl	100 µl	6.25 µl	50 µl	6.25 µl	50 µl	100 µl
		12.5 µl	**250 µl**	**12.5 µl**	**250 µl**	**12.5 µl** (6.25mg)	**100 µl** (50mg)	**12.5 µl** (6.25mg)	**100 µl** (50mg)	**100 µl**
Exp. 3	*P.aeruginosa*(P)	12.5 µl	250 µl	12.5 µl	250 µl	12.5 µl	100 µl	12.5 µl	100 µl	100 µl
	S.aureus(S)	12.5 µl	250 µl	12.5 µl	250 µl	12.5 µl	100 µl	12.5 µl	100 µl	100 µl
	E.coli(E)	12.5 µl	250 µl	12.5 µl	250 µl	12.5 µl	100 µl	12.5 µl	100 µl	100 µl
		25 µl	**500 µl**	**25 µl**	**500 µl**	**25 µl** (12.5mg)	**200 µl** (100mg)	**25 µl** (12.5mg)	**200 µl** (100mg)	**100 µl**
Exp. 4	*P.aeruginosa*(P)	25 µl	500 µl	25 µl	500 µl	25 µl	200 µl	25 µl	200 µl	100 µl
	S.aureus(S)	25 µl	500 µl	25 µl	500 µl	25 µl	200 µl	25 µl	200 µl	100 µl
	E.coli(E)	25 µl	500 µl	25 µl	500 µl	25 µl	200 µl	25 µl	200 µl	100 µl
Control1	*Only media for all experiments to check the contamination*									

Note: DMSO and Methanol used in all experiments was 50% v/v. LB broth used was 100µl. Control 1: LB broth medium only; Control 2: LB broth medium with bacterial culture only.

Results

Antibacterial Activity of Olive Oil and White Cuminum Seeds

The maximum antibacterial activity was seen in experiment 1 in which minimum concentrations of the extracts were used. And the minimum antibacterial activity was seen in experiment 4 which increased concentrations of the extracts were used. In first experiment (in which minimum concentrations of extract were used), the maximum antibacterial activity (OD: 0.006) was seen in the bacterial culture of *P. aeruginosa* with methanol extracts of Olive oil (50 µl concentration) and cumin (25 µl/12.5 mg concentration). In the same experiment, the maximum antibacterial activity (OD: 0.004) was seen in the bacterial culture of *S. aureus* with methanol extract of cumin (25 µl/12.5 mg concentration). In the same experiment, the maximum antibacterial activity (OD: 0.007) was seen in the bacterial culture of *E. coli* with methanol extract of cumin (3.1 µl/1.55 mg concentration). The details of all four experiments are mentioned in **Table 2.** Overall, among all four experiments, the maximum antibacterial activity (OD: 0.002) was observed with increased concentrations with methanol extracts of cumin(i.e., 100µl/50 mg & 200 µl/100 mg) and methanol extract olive oil (i.e., 500µl) against *S. aureus* and *P. aeruginosa* respectively. Overall, *Cuminum cyminum* (methanol extracts) was found more effective as antibacterial agent as compared to *Olea europaea*.

Table 2.Antibacterial effects of *O. europaea* and *C. cyminum* at different concentrations.

OPTICAL DENSITY RESULTS										
Exp. No.	Bacterial Culture (µl)	Olive Oil				White Cumin Seeds				Control
		DMSO		Methanol		DMSO		Methanol		
		3.1 µl	50 µl	3.1 µl	50 µl	3.1 µl (1.55 mg)	25 µl (12.5 mg)	3.1 µl (1.55 mg)	25 µl (12.5 mg)	
Exp. 1	*P. aeruginosa* (P)	0.069	0.025	0.017	0.006*	0.059	0.076	0.007	0.006*	0.12
	S. aureus (S)	0.018	0.057	0.027	0.007*	0.060	0.035	0.010	0.004*	0.10
	E. coli (E)	0.010	0.008*	0.029	0.016	0.078	0.046	0.007*	0.037	0.11
Exp. 2		**6.25 µl**	**100 µl**	**6.25 µl**	**100 µl**	**6.25 µl** (3.1 mg)	**50 µl** (25 mg)	**6.25 µl** (3.1 mg)	**50 µl** (25 mg)	
	P. aeruginosa (P)	0.008	0.014	0.015	0.004*	0.058	0.050	0.007*	0.034	0.13
	S. aureus (S)	0.016	0.005*	0.013	0.006	0.10	0.080	0.006	0.003*	0.11
	E. coli (E)	0.008	0.004*	0.006	0.004*	0.004	0.003*	0.035	0.006	0.10
Exp. 3		**12.5 µl**	**250 µl**	**12.5 µl**	**250 µl**	**12.5 µl** (6.25 mg)	**100 µl** (50 mg)	**12.5 µl** (6.25 mg)	**100 µl** (50 mg)	
	P. aeruginosa (P)	0.090	0.003*	0.008	0.004	0.008	0.005	0.006	0.004	0.12
	S. aureus (S)	0.080	0.004*	0.009	0.005	0.009	0.004	0.005	0.002*	0.11
	E. coli (E)	0.009	0.004*	0.090	0.007	0.080	0.004	0.005	0.003*	0.11
Exp. 4		**25 µl**	**500 µl**	**25 µl**	**500 µl**	**25 µl** (12.5 mg)	**200 µl** (100 mg)	**25 µl** (12.5 mg)	**200 µl** (100 mg)	
	P. aeruginosa (P)	0.005	0.003	0.006	0.002*	0.006	0.004*	0.007	0.006	0.11
	S. aureus (S)	0.010	0.004	0.009	0.002*	0.004	0.003	0.009	0.002*	0.11
	E. coli (E)	0.009	0.005	0.005	0.003*	0.007	0.004	0.008	0.003*	0.10

***Grey color** in table shows the maximum antibacterial activity (OD value) of *Cuminum cyminum* and *Olea europaea* against *Psedumonas aeruginosa*, *Staphylococcus aureus* and *E. coli* for each concentration of extract. DMSO and methanol used in all experiments was 50% v/v. OD was taken at 600 nm. Least concentration in exp 1 (most effective) and maximum in exp 4

Antifungal Activities of Olive Oil and White Cumin Seeds: The extracts of *C. cyminum* and *O. europaea* showed considerable antifungal activities (Tables 3 & 4). The antifungal effects at different concentrations of plant extracts were recorded. A minimum concentration of 151.20 µl (75 mg) of *Cuminum cyminum* methanol extract showed greater fungal inhibition (radial growth inhibition diameter-RGID: 1.6 cm), against *A. niger*. With same minimum concentration of 151.20 µl (75 mg) of *C. cyminum* methanol extract, a second maximum fungal inhibition (RGID: 1.8 cm) was observed against *C. albicans*. A third maximum radial growth inhibition was observed against *F. oxysporum* with DMSO concentration of 151.20 µl (75 mg). A gradual decrease in radial growth diameters of all three fungi was observed as the concentration of the cumin extracts increased from 75 mg to 300 mg.With minimum concentration of 150 µl of *O. europaea* methanol extract showed greater fungal inhibition (RGID: 1.3 cm), against *C. albicans*. With same minimum concentration of 150 µl of *O. europaea* methanol extract, a second maximum fungal inhibition (RGID: 1.9 cm) was observed against *A. niger*. *O. europaea*'s DMSO extract (150 µl) has also shown a second maximum fungal inhibition (RGID: 1.9 cm) against *F. oxysporum.* Gradual decrease in radial growth diameter of all three fungi was observed as the concentration of the olive oil extracts increased from 150 µl to 600 µl. Overall, methanolic extracts of both *C. cyminum* and *O. europaea* were more effective in antifungal activities in terms of maximum inhibition as compared to DMSO extracts. The fungal assay the extract of *O. europaea* was more effective in solvent methanol on the growth of *C. albicans* with radial growth inhibition diameter: 1.3 cm (150 µl) as compared to *C. cyminum.*

Table 3. Radial growth inhibition diameter of both solvents extracts of *Cuminum cyminum* against the selected fungal isolates

Fungi species	Methanol Solvent (50% v/v)	DMSO Solvent (50% v/v)	151.20 (µl) (75 mg)		302.40 (µl) (150 mg)		604.80 (µl) (300 mg)	
	Control	Control	Methanol	DMSO	Methanol	DMSO	Methanol	DMSO
A. niger	No inhibition	No inhibition	1.6 cm*****	2.2cm	1.2 cm	1.9 cm	0.8 cm	1.2 cm
C. albicans	No inhibition	No inhibition	1.8 cm*****	2.1 cm	1.4 cm	1.6 cm	1.1 cm	1.1 cm
F. oxysporum	No inhibition	No inhibition	2.6 cm	2.4 cm*****	2.1 cm	2.2 cm	1.3 cm	1.5 cm

***Grey color** highlights the maximum radial growth inhibition value of *Cuminum cyminum* in least concentration (75mg) against *A. niger*in solvent methanol. While, second maximum radial growth inhibition value of *Cuminum cyminum* in least concentration (75mg) was observed against *C. albicans* (**Green color**) and third maximum radial growth inhibition was observed against *F. oxysporum*highlighted with **Blue color**.
A gradual decrease in radial growth diameter of all three fungi was observed as the concentration of the cumin extracts increased from 75 mg to 300 mg.

Table 4. Radial growth inhibition diameter of both solvents extracts of *Olea europaea* against the selected fungal isolates.

Fungi species	Methanol Solvent (50% v/v)	DMSO Solvent (50% v/v)	150 (µl)		300 (µl)		600 (µl)	
	Control	Control	Methanol	DMSO	Methanol	DMSO	Methanol	DMSO
A. niger	No Inhibition	No inhibition	1.9 cm*****	2.6 cm	1.2 cm	1.7 cm	0.9 cm	1.2 cm
C. albicans	No Inhibition	No inhibition	1.3 cm*	1.8 cm	1.1 cm	1.1 cm	0.7 cm	0.9 cm
F. oxysporum	No Inhibition	No inhibition	2.6 cm	1.9 cm*****	2.0 cm	1.5 cm	1.7 cm	1.0 cm

***Grey color** highlights the maximum radial growth inhibition value of *Olea europaea* oil in least concentration contained in 150 µl against *C. albicans* insolvent methanol. While second maximum radial growth inhibition value of *Olea europaea* oil in least concentration was observed against *A. niger* and *F. oxysporum*highlighted with Green color.
A gradual decrease in radial growth diameter of all three fungi was observed as the concentration of the olive oil extracts increased from 150µl to 600 µl.

Discussion

Plants' antibacterial activity assay (*in vitro*) are the crucial sources of the development of new natural drugs for several infections (Tona *et al.*, 1998). We obtained positive results as inhibitory actions were observed on the tested organism's growth. The antimicrobial and antifungal activities of *Cuminum cyminum* (white seeds) and *Olea europaea* (oil) were clearly observed against the tested bacterial and fungal species. Overall, methanol extracts were found more effective in antibacterial activity, than DMSO. Overall, *Cuminum cyminum* (methanol extracts) was found more effect as antibacterial agent as compared to *Olea europaea*.Cumin has lots of dietary benefits such as digestion due to its composition (Milan *et al*., 2008).The antimicrobial capability of methanolic, dimethylsulphoxide and water extracts of cuminseeds against bacteria *E.coli, P.aeruginosa*and *S. aureus* has been tested. Cumin with an excessive phenolic content and excellent antioxidant potential can be supplemented in such conditions (Thippeswamy and Naidu, 2005). It is now emphasized that the consumption of antioxidant spices can prevent from various infections and health conditions (Dragland *et al.,* 2003). Heinonen *et al.,* (1998) examined antimicrobial activity (*in vitro*) of olive leaves and reported that due to phenolic compounds of olive leaves, the extracts showed antimicrobial effect against gram negative and gram positive bacteria and fungi

(Heinonen *et al.*, 1998). Cumin oils have been extensively studied for its antioxidant (Martinez-Tome *et al*., 2001), antimicrobial (Allahghadri *et al*., 2010) and anticancer (Bukhari *et al*., 2009) effects. Pereira *et al*., (2007) reported evaluated the phenolic composites of *Olea europaea* and tested them against respiratory and intestinal infections from gram positive (*B. cereus, B. subtilis* and *S. aureus*), gram negative (*P. aeruginosa*, *E. coli* and *K. pneumoniae*) and fungi *C. albicans* and *C. neoformans.* They found that even at reduced concentrations, the extracts of olives exhibited both antifungal and antibacterial activities. It was recommended that olive leave extracts can be advised as natural medicine for the treatment of various infections. Sudjana *et al.,* (2009) also investigated extracts of *Olea europaea* against broad spectrum of microorganisms in terms of minimum inhibitory concentrations. The olive extracts were found more effective only against following: *H. pylori, S. aureus* and *C. jejuni*. Mekawey *et al.,* (2009) concluded through release kinetic study, that cumin's essential oil's efficiency to inhibit bacterial growth can be improved by encapsulation. A biological active compound Phenol (EHP) of cumin was extracted by benzene to study for its antifungal activity and anti-tumor agent for six types of tumor cell-lines: HEPG2, HELA, HCT116, MCF7, HEP2, CACO2. This extract was found more effective in MCF7. This cumin's compound's antibacterial activity was also assessed for gram-positive and gram-negative bacterial (Mekawey *et al.,* 2009).

Overall, methanolic extracts of both *C. cyminum* and *O. europaea*were more effective in antifungal activities in terms of maximum inhibition as compared to DMSO extracts. Anti-fungal effects of olive leaves has alsobeen demonstrated by pharmacies (Bennani *et al*., 2000). Among *Candida* species, *C. albicans* is more exclusively responsible for fungal infections (Al Mosaid *et al.,* 2003; Weinstein *et al.,* 1997). The cumin's crucial oils are known as disinfectant and anti-fungal. The aqueous extract of cumin is suggested to inhibit the growth of many pathogens which includes *Escherichia coli*,*Staphylococcus aureus*, *Salmonella species*, *Bacilluscereus* and *Aspergillus niger* (Dua *et al.,* 2013). Cumin's oil, its water extracts and other derived extracts have shown an effective antimicrobial actions. This antibacterial activity has been evaluated in pathogenic gram-positive and gram-negative bacterial strains (Shetty *et al.,*1994; Shetty &Bhat, 1997). Cumin oil's alcoholic extract inhibited the growth of *Klebsiella pneumoniae*. Its clinical isolates have been proved in cellular morphology and medicinal expressions due to its aldehyde content (Derakhshan *et al*., 2008). Faiza *et al.,* (2017)the *Olea europaea*'s extracts were prepared to assess antimicrobial activities of various microbial agents. They reported that *Olea europaea* showed both antifungal an antibacterial functions with ethyl acetate and acetone extracts. Five *Olea europaea*'s extracts in solvents ethanol, water, acetone and ethyl acetate. These extracts were tested against following: *K. apiculata*, *S. cerevisiae*, *S. pombe*, *C. oleophila*, *S. uvarum*and *M. fructicola*. All five extracts found effective in varying quantities (Korukluoglu *et al.,* 2006). Markin *et al.,* (2003) reported that the water extract of *Olea europaea* was most effective against *C. albicans* (Korukluoglu *et al.,* 2006). The aldehydes of olive fruit were found effective for antifungal activities for *Microsporumcanis*, *Tricophytonmentagrophytes* and *Candida* spp. (Battinelli *et al.,* 2006). Zhaveh *et al.,* (2015) used encapsulation chitosan-caffeic acid nanogel in order to improve antimicrobial function of *Cuminum cyminum* against *A. flavus.* Naeini *et al.,* (2014) used alcoholic extract of cumin (*in vitro*) to find its effectiveness against *C. parapsilosis*, *C. krusei*, *C. albicans*, *C. dubliniensis* and *C. glabrata.* It was found that *C. cyminum* exhibited maximum MIC (minimal inhibitory concentration) in wide range of fungal pathogenic *Candida* sp. (Naeini *et al.,* 2014).

Conclusion and Recommendation

The antimicrobial and antifungal activities of *Cuminum cyminum* (white seeds) and *Olea europaea* (oil) were clearly observed against the tested bacterial and fungal species. Overall, methanol extracts were found more effective in antibacterial activity, than DMSO.According to all the results and from different researches about *O. europaea* and *C. cyminum*, both have positive antibacterial and antifungal compounds which inhibit the microbial growths and we can use these two plants for remedy of different pharmaceutical, antifungal and antibacterial related problems. Future '*in vivo'* studies should determine how exactly such nutraceutical agents should be applicable clinically as antimicrobial drugs. Moreover, plant crude extracts should be evaluated in detail with regard to their specific phenolic components.

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