ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synergistic antimicrobial effects and GC-MS analysis of phytocomponents of *Commiphora quadricincta.*

Nehad M Gumgumjee[1]*, Nariman A H Aly[2], Yahya S Masrahi[3], and Fawziah H Malawi[1].

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, KSA
[2]National Research Centre, Microbial Genetics Department, Cairo, Egypt
[3]Biology department, Faculty of Science, Jazan University, KSA

ABSTRACT

The *Commiphoraquadricincta*, a small tree, belong to *Burseraceae* family is traditionally known for its medicinal properties. The present study was therefore carried out to investigate the synergistic antimicrobial activities and the Phytochemicals of the bioactive components in the extract of this plant species. The antimicrobial activities of stem, bark and leaves extract was investigated against 7 medically important bacterial strains, namely *Bacillus subtilius* , MRSA, *Micrococcus* ,*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aurue*s and *Klebsellapneumonia*e and five fungi (*Aspergillusniger, A.fumigatus, A.flavus, Candida albicansand Saccharomyces spp*).The antibacterial activity was determined using agar well diffusion method. The most susceptible bacteria to this extract was*Escherichia coli*, while the most susceptible fungi was *A.flavus*. GC-MS analysis revealed that the ethanol extract of *Commiphoraquadricincta* contained mainly; 2Methyl3pentanol (2.84%); Butyl hydroxytoluene (22.32%);9,12,15Octadecatrienoic acid, 2-phenyl-1, 3-dioxan-5-yl ester (5.90); Ethyl isoallocholate(11.21%); àAmyrin (2.22%)and Flavone 4'oh,5oh,7dioglucoside(11.21%). Most identified compounds are known to have antimicrobial activity.

Keywords: *Commiphoraquadricincta*: antimicrobial activities, phytochemicals, GC-MS analysis

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The highly rise worldwide in the bacterial stains showing multidrug resistance are associated with reducing in the effectiveness of the global emergence of multi-drug resistant bacterial strains is increasingly limiting the effectiveness of the present drugs and leading mostly to failure in the treatment program [1]. The obtained data worldwide revealed to elevation in the incidence of dangerous diseases due to pathogenic bacteria and leading to increase in both morbidity and mortality rates in immunocompromised individuals particularly in developed countries [2]. Therefore, it is very important to find a methods to overcome the resistant of bacteria against the antibacterial drugs through improving the usage of antibiotic and control in the infections among individuals in the hospitals [3]. The urgent need for detection and formulating new substances capable of overcoming of the bacterial resistance via blocking The discovery and development of new compounds that either block or avoiding the bacterial resistant mechanisms , thus could improve the control , treatment, and suppression of resistant bacterial strains [4]. Thousands of medical herbs having different kinds of secondary metabolites, like tannins, terpenoids, flavonoids, phenol and quinones[5 and 6]. *Commiphoraquadricincta* (Schweinf ex. Engl.) has a resin-gum that is widely used as a folk medicine. Extracts of the resin-gum, leaves and root have been studied for their antibacterial activity. Commiphoraquadricincta essential oil have different fractions were obtained by fractionation on GC-MS. A high number of the detected substances were terpenoids. Volatiles harvested before the rains were comparatively higher, especially in the more volatile portions such as α-pinene, camphene, β-pineneand β-myrcen[7]. The current work was aimed to analyze the ethanol extract of the leaves of *Commiphoraquadricincta* and also to determine the most active compounds by using the efficient technique (GC-MS) that can be used in theanalyze of the leaves extract .

MATERIAL AND METHODS

Samples collection and preparation

Plant samples of *Commiphoraquadricincta* were obtained from Jizanregionrocky habitat, east of Abu Arish in Saudi Arabia during summer 2016. Determination of the plant species was performed at Faculty of Sciences Herbarium, King Abdulaziz University, Jeddah. The plant leaves was took to the laboratory, Debris and dusts was removed by washing several times with fresh tap and rinsed in distilled water for 5 min, dried from water and complete dryness at room temperature and under shade.

Bacterial and fungal strains

Seven bacterial strains were used, three were Gram- negative: *Klebsiellapneumoniae* (ATCC700603);*Escherichia coli* (ATCC8739) and *Pseudomonas aeruginosa* (ATCC27853) and four were Gram-positive:;*Staphylococcus aureus* (ATCC29213); Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC977); *Micrococcus luteus*(ATCC4698) and *Bacillus subtilis* (ATCC11774). Those strains were supplied byMicrobiologics® USA. The bacteria were got from King Abdul Aziz Hospital, Jeddah, Saudi Arabia. The tested fungi were, *Aspergillus fumigatus* (ATCC204305); (*Aspergillusflavus*(ATCC200026); *Aspergillusniger*(ATCC1015) and *Candida albicans*(ATCC10231)*Saccharomyces spp* were obtained from Biology Department, Fac. Science, KAU, Jeddah. The test microorganisms were cultured on nutrient agar slants for bacteria, Sabouroud dextrose agar for fungi and yeasts then incubated. The agar slants were maintained at 4°C.

Antimicrobial activity

The agar well diffusion technique was used for determination of the antimicrobial activity [8]. In this method, DMSO was taken as a negative control, the plates were prepared in triplicate. Antimicrobial activity was determined by measurement zone of inhibition [9].

Antibiotics used

Different types of antimicrobial drugs were used as : Erythromycin 5µg, Chloramphenicol 25µg Methicillin 10µg,, Fusidic Acid 10µg, Penicillin G 1unit,Novobiocin 5µg, Tetracycline 25µg, Streptomycin 10µg, Cephalothin 5µg, Ampicillin 10µg, Colistinsulphate 25µg, Gentamicin 10µg, Cotrimoxazole 25µg and Sulphatriad 200µg,. From Mast Diagnostics Mast Group Ltd. Merseyside U.K. Ketoconazole 200mg and

Itraconazole 100mg from Janssen-Cilag. Standard antifungal agent Ketoconazole 200mg and Itraconazole 100mg were prepared .

Extraction

According to [10] Ten Grams of dried Samples of *Commiphoraquadricincta*(stem, bark and leaves) were used and extracted by adding of either 100 ml distilled water or 100ml of organic solvents (ethanol and chloroform) (1:10W/V) to 10gm of the plant and putted in a separating funnel , shaking for 72 hrs at room temperature. Decant the supernatant layer and filtered using Whatman filter paper (No.1) and the organic solvents were evaporated under reduced pressure at 40°C until dryness. The obtained extracts were dissolved in suitable volume of DMSO and kept in small closed vials at low temperature 4°C. The compounds and structures of major compounds were analyzed by GC- MS (Perkin Elmer).

Synergism between plant extract and antibiotics

Nutrient agar was prepared according to the traditional method and each of the selected bacteria was inoculated on the surface of the nutrient Agar plates. Then, the disks (diameter=5mm) containing different antibiotics with standard concentrations was putted on the surface of each inoculated plate and also, 20 µl of the tested plant extract was added at concentration of 200mg/ml. In order to demonstrate the synergistic effect of plant extract with different antibiotics, the plant extract was mixed with antibiotics and placed on a filter paper disk, then left for one hour to dry. After drying, the plates were incubated at 37º C for 24 h. The diameters of inhibition zones were determined [11].

Gas chromatography- mass spectrum analysis (GC-MS)

Apparatus Name; Thermo Scientific, Trace GC Ultra and ISQ Single Quadruple MS. Capillary column: TG-5MS fused silica. Carrier Gas: Helium. Temperature program 40 ºC for 3 min then to 280 ºC at a rate 5 ºC/min then isothermal at 280 ºC for 5 min.

Extraction of the volatile constituents

A total of 200g of fresh leaves of *Commiphoraquadricincta* was extracted with ether [12]. The extract was filtered and the solvent was removed under reduced pressure at 30°C after dehydration over anhydrous sodium sulphate. The yielded quantity was subjected to GC/MS spectrometer.

Investigation of the volatile constituents

Volatile constituents were assayed by using GC/MS .The components were recognized by matching their retention times and mass fragmentation forms with the database libraries (Wiley Int.USA) and the published data [13]. Quantitative measurements were performed depending on computerized peak area dimensions.

Statistical analysis

Triplicates determinations for each treatment were performed in the current study. Means of variable ± standard error was estimated by using the software SPSS to confirm the significant differences between both the pathogenic bacteria & fungi and plant extract.

RESULTS AND DISCUSSION

Antimicrobial activities

Commiphoraquadricincta extracts were found effective with inhibition zone ranged from 12.00-32.33 mm against all tested bacteria with ethanol and chloroform extracts. The previous aqueous extracts of stem and leaves showed no activity on all tested bacteria. However, the aqueous extract of bark showed antimicrobial activity against *Bacillus subtilis*, Methicilin resistant S*taphylococcus aureus* (MRSA) and *Staphylococcus aureus*.From the previous results, *C. quadricinctastem* extracts have shown higher

antimicrobial activity than bark and leaves with ethanol and chloroform extracts against Gram- positive and negative bacteria. Antibacterial activities of *C. quadricincta* extracts using three types of solvents (ethanol, chloroform, water) of stem, bark and leaves as shown in (Table 1). The data indicated that ethanol extracts was the strongest one. The data indicated that *E.coli* was the most sensitive strain of the tested Gram-negative bacteria using the ethanol stem extract of *C. quadricincta* with the biggest inhibition zone (32.33 mm). For fungi the mean diameter of inhibition zone (mm) obtained by *Commiphoraquadricincta* of stem, bark and leaves extract indicated that ethanol solvent is the strongest one in all tested fungi and yeasts except with *Saccharomyces* spp. *Commiphoraquadricincta* aqueous extract showed no activity against all fungi and yeasts. In the ethanol extract the diameter of inhibition zones ranged from 18.00 to 26.33 mm and the leaves extract displayed higher antifungal activity than the bark and stem extracts in all tested fungi, where the highest inhibition zone obtained for *Aspergillus flavus* was 26.33 mm as showed in (Table 2). For yeasts the mean diameter of inhibition zone (mm) ranged from 12.00 to 21.00 mm, where the highest inhibition zone obtained for *Candida albicans* was 21.00 mm. The lowest inhibition zone was obtained for *Saccharomyces*spp 12.00 mm.In the current study, *C. quadricincta* extracts using two types of solvents (ethanol, chloroform) and water of stem, bark and leaves. Ethanol extracts is the strongest one. E. coli was the most sensitive strain of the tested Gram- negative bacteria using the ethanol stem extract with the biggest inhibition zone (32.33mm). Similar activity for this plant was recorded by [14] who reported that the antibacterial activity of the ethanolic extract of *C. quadricincta* was against *Yersinia enterocolitica*, *Staphylococcus epidermidis* and *E.coli*, and also reported that *C. quadricincta* showed higher activity of resin against *Yersinia enterocolitica*, *Staphylococcus epidermidis* and *E.coli*.

From the results of two previous studies, extract of *C. quadricincta* showed inhibitory effects on different antibiotic resistant isolates. This may due to the presence of different active compounds which inhibited bacterial growth. Thus extract of the previous plant can be used in traditional medicine to treat many bacterial diseases especially that which were caused by resistant bacteria.*C. quadricincta* showed strong antifungal activities with inhibition zone ranged from 12.00 to 26.33mm. The ethanol solvent was the stronger one. *C. quadricincta* aqueous extract showed no activity against all fungi and yeasts. The highest inhibition zone obtained for *A. flavus* was 26.33 mm. The lowest inhibition zone was obtained for *Saccharomycesspp* was 12 mm.

Table 1: Antimicrobial activity of medicinal plant *Commiphoraquadricincta* extracts against Gram- positive and negative bacteria, tested using well diffusion assay.

	Diameter of the inhibition zone (mm) Mean ± SD								
	Ethanol			Chloroform			Water		
Tested Bacteria	Stem	Park	Leaves	Stem	park	Leaves	Stem	Park	Leaves
Bacillus subtilis ATCC11774	21.66± 0.58	18.00 ±1.00	16.00± 1.00	15.33± 0.58	15.00± 0.0	13.00 ±0.0	0.0± 0.0	12.67± 0.58	0.0± 0.0
MRSA ATCC977	23.67± 1.16	19.67± 0.58	16.33± 0.58	17.67± 0.58	16.33± 0.58	15.00 ±1.00	0.0± 0.0	15.00± 0.0	0.0± 0.0
Micrococcus luteus ATCC4698	24.67± 0.58	19.67± 0.58	14.67± 0.58	14.00± 1.00	13.33± 0.58	12.00± 1.00	0.0± 0.0	0.0± 0.0	0.0± 0.0
Staphylococcusaureus ATCC29213	22.33± 1.53	20.00± 1.00	19.00± 1.00	20.67± 0.58	16.66± 0.58	16.33± 0.58	0.0± 0.0	14.67± 0.58	0.0± 0.0
Escherichia coli ATCC8739	32.33± 0.58	23.33± 0.58	18.00± 1.00	14.33± 1.15	14.00± 1.00	12.33± 0.58	0.0± 0.0	0.0± 0.0	0.0± 0.0
Klebsiellapneumoniae ATCC700603	19.67±	17.67±	14.33±	15.00±	13.33±	12.67±	0.0±	0.0±	0.0±

ISSN: 0975-8585

	0.58	0.58	0.58	1.00	0.58	0.58	0.0	0.0	0.0
Pseudomonasaeruginosa ATCC27853	20.33± 1.53	16.33± 0.58	15.33± 0.58	17.67± 0.58	13.66± 0.58	12.33± 0.58	0.0± 0.0	0.0± 0.0	0.0± 0.0

Table 2: Antifungal activity of medicinal plant *Commiphoraquadricincta* extracts against fungi species tested using well diffusion assay

	Diameter of the inhibition zone (mm)± SD								
	Ethanol			Chloroform			Water		
Fungi tested	Stem	Bark	Leaves	Stem	Bark	Leaves	Stem	Park	Leaves
Aspergillus flavus (ATCC200026)	20.00± 0.0	22.33± 0.58	26.33± 0.58	16.30± 0.58	16.67 ±1.15	20.33± 0.58	0.0± 0.0	0.0± 0.0	0.0 ±0.0
Aspergillus fumigates (ATCC204305)	20.00± 0.0	20.00± 1.00	20.67± 1.53	20.00± 0.0	15.33 ±0.58	18.00± 0.00	0.0± 0.0	0.0± 0.0	0.0± 0.0
Aspergillusniger (ATCC1015)	18.00± 0.0	20.33± 0.58	20.67±0 .58	15.70± 0.58	18.67 ±0.58	18.33± 0.58	0.0± 0.0	0.0± 0.0	0.0± 0.0
Candida albicans (ATCC10231)	19.30± 0.58	19.67± 0.58	21.00± 1.00	16.70± 0.58	17.33 ±0.58	17.67± 0.58	0.0± 0.0	0.0± 0.0	0.0± 0.0
Saccharomyces spp	13.50± 0.71	12.00± 1.41	12.50± 0.71	12.50± 0.71	12.50 ±0.71	13.50± 0.71	0.0± 0.0	0.0± 0.0	0.0± 0.0

Evaluation the synergistic effect

In vitro synergism between the most active part of plant of *Commiphoraquadricincta*, and antimicrobial drugs utilized against *E.coli*. As shown in (Table 3) *C. quadricincta* extract is possessed a synergistic activity with several of the tested antibiotics. The maximum synergistic action of the most active ethanolic extract was with Sulphatriad, Cotrimoxazole, Gentamicin, Cephalothin, Streptomycin, Penicillin G, Colistinsulphate and Methicillin (25mm, 24mm, 20mm. 16mm, 16mm, 15mm, 13mm, and 11mm Respectively). The rest of antibiotics have shown antagonism effect.

As shown in (Table 4) the interaction of *Commiphoraquadrienicta* with Itraconazole have had antagonism effect at varying inhibition degrees. The most interaction of Ketoconazol with ethanolic extract of *Commiphoraquadrienicta* was synergism the inhibition zone was (41mm). The present study aimed to estimate the capability of the plant extract to suppress the multiplication of pathogenic microorganisms either alone or in combination with other antibiotics and to show the synergistic action with antimicrobial drugs . In our study, tested plant extracts showed antibacterial activity against *E.coli*. The synergistic action of plant extracts to suppress the microbial growth depends mainly on the process of extraction and the solvents used . The test plant extracts in the current study had possessed antimicrobial activity and this action is increased when supplemented with standard antibiotics [15]. One of the most effect tool to avoid or overcome the resistant induced by bacteria against antibiotics by discovering new substances or synthetizing a new drugs having antibacterial activity or through adding of combinations of drugs to potentiate antibacterial effect [16]. The synergistic action between the known drugs and the bioactive ingredients in plant extracts is the subject of research worldwide , due to the befits of this synergism among known therapies and bioactive plant extracts either due to additive interaction or synergistic action or due to antagonistic action (Toxic or inactive substances) [17]. In present study, synergism between the most active plant of *C. quadricincta* and antimicrobial drugs utilized against *E. coli* using disc diffusion method showed beneficial synergistic or additive interaction such as Methicillin, Penicillin G, Streptomycin, Cephalothin, Colistinsulphate, Gentamicin and

Cotrimoxazole. Deleterious (antagonistic) such as Fusidic acid, Novobiocin and Ampicillin. The rest of antibiotic have shown both synergistic and antagonistic effects. Similar to our results, [17] showed the synergistic or antagonistic effect between antibiotic and bioactive plant extracts of *Psidiumguajava, Rosmarinusofficinalis, Salvia fruticsa, Majoranasyriaca, Ocimumbasilucum, Syzygiumarromaticum, Laurusnobilis and Rosa damascene*. The obtained data from the current study were coordinated with former In vitro researches , where they found synergistic action with high decrease in MICs of the antibiotics as a result of addition of different antibacterial drugs to different crude plant extracts against *S. aureu*s strains [18,19,20, 21, 22 and 23]. And stand out as veritable sources of potential resistance transforming substances [24].

In our research, synergism between C*. quadricincta* extracts and antibiotics (Itraconazole and Ketoconazol) utilized against *A. flavus* showed antagonism effect with Itraconazole. On the other hand, Ketoconazol showed with *C. quadricincta* synergism effect against *A. flavus*.

Our findings agree with those reported by [25], who reported that synergy observed between natural products and Ketaconazole, Clorimazole, Miconazole and Amphotericin B toward *A. niger, A. flavus, C. albicans, F. verticillioides* and *T. rubrum*. In a study in which the fruits of *Melia azedarach* L. was used for extraction of scopoletin (hydroxyl coumarin) , this extract was used with two known synthetic drugs mancozeb and carboxin for studying of the synergistic activity of this combination against *Fusarium verticillioides*[26]. In addition, the synergistic interaction against *Candida albicans* was studied through adding known antimycotics such as amphotericin B and fluconazole with EGCg against *C. albicans*. The mechanism of action of EGCg on *C.albicans* may be via attacks the cell membrane and causes cell lysis [27], whereas, amphotericin B lower than the minimum fungicidal concentration (MFC) is recognized to improve the permeability of catechin via the fungal membrane, in that way it enhance its penetration into the cell. A few herbal essential oils [28] particularly estragole, an oil from Agastacherugresa [29], Tea tree (*Melaleuca alternifolia*) oil [30] and volatile oils from Allium plants and Euphorbia characigs[31] have demonstrated significant synergism with ketaconazole against certain fungal species. In a recent study by [32], a synergistic effect of grape seed extract (GSE) with amphotericin B was observed in both In vitro and in murine model of disseminated candidacies due to *Candida albicans*.

Table 3: Synergism between Antibiotics and Ethanolic Extracts of Plant against *E.coli*

Antibiotic	Antibiotics alone	*Commiphoraquadricincta*	
		E	E+A
Chloramphenicol	29	32.33	26
Erythromycin	25		20
Fusidic Acid	35		20
Methicillin	0		11
Novobiocin	33		24
Penicillin G	0		15
Streptomycin	10		16
Tetracycline	28		20
Ampicillin	28		16
Cephalothin	0		16
Colistinsulphate	0		13
Gentamicin	12		20
Sulphatriad	21		25
Cotrimoxazole	22		24

Table 4: Synergistic effects of Itraconazole and Ketoconazol Antibiotics with Ethanolic Extracts of *Commiphoraquadricincta* against *A. flavus*

Antibiotic	Antibiotic alone	*Commiphoraquadricincta*	
		E	E+A
Itraconazole	21	26.33	18
Ketoconazol	40		41

GC-MS analysis

GC-MS chromatogram of the ethanolic extract of *Commiphoraquadrienicta* was showed in table 5. In this study the data obtained from GC-MS , concerning the mass spectra of different plant extract constituents were plotted against the NITS library data ,it showed that six peaks were obtained; all the phytocomponents were characterized and identified (Table 5). The retention times (RT) are in minutes.These compounds in the previous plant extract were :2Methyl-3pentanol, Butyl Hydroxy Toluene,9,12,15-Octadecatrienoic acid, 2-phenyl-1, 3-dioxan-5-yl ester, Ethylisoallocholate, α Amyrin and Flavone 4'OH,5OH,7 dioglucoside. In this study, the activity of plant extracts lead us to suggest that the biological activity against microorganisms may attributed to the presence of some secondary metabolites in the extract. The *C. quadricincta* extract revealed the presence ofMethyl3pentanol,butylhydroxyltoluene,octadecatrienoicacid, 2-phenyl-1,3-dioxan-5-ylester, ethylisoallocholate, α Amyrin and flavone. Moreover, [33] reported the presence of terpenoids compounds in the crude extracts of the plant as determined by GC-mass spectrometry. Concerning essential oils extracted from *Commiphoraquadricinctaa* by using GC-MS, the results pointed to the presence of large numbers of compounds in the extracted oil from *Commiphoraquadricinctaa* . In this study , terpenoids represent the main compound in the essential oil obtained from the plant extract . In another study, volatile oils harvested before the rains were relatively higher than that obtained in other occasion ,specially in the more volatile portions such as α-pinene, camphene, β-pineneand β-myrcen[7].

Table 5: Phytocomponents identified in the ethanolic extract of *Commiphoraquadricincta* by GC-MS

NO.	RT	Compound	Molecular Formula	MW	Peak Area%	Biological activity
1	5.03	2-Methyl-3pentanol	C6H14O	102	2.84	
2	28.47	Butyl Hydroxy Toluene	C15H24O	220	22.32	Antioxidant [34]
3	51.24	9,12,15-Octadecatrienoic acid, 2-phenyl-1, 3-dioxan-5-yl ester	C28H40O4	440	5.90	
4	51.34	Ethyl isoallocholate	C26H44O5	436	11.21	
5	23.88	àAmyrin	C30H50O	426	2.22	
6	51.34	Flavone4'OH,5OH,7Dioglucoside	C27H30O15	594	11.21	

REFERENCES

[1] Hancock, E.W. (2005). Mechanisms of action of newer antibiotics for Gram-positive pathogens. Lancet Infectious Diseases 5(4): 209-218.

[2] Al-Bari, M.A.A., Sayeed, M.A. and Rahman, M.S. (2006). Characterization and antimicrobial activities of a phenolic acid derivative produced by Streptomyces bangladeshiensis: A novel species collected in Bangladesh. Research Journal of Medical Sciences, 1:77-81.

[3] French, G.L. (2005). Clinical impact and relevance of antibiotic resistance. Journal of Advanced Drug Delivery Reviews, 57 (15):14-27.

[4] Oluwatuyi, M., Kaatz, G.W. and Gibbons, S. (2004). Antibacterial and resistance modifying activity of Rosmarinusofficinalis. Phytochemistry, 65 (24): 3249-3254.

[5] Al-Zubaydi, S.R., Al-Hmdany , M.A. and Raesan, S.J. (2009). Antibactrial effect of some medicinal plant extracts against some pathogenic bacteria strains. Journal of Duhok University, 12(1): 244-249.

[6] Leon, J., E. Rojo and Sanchezerrano, J. (2001). Wound signling in plants. Journal of Experimental Botany, 52: 1-9.

[7] Assad, Y., Torto, B., Hassanali, A., Njagi, P., Bashir, N., and Mahamat, H. (1997). Seasonal variation in the essential oil composition of Commiphoraquadricincta and its effect on the maturation of immature adults of the desert locust, Schistocercagregaria. Phytochemistry, 44(5): 833-841.

[8] Holder IA and Boyce ST (1994). Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns, 20: 426-429.

[9] Agwa, A., Aly, M., and Bonaly, R. (2000). Isolation and characterization of two Streptomyces species produced non polyenic antifungal agents. Journal Union Arab Biologi, 7, 62-82.

[10] Boeru, V., and Derevici, A. (1978). Some chemical and physical data on Romania propolis. Apimondia" propolis" Bucharest, 19-26.
[11] Mahmoud, M.J. (2013). The Antibacterial Effect of Some Medicinal Plant Extracts and their Synergistic Effect with Antibiotic and Non-antibiotic Drugs. Thesis, Islamic University-Gaza, Palestine.
[12] Harborne, J. (1973). Phytochemical methods, a guide to modern techniques of plant analysis, Jeffrey Barry Harborne.
[13] Adams, R.P., (1995). Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publishing Corporation, Carol. USA.
[14] Salamah, A.A., Zaid, A.M. (2000). Antimicrobial activity of Commiphoraquandricincta from Saudi Arabia. Journal of King Saud University, 12: 1-10.
[15] Rakholiya, K., and Chanda, S. (2012). In vitro interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. Asian Pacific Journal of Tropical Biomedicine, 2(2): S876-S880.
[16] Stefanovic, O., Stankovic, M. S., and Comic, L. (2011). In vitro antibacterial efficacy of Clinopodiumvulgare L. extracts and their synergistic interaction with antibiotics. Journal of Medicinal Plants Research, 5(17), 4074-4079.
[17] Adwan, G. and Mhanna, M. (2008). Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. Middle-East Journal of Scientific Research, 3(3), 134-139.
[18] Betoni, J. E. C., Mantovani, R. P., Barbosa, L. N., Di Stasi, L. C., and Fernandes Junior, A. (2006). Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Memorias do InstitutoOswaldo Cruz, 101(4): 387-390.
[19] Esimone, C., Iroha, I., Ibezim, E., Okeh, C., and Okpana, E. (2006). In vitro evaluation of the interaction between tea extracts and penicillin G against Staphylococcus aureus. African Journal of Biotechnology, 5(11).
[20] Yang, Z.-C., Wang, B.-C., Yang, X.-S., Wang, Q., and Ran, L. (2005). The synergistic activity of antibiotics combined with eight traditional Chinese medicines against two different strains of Staphylococcus aureus. Colloids and surfaces B: Biointerfaces, 41(2): 79-81.
[21] Aqil, F., Khan, M. S. A., Owais, M., and Ahmad, I. (2005). Effect of certain bioactive plant extracts on clinical isolates of β-lactamase producing methicillin resistant Staphylococcus aureus. Journal of Basic Microbiology, 45(2): 106-114.
[22] Braga, L. C., Leite, A. A. M., Xavier, K. G. S., Takahashi, J. A., Bemquerer, M. P., Chartone-Souza, E. and Nascimento, A. M. A. (2005). Synergic interaction between pomegranate extract and antibiotics against Staphylococcus aureus. Canadian Journal of Microbiology, 51, 541-547.
[23] Yam, M.F., Basir, R., Asmawi, M.Z., Rosidah, Ahmad, M. and Akowuah, G.A. (2008). Antioxidant and hepatoprotective activities of Elephantopustomentosus ethanol extract. Journal of Pharmaceutical Biology, 46:199-206.
[24] Sibanda, T., and Okoh, A. (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. African Journal of Biotechnology, 6(25).
[25] Hemaiswarya, S., Kruthiventi, A. K., and Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. Phytomedicine, 15(8): 639-652.
[26] Carpinella, M. C., Ferrayoli, C. G., and Palacios, S. M. (2005). Antifungal synergistic effect of scopoletin, a hydroxycoumarin isolated from Melia azedarach L. fruits. Journal of agricultural and Food Chemistryistry, 53(8): 2922-2927.
[27] Toyoshima, Y., Okuba, S., Toda, M., Hara, Y. and Shimamura, T., (1993). Effect of catechin on the ultrastructure of Trichophyton mentagrophytes. KansenshogakuZasshi, 68: 295–303.
[28] Shin, S., and Lim, S. (2004). Antifungal effects of herbal essential oils alone and in combination with ketoconazole against Trichophyton spp. Journal of applied microbiology, 97(6): 1289-1296.
[29] Shin, S., and Kang, C. A. (2003). Antifungal activity of the essential oil of AgastacherugosaKuntze and its synergism with ketoconazole. Letters in applied microbiology, 36(2): 111-115.
[30] Hammer, K., Carson, C., and Riley, T. (2000). In vitro activities of ketoconazole, econazole, miconazole, and Melaleuca alternifolia (tea tree) oil against Malassezia species. Antimicrobial agents and chemotherapy, 44(2): 467-469.
[31] Giordani, R., Regli, P., Kaloustian, J., Mikail, C., Abou, L., and Portugal, H. (2004). Antifungal effect of various essential oils against Candidaalbicans. Potentiation of antifungal action of amphotericin B by essential oil from Thymus vulgaris. Phytotherapy research, 18(12): 990-995.

[32] Han, Y. (2007). Synergic effect of grape seed extract with amphotericin B against disseminated candidiasis due to Candida albicans. Phytomedicine, 14(11): 73.

[33] Hassan, S.W., Bilbis, F.L., Ladan, M.J., Umar, R.A., Dangoggo, S.M., Saidu, Y., Abubakar, M.K. and Faruk, U.K. (2006). Evaluation of antifungal activity and phytochemical analysis of leaves, roots and stem barks extracts of Calotropisprocera (Asclepiadaceae). Pakistan Journal of Biological Sciences. 9(14):2624-2629.

[34] Murugesan, S., Senthilkumar, N., Rajeshkannan, C. and Vijayalakshmi K. B. (2013). Phytochemical characterization of Meliadubia for their biological properties. Der ChemicaSinica, 4(1):36-40.