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ANTIMICROBIAL EFFECTS OF THREE MEDICINAL PLANTS EXTRACTS CULTIVATED AT HIGH ALTITUDE AGAINST VARIOUS PATHOGENIC BACTERIA AND FUNGI

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ABSTRACT

The medicinal plants indicated multiple health advantageous properties and have antimicrobial (bacteria and fungi) effects. Moreover, the extracts of these plants are less harmful to the environment, low cost, and their use indicate their low toxicity to humans. The aim of this study was to evaluate the effectiveness of the ethanolic extracts of three famous medicinal plants *Mentha piperita*, *Mentha longifolia*, and *Ocimum basilicum* from Ranyah distinct, KSA (about 2000 meters above level sea) against six pathogenic bacteria and sex pathogenic fungi. This evaluation was carried out through biofilm estimation and membrane filter estimation. The obtained results indicated that the pathogenic bacteria; *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Staphylococcous aureus, Escherichia coli* and *Pseudomonas aeruginosa* were affected by all extracts of the tested plants especially with high concentration of 100%. In the same manner, the pathogenic fungi; *Candida albicans*, *Aspergillus niger*, *Aspergillus ochraceus Aspergillus flavus*, *Penicillium funiculosum*, and *Penicillium ochrochloron* were strongly affected by *M. piperita, M. longifolia,* and *O. basilicum* extracts. It could be concluded that the ethanolic extracts of these medicinal plantswhich collected from high altitude region showed high activities on pathogenic bacteria and fungi. Therefore, it can recommend that these medicinal plants could be usefull as herbalmedicinal treatment after the pharmaceutical preparations.

KEYWORDS:
Medicinal plants, Pathogenic bacteria, Pathogenic fungi, Biofilm, Membrane filter

INTRODUCTION

Plants especially medicinals produce chemical substances and secondary metabolites to protect themselves from the attack of pests and pathogens [1]. Moreover, these plant extracts are generally less harmful to the environment, low cost, and their use indicate their low toxicity to humans [2, 3].

During 1990s, World Health Organization stated sustainable way to bring chief inhabitants fitness in emerging countries of ordinary herbs drugs using [4]. About 25% manufactured, medicinal yields came straight or disjointedly from plants [5].

Therapeutic plants will be the usual basis obtainable of mixes that had the ability to supervisor as antipathogenic bacteria. The pathogenic microorganisms are leading to infective diseases, can develop multi drug resistance which posing as health threats [1]. Terpenoids and flavonoids made their belongings by disturbance of pathogenic bacterial membranes and connection polypeptides embarrassment of pathogenic bacterial proteins to host polysaccharide receptors and alkaloids complexes effected by constraining of efflux pump [7].

Generally, there are different factors that affect the total components and types of phenols and flavonoids extracted from these medicinal plants, as plant origin, the extraction method, solvent type, and the season of harvesting [8].

Lamiaceae is an important family from the medicinal plants which includes more than 200 genera and 3000 species. The genus *Mentha* included more than 25 species. Extracts of genus *Mentha* have impacts against pathogenic bacteria marked interaction in natural drugs, due to their active contents [9]. . *Mentha longifolia* and *M. piperita* commonly known as wild mint and peppermint, respectively, are frequently cultivated in many region of the world [10, 11]. Basil (*Ocimum* spp.) also belongs to the same family and it is cultivated and widely distributed throughout the world and has various chemical compounds such as phenols and flavonoids [12].

Due to the variation of chemical composition of

plant extracts, the biological properties such as insecticidal, antimicrobial activity, antioxidant and anti-inflammatory were variable [13].

Therefore, in this study, we aimed to evaluate the ethanolic extract of three medicinal plants; *Mentha longifolia, Mentha piperita* and *Ocimum basilicum*, grown in high altitude of Taif region, KSA against different pathogenic microorganisms (six species of bacteria and six species of fungi).

MATERIALS AND METHODS

Plant Material Preparation. At May 2021, *M. piperita, M. longifolia* and also *O. basilicum* plants were collected, which growing in Ranyah area southern KSA. The samples were washed, then leaves were collected and dried for 2-3 days at room temperature. Dried leaves were crushed by sterile device to get powders and kept [11]. The plant leaves powder (30 g) had mixed with (300 ml) of absolute ethanol alcohol, (Sigma Aldrich, St. Louis, MS, United States) then put in shaker for overnight at 4°C. The suspension were filtrated and the supernatants were located in Eppendorf tubes then stored at 4°C [14]. The plant extracts were prepared and were used in 25%, 50%, and 100% concentrations [15].

Microorganisms Preparation. The pathogenic bacteria (Gram positive) were *S. aureus*, *S. pneumoniae*, *L. monocytogenes*, while Gram negative were *P. aeruginosa, E. coli,* and *K. pneumoniae*. The pathogenic fungi were *C. albicans*, *A. flavus*, *A. niger*, *A. ochraceus*, *P. funiculosum* and *P. ochrochloron.* The isolates were cultured on Nutrient Agar (Oxoid Ltd., Hampshire, United Kingdom) and Mueller-Hinton Agar (Oxoid, Basingstoke, UK). The plates were incubated at 37°C for 24 hours. The colonies were suspended in Nutrient Broth (Oxoid Ltd., Hampshire, United Kingdom) and Peptone Water (Oxoid, Basingstoke, UK). The suspensions matched 0.5 "McFarland Turbidity Standard" equal to ($1.5X10^8$ CFU / mL) [16]. Each microorganism species was prepared in 6 test tubes.

Biofilm Estimation. The suspensions of pathogenic isolates were used in three test tubes as replicates) for each plant concentration. The isolate suspensions (2 ml) had mixed with 2 ml from each concentrations of each plant extract. All test tubes were incubated at 37°C for 24 hours. Then, each test tube was passed to "Biofilm Estimation" by biofilm formation turbidity used Spectrophotometer at 350 nm [15].

Membrane filter Estimation. The other three test tubes were passed through bacterial membrane filter.

The filtrates were transferred to the Nutrient Agar (Oxoid Ltd., Hampshire, United Kingdom) for bacterial strains, while for fungal strains, Potato Dextrose Agar (Scharlau, Eur. Pharm. 01-483-500) was used. The cultured-plates were incubated for 24 hours at 37°C. Then, the colonies growth percentages were recorded by this equation [Colony number/300 X 100] [17].

Statistical Analysis. One-Way Analysis Of Variance (ANOVA) was used to comare among all means with Duncan's test. All data were manged by "IBM SPSS Statistics, Software Version 23 [18].

RESULTS AND DISCUSSION

Biofilm estimation turbidity and growth percentage for pathogenic bacteria. The antibacterial activities of 25, 50 and 100% concentrations of ethanolic extracts of *M. piperita, M. longifolia* and *O. basilicum* were estimated through biofilm estimation turbidity and are shown in Table (1), Also, these activities were evaluated through growth percentage and shown in Table (2) . In estimation of biofilm turbidity, all extracts and concentrations had significant antibacterial activities while this activity increased with the increase of concentration from 25 to 100%. These increases in antibacterial activities were significantly different among all plant extracts and concentrations ($P<0.001$) except of *S. pneumonia* where it was not affected significantly by different plant extracts and concentrations ($P=0.228$). Also, there were significant differences among all tested pathogenic bacteria ($P<0.001$) with the same concentration of each plant extract except of the concentration (50%) of *M. longifolia* ($P=0.336$). Regard to growth percentages of bacteria, the same increases in antibacterial activities (lower growth percentages) were significantly different among all plant extracts and concentrations ($P<0.001$). Morover, there were significant differences among all tested pathogenic bacteria ($P<0.001$) with the same concentration of each plant extract.

In general, both of *M. longifolia* and *O. basilicum* extracts had a high effect more than *M. piperita* extract through both estimations of biofilm turbidity and growth percentages against all tested pathogenic bacteria. Previous investigation stated that the extracts of *Mentha* species had pharmacological antibacterial effects [19]. The most phenol compounds have an antimicrobial activity [20]. They caused surface webbing, RNA and protein chemical change inhibition for pathogenic bacterial cells [21]. From north Saudi Arabia, the ethanolic extracts of *M. piperita* and *M. longifolia* had the polyphenol visibility and biological activities which including phenolic acids: cynaroside, chloro-genic acid-rosmarinic acid, crypto-chloro-genic acid, flavonoids and naringin. The poly-phenols were: rosmarinic acid, chloro-genic acid, p-coumaric acid, m-coumaric acid,

and crypto-chlorogenic acid. The antibacterial effects of leaf extracts against *P. aeruginosa*, was related to poly-phenols. Also, it had antifungal activities against *A. flavus* related to caeic acid, crypto-chloro-genic acid, and naringin. [22]. *M. piperita* ethanol extract showed act against *S. aureus, S. pneumoniae, K. pneumoniae,* and *E. coli*. [23]. Moreover, *Mentha* had antibacterial effects on *S. aureus, E. coli*, *P. aeruginos*, and *Streptococcus* sp. [21]. *M. piperita* had antibacterial activity on *S. aureus* and *Streptococcus* sp. [24]. Also it had antibacterial activity on *E. coli*, *S aureus*, *P. aeruginosa*, and *K. pneumonia* [25]. It had powerful action against *S. aureus* and *E. coli* [26]. *M. piperita* at KSA had poly-phenol as antibacterial activity against *P. aeruginosa* [22] as well as against *S. pneumoniae, S. aureus, E. coli,* and *K. pneumoniae* [27]. Also, it had a good impact against human pathogenic bacteria such as *E. coli, P. aeruginosa,* and *K. pneumoniae* [23].

M. longifolia from north KSA had poly-phenol as antipathogenic bacterial effects on *P. aeruginosa* [22, 28], as well on *E. coli* [28], and *S. aureus* [29].

In the present study, *O. basilicum* leaf extract had a high impact on the tested bacterial strains. Other studies indicated the same results especially from KSA where it had 3,4-di-hydroxy-phenyl-acetic acid (α-hydroxy-di-hydro-caffeic acid) and caffeic acid and had an effect 3 times higher than those of Romanian origin where the leaf extracts had strong antibacterial on *S. aureus* related to poly-phenol [30]. Also, from different regions of KSA, it had antibacterial activity against *Streptococcus sp., S. aureus, K. pneumoniae* and *E. coli* [31, 32].

Biofilm estimation turbidity and growth percentage for pathogenic fungi. The antifugal activities of the three tested concentrations of ethanolic extracts of the three tested medicinal plants were estimated also through both of biofilm estimation turbidity that are shown in Table (3), and growth percentage that shown in Table (4). The biofilm turbidity indicated that all extracts and concentrations had significant antifungal activities where this activity increased with the increase of concentration from 25 to 100%. These increases in antifugal activities were significantly different among all plant extracts and concentrations (P<0.001). Also, there were high significant differences among all tested pathogenic bacteria (P<0.001) with the same concentration of each plant extract except of the concentration (25%) of *M. longifolia* which had low significant differences (P=0.015) (Table 3). Estimation of growth percentages of tested fungi, the same increases in antifungal activities (lower growth percentages) were significantly different among all plant extracts and concentrations (P<0.001) (Tale 4). Morover, there were significant differences among all tested pathogenic bacteria (P<0.001) with the same concentration of each plant extract.

In this regard, all of *M. longifolia, M. piperita* and *O. basilicum* extracts at the same concentration approximately had the same effect on all tested pathogenic fungi through both estimations of biofilm turbidity and growth percentages. On the other hand, *Mentha* species have potential source as antifungal activity against the pathogenic fungi [25]. The broad-spectrum antifungal activity of *Mentha* was recorded according to its contents as carvone, menthone, piperitenone oxide, menthol [33], *M. piperita* had significant antifungal activity against *Candida* sp. [34]. The antifungal activities associated to plasma membrane rupture, as depolarization and accrued permeable on fungal cells [35]. *M. longifolia* antipathogenic fungi act on *Aspergillus* sp., *Penicillium* sp., *P. ochrochloron* [33], and *A. niger* [36]. The antifungal activity of *O. basilicum* leaf extracts reported against several fungi [37]. It associated with poly-phenols, including 3,4-di-hydroxy-phenyl-acetic, and rutosidegentisic acid [38], rutoside, and caeic acid [39]. *O. basilicum* from Riyadh, KSA had major poly-phenol also 3,4-di-hydroxy-phenyl-acetic acid and rutoside, connected with rosmarinic acid as antifungal activity [40]. Also, the basal oils of *O. basilicum* from Asir, KSA had antipathogenic fungi act on *C. albicans* [41].

TABLE 1
Biofilm estimation turbidity for pathogenic bacteria treated with ethanol extract

Pathogenic bacteria	*Mentha piperita*			*Mentha longifolia*			*Ocimum basilicum*			$F_{df\,(8,18)}$	*P (0.05)*
	25%	50%	100%	25%	50%	100%	25%	50%	100%		
Staphylococcous aureus	0.29±0.01^{Abc}	0.16±0.02^{Abc}	0.80±0.05^{Aa}	0.16±0.01^{C} bc	0.32±0.41^{Ab}	0.04±0.02 BCc	0.19±0.05 Bbc	0.09±0.01 Bbc	0.04±0.01 Bc	8.574	<0.001
Streptococcus pneumoniae	0.27±0.02^{Ca}	0.14±0.01^{Ca}	0.28±0.04^{Ba}	0.20±0.01^{B} a	0.11±0.02^{Aa}	0.06±0.03 Ba	0.22±0.02 Aa	0.10±0.01 Aa	0.06±0.01 Aa	1.492	0.228
Listeria monocytogenes	0.21±0.03^{Da}	0.11±0.01^{Ed}	0.52±0.01^{Be}	0.14±0.01^{D} c	0.06±0.02^{Ae}	0.02±0.01 Cf	0.17±0.02 CDb	0.09±0.01 Bd	0.02±0.01 Cf	106.82	<0.001
Escherichia coli	0.28±0.02^{Ba}	0.14±0.01^{Bc}	0.82±0.01^{Be}	0.12±0.01^{E} d	0.05±0.01^{Ag}	0.01±0.01 Ci	0.16±0.01 Db	0.07±0.01 Cf	0.02±0.01 Ch	14985.45	<0.001
Pseudomonas aeruginosa	0.29±0.01^{Aa}	0.16±0.01^{Ab}	0.90±0.01^{Be}	0.11±0.01^{F} d	0.05±0.01^{Ag}	0.03±0.01 Ch	0.13±0.01 Ec	0.06±0.00 Df	0.02±0.01 Ci	1135.932	<0.001
Klebsiella pneumonia	0.22±0.02^{Db}	0.12±0.01^{Dd}	0.06±0.01^{Bf}	0.24±0.02^{A} a	0.20±0.02^{Ac}	0.09±0.01 Ae	0.18±0.03 BCc	0.07±0.01 Cf	0.05±0.01 Bg	246.642	<0.001
$F_{df\,(5,12)}$	992.66	658.380	11.630	304.335	1.200	14.894	27.336	34.964	40.569		
P (0.05)	<0.001	<0.001	<0.001	<0.001	0.336	<0.001	<0.001	<0.001	<0.001		

Means followed with different Capital letters within each column and small letters within each row are not significantly different according to Duncan test ($P = 0.05$).

TABLE 2
Growth percentage of pathogenic bacteria treated with ethanol extract

Pathogenic bacteria	*Mentha piperita*			*Mentha longifolia*			*Ocimum basilicum*			$F_{df\ (8,18)}$	P (0.05)
	25%	50%	100%	25%	50%	100%	25%	50%	100%		
Staphylococcous aureus	60 ± 2.0^{Aa}	31 ± 1.0^{Ac}	15 ± 1.0^{Bd}	34 ± 4.0^{Cc}	17 ± 2.0^{Cd}	8 ± 2.0^{Ce}	39 ± 1.0^{Bb}	17 ± 0.0^{BCd}	7 ± 1.0^{BCe}	250.805	<0.001
Streptococcus pneumoniae	50 ± 1.0^{Ba}	24 ± 0.0^{Dd}	10 ± 1.0^{Ce}	39 ± 2.0^{Bc}	23 ± 2.0^{Bd}	12 ± 2.0^{Be}	45 ± 3.0^{Ab}	21 ± 0.0^{Ad}	13 ± 3.0^{Ae}	186.047	<0.001
Listeria monocytogenes	42 ± 1.0^{Ca}	21 ± 0.6^{Ed}	9 ± 0.0^{Cg}	26 ± 1.0^{Dc}	13 ± 1.0^{Df}	5 ± 0.0^{Di}	32 ± 3.0^{CDb}	17 ± 2.0^{BCf}	5 ± 2.0^{Ci}	212.721	<0.001
Escherichia coli	59 ± 2.0^{Aa}	27 ± 1.0^{Cc}	15 ± 2.0^{Be}	25 ± 0.0^{Dc}	13 ± 0.0^{De}	4 ± 1.0^{Df}	34 ± 1.0^{Cb}	18 ± 2.0^{Bd}	6 ± 0.0^{Cf}	511.200	<0.001
Pseudomonas aeruginosa	61 ± 1.0^{Aa}	29 ± 1.0^{Bb}	17 ± 0.0^{Ae}	21 ± 2.0^{Ed}	11 ± 1.0^{D}	4 ± 0.0^{Dg}	29 ± 1.0^{Db}	15 ± 0.0^{Cf}	4 ± 2.0^{Cg}	691.188	<0.001
Klebsiella pneumonia	41 ± 2.0^{Cb}	22 ± 10^{Ed}	10 ± 0.0^{Cf}	49 ± 2.0^{Aa}	32 ± 1.0^{Ac}	19 ± 1.0^{Ae}	40 ± 0.0^{Bc}	$19\pm1.0A^{Be}$	10 ± 0.0^{ABf}	451.375	<0.001
$F_{df\ (5,12)}$	100.520	68.569	34.400	67.945	105.000	63.120	29.743	8.333	11.500		
P (0.05)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001		

Means followed with different Capital letters within each column and small letters within each row are not significantly different according to Duncan test ($P = 0.05$).

TABLE 3
Biofilm estimation turbidity for pathogenic fungi treated with ethanol extract

Pathogenic fungi	*Mentha piperita*			*Mentha longifolia*			*Ocimum basilicum*			$F_{df\ (8,18)}$	*P (0.05)*
	25%	50%	100%	25%	50%	100%	25%	50%	100%		
Candida albicans	0.40±0.01 Eb	0.21±0.01 Cd	0.09±0.01 De	0.43±0.03 Ba	0.22±0.01 Bcd	0.10±0.01 De	0.44±0.01 Ea	0.23±0.01 Cc	0.10±0.01 De	552.187	<0.001
Aspergillus niger	0.42±0.03 DEb	0.23±0.01 Cc	0.10±0.01 CDd	0.44±0.04 Bab	0.22±0.01 Bc	0.10±0.01 CDd	0.46±0.02 DEa	0.24±0.01 Cc	0.12±0.01 CDd	183.732	<0.001
Aspergillus flavus	0.48±0.02 ABa	0.29±0.01 Ab	0.19±0.01 Ac	0.49±0.01 Aa	0.28±0.01 Ab	0.20±0.03 ABc	0.50±0.01 ABa	0.30±0.01 Ab	0.20±0.02 Ac	266.041	<0.001
Aspergillus ochraceus	0.46±0.01 CDa	0.24±0.04 BCb	0.12±0.01 Cc	0.46±0.01 ABa	0.23±0.01 Bb	0.12±0.02 CDc	0.50±0.01 CDa	0.25±0.01 BCb	0.13±0.02 Cc	225.981	<0.001
Penicillium funiculosum	0.50±0.01 Aa	0.30±0.01 Ab	0.19±0.03 Ac	0.49±0.01 Aa	0.29±0.01 Ab	0.18±0.02 Ac	0.50±0.01 Aa	0.30±0.01 Ab	0.20±0.01 Ac	349.823	<0.001
Penicillium ochrochloron	0.46±0.02 BCa	0.27±0.01 ABb	0.16±0.01 Bc	0.48±0.01 Aa	0.27±0.04 Ab	0.14±0.01 BCc	0.50±0.01 BCa	0.27±0.03 Bb	0.16±0.01 Bc	180.383	<0.001
$F_{df\ (5,12)}$	14.656	12.064	37.642	4.501	10.479	10.689	13.176	16.600	24.578		
P (0.05)	<0.001	<0.001	<0.001	0.015	<0.001	<0.001	<0.001	<0.001	<0.001		

Means followed with different Capital letters within each column and small letters within each row are not significantly different according to Duncan test ($P = 0.05$).

TABLE 4
Growth percentage of pathogenic fungi treated with ethanol extract

Pathogenic fungi	*Mentha piperita*			*Mentha longifolia*			*Ocimum basilicum*			F_{df}	*P*
	25%	50%	100%	25%	50%	100%	25%	50%	100%	*(8,18)*	*(0.05)*
Candida albicans	35 ± 2.0^{Cb}	18 ± 0.1^{Cd}	5 ± 0.0^{Cf}	42 ± 2.0^{BCa}	22 ± 1.0^{Bc}	8 ± 0.0^{Ce}	43 ± 1.0^{BCa}	21 ± 0.0^{Bc}	6 ± 1.0^{Cf}	542.318	<0.001
Aspergillus niger	36 ± 3.0^{Cb}	17 ± 2.0^{Cc}	6 ± 1.0^{Cd}	40 ± 0.0^{CDa}	16 ± 1.0^{Cc}	7 ± 0.0^{Cd}	41 ± 1.0^{Ca}	18 ± 2.0^{Cc}	7 ± 1.0^{BCd}	265.463	<0.001
Aspergillus flavus	42 ± 1.0^{Bb}	25 ± 0.0^{Ac}	13 ± 1.0^{Ad}	44 ± 3.0^{ABa} b	24 ± 1.0^{A} Bc	11 ± 0.0^{Bd}	45 ± 1.0^{Ba}	24 ± 0.0^{Ac}	13 ± 2.0^{Ad}	299.294	<0.001
Aspergillus ochraceus	40 ± 0.01^{Bb}	19 ± 1.0^{Cc}	9 ± 1.0^{Be}	38 ± 2.0^{Db}	16 ± 1.0^{Cd}	9.0 ± 0.0^{C} e	43 ± 3.0^{BCa}	21 ± 1.0^{Bc}	8 ± 0.0^{BCe}	317.294	<0.001
Penicillium funiculosum	50 ± 0.0^{Aa}	23 ± 1.0^{Bc} d	12 ± 2.0^{Af}	47 ± 2.0^{Ab}	25 ± 0.0^{Ac}	15 ± 0.01 Ae	50 ± 1.0^{Aa}	22 ± 2.0^{ABd}	13 ± 1.0^{Aef}	460.400	<0.001
Penicillium ochrochloron	42 ± 2.0^{Ba}	19 ± 1.0^{Cc}	14 ± 1.0^{Ad}	40 ± 0.0^{CDa}	23 ± 3.0^{A} Bb	12 ± 0.01 Bd	41 ± 1.0^{Ca}	18 ± 1.0^{Cc}	9 ± 0.0^{Be}	277.853	<0.001
$F_{df\ (5,12)}$	28.967	25.114	31.875	9.057	22.154	27.241	14.614	9.840	23.314		
P (0.05)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Means followed with different Capital letters within each column and small letters within each row are not significantly different according to Duncan test ($P = 0.05$).

CONCLUSION

It could be concluded that the ethanolic extracts of medicinal plants grown in high altitude showed the effects on pathogenic bacteria and fungi especially with higher concentration. Therefore, these medicinal plants could be useful as medicinal treatment after the pharmaceutical preparations.

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