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Original article

Evaluation of antimicrobial and synergistic effects of some medicinal plant extracts on antimicrobial resistant organisms

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ABSTRACT

Background: To evaluate antimicrobial activity of ten plant extracts on antimicrobial resistant organisms and investigate interactions of these extracts with antimicrobials against resistant organisms. **Methods:** The antimicrobial potential of ethanolic leaf extract of *Aberia caffra* Hook. f. & Harv., *Azadirachta indica* L., *Dodonaea viscosa* L., *Ficus nitida* L., *Lanatana camara* L., *Myrtus communis* L., *Olea europaea* L., *Ruta graveolens*, *Schinus terebinthifolius* Raddi and *Vitex agnus-castus* L. was investigated against eleven drug resistant organisms by agar well diffusion method. The interaction of these extracts with antimicrobials was assessed. **Results:** Ethanolic extracts of *Dodonaea viscosa* leaves had antibacterial activity against ESBL-producing *Escherichia coli (E. coli)* quinolone resistant *Salmonella typhi (S. typhi)* and ESBL and carbapenemase-producing *Klebsiella pneumonia (K. pneumonia). Schinus terebinthifolius* Raddi leaves showed antibacterial activity against ESBL and carbapenemase-producing *K. pneumoniae* and fluconazole resistant *C. albicans. Myrtus communis* leave extracts demonstrated activity against ESBL-producing *E. coli* and fluconazole resistant *A. fumigatus. Ruta graveolens L.* revealed activity against quinolone resistant *S. typhi* and fluconazole resistant *A. fumigatus. Vitex agnus-castus, Aberia caffra* Hook. f. & Harv. and *Azadirachta indica* L. leaf extracts had activity against ESBL-producing *E. coli. Olea europaea* L. had activity against quinolone resistant *A. baumannii. Ruta graveolens* L. and *Myrtus communis* L. were the only extracts showing synergistic effect in association with voriconazole against fluconazole resistant *A. fumigatus.* **Conclusion:** Tested plant leaf extracts had great potential as antimicrobial agents against at least one drug resistant microorganism. Isolation of active components and further studies are needed to evaluate the efficacy and toxicity of these products.

Introduction

The rise in bacterial resistance to currently available antibiotics is a global concern that emphasises the need for novel antibacterial classes. Natural-source compounds can be used to create new chemical scaffolds for antibiotic development. Unlike synthetic medications, natural-source compounds have fewer side effects, are less expensive, come from renewable sources, and have a higher level of acceptance due to their long history of use. Many of today's medications are based on

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synergistic interactions between antibiotics that target various aims. According to studies, combination of plant extracts with antimicrobials reduces the minimum inhibitory concentrations (MICs) of antibiotics for bacterial strains [1].

Medicinal plants are rich in secondary metabolites, which may be potential natural drugs and serve as alternative, less expensive and safe antimicrobials for treatment of common ailments [2]. The plant-derived phytochemicals or secondary metabolites, as the phenolic compounds and volatile oil are responsible for the antimicrobial traits [2]. Hence, more studies are needed to investigate the use of plant extracts to control antimicrobial resistant organisms. *Aberia caffra* Hook. f. & Harv. (*Dovyalis caffra* (Hook.f. & Harv.) Sim) is belonging to family Flacourtiaceae. It is a source of different secondary metabolites as alkaloids, flavonoids, phenolic glucosides, terpens and sterols. Researches support its antioxidant, anti-inflammatory, antibacterial and anticancer activities [3]. *Azadirachta indica* L. (Neem) family Meliaceae is rich in secondary metabolites as terpens, proteins, carbohydrates, sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin, tannins, aliphatic compounds and phenolic acids. Neem showed different biological activities as antioxidant, antimicrobial, hepatoprotective and cancer chemopreventive [4]. *Dodonaea viscosa* L., family Sapindaceae, the reported secondary metabolites are tannins, saponins, flavonoids and terpenoids and reported for its antibacterial, analgesic, antiviral, anti-inflammatory, antiulcer and antioxidant activities [5]. *Ficus nitida* L. is belonging to family Moraceae. It is rich in polyphenolic metabolites and showed antioxidant, antibacterial, and antiviral properties [6]. *Lanatana camara* L., family Verbenaceae, the reported secondary metabolites are terpens, iridoid glycosides, furanonaphthoquinones, flavonoids, and phenylethanoid glycosides [7]. *Lantana camara* L. has broad antibacterial, antifungal, antiviral, and antiparasitic activities [7, 8]. *Myrtus communis* L. family Myrtaceae has been used in traditional medicines for the treatment of lung disorders and rich in phenolic acids, flavonoids, tannins, volatile oils and fatty acids. *Myrtus communis* has recently been shown to have antioxidant, analgesic, antibacterial and antifungal activities [9]. *Olea europaea* L. (olive), family Oleaceae is rich in triacylglycerols, fatty acids in addition to alkyl esters, phytosterols, polyphenols, tocopherols, tocotrienols, pigments and minerals. It showed cholesterol-lowering, hypoglycemic, cytotoxic, antibacterial, neuroprotective, antioxidant, anti-inflammatory and hypotensive activities [10]. *Ruta graveolens* L., family Rutaceae, is a scented medicinal plant well-known for its therapeutic uses against inflammation, pain, pulmonary diseases and cancer. Reported secondary metabolites of *Ruta graveolens* are terpenoids, flavonoids, alkaloids, coumarins, aliphatic acids and ketones [11]. *Schinus terebinthifolius* Raddi, family Anacardiaceae is known as Brazilian pepper tree, traditionally used for treating infections in the respiratory, digestive and urinary tracts [12]. *Vitex agnus-castus* L., family Verbenaceae, its major secondary metabolites are flavonoids, essential oils, diterpenes, and glycosides. It is commonly used for many gynecological complaints and showed antibacterial activity [13, 14].

Several methods, such as disc diffusion, well diffusion, and broth or agar dilution, are well-known and widely used, but others, such as flow cytofluorometric and bioluminescent methods, are not widely used because they require specialized equipment and further evaluation for reproducibility and standardization, even though they can provide faster results and a better sensitivity [15].

The present study aimed to evaluate the antimicrobial activity of some selected medicinal plants extracts cultivated and growing in Egypt (Assiut locality) on some antimicrobial resistant organisms and investigate the interactions of these extracts with antimicrobials against resistant organisms.

Material and Methods

Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine, Assiut University (IRB. 17300576) after review and assessment of the study experimental design.

Microbial isolates

Eleven clinical antimicrobial resistant strains were collected during previous studies from patients with different sites of infections. All isolates were obtained from Assiut University Hospital, Assiut, Egypt. They were identified by the conventional phenotypic and genotypic methods and by the VITEK ® 2 system. The eleven microbial species listed below were analyzed:

1. Extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*)
2. ESBL and carbapenemase-producing *Proteus mirabilis* (*P. mirabilis*)
3. Quinolone resistant *Salmonella typhi* (*S. typhi*)
4. Quinolone resistant *Acinetobacter baumannii* (*A. baumannii*)
5. ESBL and carbapenemase-producing *Pseudomonas aeruginosa* (*P. aeruginosa*)
6. Methicillin resistant *Staphylococcus aureus* (MRSA)
7. ESBL and carbapenemase-producing *Klebsiella pneumoniae* (*K. pneumoniae*)
8. Glycopeptide and macrolide resistant coagulase-negative Staphylococci (CNS)
9. Glycopeptide and macrolide resistant *Enterococcus fecalis* (*E. fecalis*)
10. Fluconazole resistant *Aspergillus fumigatus* (*A. fumigatus*)
11. Fluconazole resistant *Candida albicans* (*C. albicans*)

The antimicrobial susceptibility of the isolated bacterial and fungal species to different antimicrobials were tested by the VITEK ® 2 system.

Preparation of plant extracts

Leaves of *Aberia caffra* Hook. f. & Harv., *Azadirachta indica* L., *Dodonaea viscosa* L., *Ficus nitida* L., *Lanatana camara* L., *Myrtus communis* L., *Olea europaea* L., *Ruta graveolens*, *Schinus terebinthifolius* Raddi and *Vitex agnus-castus* L., were collected in 2020, during the flowering stage from the Experimental Station of Medicinal Plants, Faculty of Pharmacy and the Experimental Station, Faculty of Agriculture, Assiut University, Assiut, Egypt. The plants were authenticated by Dr. Mostafa Abo el ela, Botany Department, Faculty of Science, Assiut University. Voucher specimens were kept in the herbarium, Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

The air-dried powdered leaves of the selected plants (50 g each) were exhaustively extracted with 70% ethanol by maceration at room temperature (4×150 ml). The ethanolic extract was concentrated under reduced pressure to obtain a viscous residue [16].

Preliminary phytochemical screening of the plant extracts

The major classes of secondary metabolites; alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins, sterols and /or triterpenes are screened in each plant extract according to the common phytochemical methods described by **Harborne** [17].

Screening for the antimicrobial potential of the plant extracts

Bacterial and fungal cultures were grown in Brain Heart Infusion liquid medium (HiMedia, India) at 37 °C. The agar well diffusion method was used [15].

After 6 h of growth, 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on Muller-Hinton agar plates (HiMedia, India). Using sterile agar borer, wells of 6-mm diameter were punched into the agar medium and filled with 100 µl of plant extract of 100 mg/mL concentration. Blank solvent (DMSO, Sigma-Aldrich, St. Louis, MO, USA) was used as a negative control. The antimicrobials [ciprofloxacin (0.1 µg/mL), colistin (0.125 µg/mL) or voriconazole (0.125 µg/mL), Sigma-Aldrich, St. Louis, MO, USA] were used as positive controls. The plates were incubated aerobically at 37 °C, overnight. The antimicrobial screening was evaluated by measuring the zone of inhibition (mm) around each well. Zone of inhibition measuring > 7 mm implies that the organism is susceptible to the tested plant extract [18]. The extracts that showed antimicrobial activity were further tested to determine the MIC for each of the tested organisms.

Determination of minimum inhibitory concentration

The MIC is the lowest possible concentration of the extract that can inhibit the growth of the tested organism. The test was performed with the agar well diffusion method [16].

Two-fold serial dilution of each plant extract in DMSO was utilized ranging from 100 mg/ mL to 0.012 mg/mL. One hundred µL of each extract dilution was added into the wells in Muller-Hinton agar plates, preinoculated with the test organism. The plates were incubated aerobically at 37 °C for 18-24 hours, then the inhibition zones of the different extract dilutions were measured.

Assessment of the interactions between plant extracts and antimicrobials on resistant microbial isolates

To evaluate the interactions between plant extracts and antimicrobial drugs, 50 µL of each

antimicrobial (ciprofloxacin, colistin or voriconazole) in combination with 50 µL of plants extracts (both at the MIC values) were added into the wells in Muller-Hinton agar plates, preinoculated with the test organism. The inhibition zones produced by the interaction of plant extracts with the tested antimicrobials after overnight incubation were interpreted as following : if the inhibition zone of plant extract antimicrobial mixture is equal to inhibition zone of plant extract + inhibition zone of corresponding antimicrobial, was considered addition; if the inhibition zone of plant extract antimicrobial mixture is greater than inhibition zone of plant extract + inhibition zone of corresponding antimicrobial, was considered synergism; if the inhibition zone of plant extract antimicrobial mixture is less than inhibition zone of plant extract + inhibition zone of the corresponding antimicrobial, was considered antagonism [19].

Results

Plant extracts yield and preliminary phytochemical screening of the plant extracts

The yield percentage and the phytochemical analysis are shown in **table (1)** for the selected plants extracts.

Evaluation of antimicrobial activity of the tested plant extracts

As shown **in table (2)**, all tested plant extracts showed zone of inhibition against at least one of the tested drug resistant organisms. *Schinus terebinthifolius Raddi* showed the highest activity (9/11, 81.8%) followed by *Myrtus communis* L. (8/11, 72.7%), then *Dodonaea viscosa* L. (6/11, 54.5%). The least activity was shown by *Azadirachta indica* L. (1/11, 9%). *Olea europaea* L. was active against quinolone resistant *Acinetobacter baumannii* alone. Only *Schinus terebinthifolius Raddi*. had activity against ESBL and carbapenemase-producing *Pseudomonas aeruginosa*, while *Ruta graveolens* L., *Myrtus communis* L. and *Schinus terebinthifolius Raddi* had antifungal activity. *Ruta graveolens* L. active against both fluconazole resistant *Aspergillus fumigatus* and *Candida albicans.*

Minimal inhibitory concentration of plant extracts against antimicrobial resistant organisms

The MICs of the different plant extracts against the antimicrobial resistant organisms are shown in **table (3)**. *Myrtus communis* L., *Azadirachta indica* L and *Dodonaea viscosa* L showed the lowest MIC for ESBL-producing *E. coli*. *Ruta graveolens* L. revealed the lowest MIC to quinolone resistant *Salmonella typhi. Myrtus communis* L. and *Schinus terebinthifolius* Raddi had the lowest MIC against ESBL and carbapenemase-producing *Klebsiella pneumoniae* in addition to glycopeptide and macrolide resistant *Enterococcus fecalis*, respectively.

Interactions between plant extracts and antimicrobials on resistant microbial isolates

Ruta graveolens L. and *Myrtus communis* L. were the only plant extracts showing synergistic outcome with voriconazole against fluconazole resistant *Aspergillus fumigatus*, as shown in **table (4)**. Otherwise, the remaining plant extract-antimicrobial combinations showed antagonism.

Table 1. Plant extracts yield and preliminary phytochemical screening of the plant extracts.

Plant name	Plants extracts yield percentage*	preliminary phytochemical screening					
		Alkaloids	Anthraquinones	Flavonoids	Saponins	Tannins	Sterols and /or Triterpenes
***Aberia caffra* Hook f. & Harv.**	7.7	+	-	+	-	+	+
***Azadirachta indica* L.**	7.1	+	-	+	+	+	+
***Dodonaea viscosa* L.**	11.3	-	-	+	+	+	+
***Ficus nitida* L.**	9.3	-	+	+	-	+	+
***Lanatana camara* L.**	6.8	+	-	+	+	+	+
***Myrtus communis* L.**	7.1	+	-	+	-	+	+
***Olea europaea* L.**	11.7	+	-	+	-	+	+
***Ruta graveolens* L.**	11.3	-	-	+	-	+	+
***Schinus terebinthifolius* Raddi**	6.5	+	-	+	-	+	+

(+): prescence, (-) absence, *The yield% = (weight of dry extract / weight of dry powdered plant) *100

Table 2. Antimicrobial activity of plant extracts by agar well diffusion method.

Micro-organism.	*Ruta gaveolens* L.	*Lantana camara* L.	*Myrtus communis* L.	*Azadirachta indica* L.	*Olea europaea* L.	*Vitex agnus-castus* L.	*Dodonaea viscosa* L.	*Ficus nitida* L.	*Aberia caffra Hk. f. & Harv.*	*Schinus terebinthifolius* Raddi
1	-	-	+	+	-	+	+	-	+	-
2	-	+	+	-	-	-	-	-	+	+
3	+	-	-	-	-	+	+	-	-	+
4	-	-	+	-	+	-	-	-	-	+
5	-	-	-	-	-	-	-	-	-	+
6	-	-	+	-	-	-	+	+	-	+
7	-	+	+	-	-	-	+	-	+	+
8	-	+	+	-	-	-	+	-	-	+
9	-	+	+	-	-	-	+	+	-	+
10	+	-	+	-	-	-	-	-	-	-
11	+	-	-	-	-	-	-	-	-	+

(+) susceptibility (inhibition zone > 7 mm)

(-) absence of susceptibility (inhibition zone <7 mm)

(1) ESBL-producing *Escherichia coli* (2) ESBL and carbapenemase-producing *Proteus mirabilis* (3) Quinolone resistant *Salmonella spp.* (4) Quinolone resistant *Acinetobacter baumannii* (5) ESBL and carbapenemase-producing *Pseudomonas aeruginosa*, (6) Methicillin resistant *S. aureus* (7) ESBL and carbapenemase-producing *Klebsiella pneumoniae* (8) Glycopeptide and macrolide resistant *coagulase-negative staphylococci* (9) Glycopeptide and macrolide resistant *Enterococcus fecalis* (10) Fluconazole resistant *Aspergillus fumigatus (*11) Fluconazole resistant *Candida albicans*

Table 3. Minimal Inhibitory Concentration (MIC) of plant extracts (mg/mL) against antimicrobial resistant organisms.

Micro-organism.	*Ruta gaveolens* L.	*Lantana camara* L.	*Myrtus communis* L.	*Azadirachta indica* L.	*Olea europaea* L.	*Vitex agnus-castus* L.	*Dodonaea viscosa* L.	*Ficus nitida* L.	*Aberia caffra Hk. f. & Harv.*	*Schinus terebinthifolius* Raddi
1	-	-	0.012	0.012	-	0.097	0.012	-	0.195	-
2	-	100	50	-	-	-	-	-	25	50
3	0.024	-	-	-	-	6.25	0.097	-	-	100
4	-	-	6.25	-	0.39	-	-	-	-	100
5	-	-	-	-	-	-	-	-	-	100
6	-	-	1.56	-	-	-	50	100	-	12.5
7	-	0.93	1.56	-	-	-	0.048	-	50	0.012
8	-	50	25	-	-	-	50	-	-	50
9	-	50	3.125	-	-	-	50	50	-	50
10	0.097	-	0.097	-	-	-	-	-	-	-
11	1.56	-	-	-	-	-	-	-	-	0.012

(1) ESBL-producing *Escherichia coli* (2) ESBL and carbapenemase-producing *Proteus mirabilis* (3) Quinolone resistant *Salmonella typhi* (4) Quinolone resistant *Acinetobacter baumannii* (5) ESBL and carbapenemase-producing *Pseudomonas aeruginosa*, (6) Methicillin resistant *S. aureus* (7) ESBL and carbapenemase-producing *Klebsiella pneumoniae* (8) Glycopeptide and macrolide resistant *coagulase-negative staphylococci* (9) Glycopeptide and macrolide resistant *Enterococcus fecalis* (10) Fluconazole resistant *Aspergillus fumigatus (*11) Fluconazole resistant *Candida albicans*

Table 4. Effect of the association of plant extracts with antimicrobials on resistant organisms.

Micro-organism	Tested reagent	*Ruta gaveolens* L.	*Lantana camara* L.	*Myrtus communis* L.	*Azadirachta indica* L.	*Olea europaea* L.	*Vitex agnus-castus* L.	*Dodonaea viscosa* L.	*Ficus nitida* L.	*Aberia caffra Hk. f. & Harv.*	*Schinus terebinthifolius* Raddi
		zones of inhibition mm									
1	PE	-	-	40	30	-	30	30	-	40	-
	CIP	-	-	40	40	-	40	40	-	40	-
	PE+CIP	-	-	45	45	-	40	40	-	45	-
	Outcome	-	-	Antagonism	Antagonism	-	Antagonism	Antagonism	-	Antagonism	-
2	PE	-	22	30	-	-	-	-	-	45	25
	CIP	-	67	67	-	-	-	-	-	67	67
	PE+CIP	-	15	25	-	-	-	-	-	45	20
	Outcome	-	Antagonism	Antagonism	-	-	-	-	-	Antagonism	Antagonism
3	PE	28	-	-	-	-	27	25	-	-	25
	CST	31	-	-	-	-	31	31	-	-	31
	PE+CST	32	-	-	-	-	32	34	-	-	32
	Outcome	Antagonism	-	-	-	-	Antagonism	Antagonism	-	-	Antagonism
4	PE	-	-	23	-	27	-	-	-	-	23
	CST	-	-	36	-	36	-	-	-	-	36
	PE+ CST	-	-	31	-	30	-	-	-	-	31
	Outcome	-	-	Antagonism	-	Antagonism	-	-	-	-	Antagonism
5	PE	-	-	-	-	-	-	-	-	-	25
	CST	-	-	-	-	-	-	-	-	-	40
	PE+ CST	-	-	-	-	-	-	-	-	-	25

	Outcome	-	-	-	-	-	-	-	-	-	**Antagonism**
6	**PE**	-	-	**30**	-	-	-	**30**	**26**	-	**36**
	CIP	-	-	**30**	-	-	-	**30**	**30**	-	**30**
	PE+CIP	-	-	**20**	-	-	-	**34**	**34**	-	**42**
	Outcome	-	-	**Antagonism**	-	-	-	**Antagonism**	**Antagonism**	-	**Antagonism**
7	**PE**	-	**19**	**30**	-	-	-	**20**	-	**19**	**23**
	CST	-	**30**	**30**	-	-	-	**30**	-	**30**	**30**
	PE+ CST	-	**26**	**20**	-	-	-	**26**	-	**26**	**27**
	Outcome	-	**Antagonism**	**Antagonism**	-	-	-	**Antagonism**	-	**Antagonism**	**Antagonism**
8	**PE**	-	**30**	**30**	-	-	-	**25**	-	-	**27**
	CIP	-	**35**	**35**	-	-	-	**35**	-	-	**35**
	PE+CIP	-	**30**	**35**	-	-	-	**30**	-	-	**20**
	Outcome	-	**Antagonism**	**Antagonism**	-	-	-	**Antagonism**	-	-	**Antagonism**
9	**PE**	-	**17**	**12**	-	-	-	**15**	**16**	-	**16**
	CIP	-	**30**	**30**	-	-	-	**30**	**30**	-	**30**
	PE+CIP	-	**17**	**16**	-	-	-	**17**	**18**	-	**18**
	Outcome	-	**Antagonism**	**Antagonism**	-	-	-	**Antagonism**	**Antagonism**	-	**Antagonism**
10	**PE**	**22**	-	**27**	-	-	-	-	-	-	-
	VOR	**30**	-	**30**	-	-	-	-	-	-	-
	PE+VOR	**57**	-	**58**	-	-	-	-	-	-	-
	Outcome	**Synergism**	-	**Synergism**	-	-	-	-	-	-	-
11	**PE**	**20**	-	-	-	-	-	-	-	-	**26**
	VOR	**30**	-	-	-	-	-	-	-	-	**30**
	PE+VOR	**40**	-	-	-	-	-	-	-	-	**22**
	Outcome	**Antagonism**	-	-	-	-	-	-	-	-	**Antagonism**

(1) ESBL-producing *Escherichia coli* (2) ESBL and carbapenemase-producing *Proteus mirabilis* (3) Quinolone resistant *Salmonella spp.* (4) Quinolone resistant *Acinetobacter baumannii* (5) ESBL and carbapenemase-producing *Pseudomonas aeruginosa*, (6) Methicillin resistant *S. aureus* (7) ESBL and carbapenemase-producing *Klebsiella pneumoniae* (8) Glycopeptide and macrolide resistant *coagulase-negative staphylococci* (9) Glycopeptide and macrolide resistant *Enterococcus fecalis* (10) Fluconazole resistant *Aspergillus fumigatus (*11) Fluconazole resistant *Candida albicans*

PE plant extract; CIP ciprofloxacin; CST colistin; VOR voriconazole

Synergism: Inhibition zones of combination treatment > inhibition zone of plant extract + zone of the corresponding antimicrobial

Antagonism: Inhibition zone of combination treatment < inhibition zone of plant extract + zone of the corresponding antimicrobial

Discussion

The antibacterial and antifungal activity of *Dodonaea viscosa* L. have been previously proved [5, 20,21]. Our results showed strong antibacterial activity of *Dodonaea viscosa* leave extract against ESBL-producing *E. coli*, Quinolone resistant *S. typhi* and ESBL and carbapenemase-producing *K. pneumoniae* and week activity against the tested Gram positive cocci, with no detected antifungal activity. Additionally, antagonistic activity against the previous isolates was detected when *Dodonaea viscosa* L. extract interacted with ciprofloxacin or colistin. Similarly, an earlier study reported notable antimicrobial activity against both Gram positive and negative organisms [20]. Meanwhile, **Getie et al.** [5] observed activity against gram positive bacteria with no activity against neither gram negative bacteria nor fungi. On the other hand, the antifungal activity of *Dodonaea viscosa* was previously reported against *C. albicans* [21]. The leaves contain 5,6,8-Trihydroxy-7,4-dimethoxyflavone which has antimicrobial activities against common pathogens and unicellular fungi [22].

Several studies described a wide antibacterial activity of *Schinus terebinthifolius* Raddi leaves and fruits extract and attributed this activity to essential oil content in addition to phenols, flavonoids, xanthone and lectin [12, 23, 24]. In the present study, the extract of *Schinus terebinthifolius* Raddi leaves showed antibacterial activity against all the studied pathogens except ESBL-producing *E. coli* and fluconazole resistant *A. fumigatus.* However, inhibition was only strong against ESBL and carbapenemase-producing *K. pneumoniae* and fluconazole resistant *C. albicans.* Additionally, the combination of *Schinus*

terebinthifolius Raddi extract with antimicrobials revealed antagonistic activity. Previous studies reported the capacity of the ethanol extract and essential oil of leaves of *Schinus terebinthifolius* to inhibit the growth of *S. aureus* [24], *P. aeruginosa* [23], *Yersinia enterocolitica, E. coli, Acinetobacter calcoaceticus, K. pneumoniae* and *Bacillus subtilis* [23]. Meanwhile, Jessica and colleagues [25] described an excellent antimicrobial activity of ethanolic extract of *Schinus terebinthifolius* leaves against *E. coli* with no inhibitory effect on *C. albicans*.

The leaf extract of *Myrtus communis* had shown antibacterial and antifungal activities against some pathogenic bacterial and fungal strains [26]. In our study, *Myrtus communis* leaf extract demonstrated strong activity against ESBL-producing *E. coli* and fluconazole resistant *A. fumigatus* and moderate to weak activity against the remaining microbes, except quinolone resistant *S. typhi*, ESBL and carbapenemase-producing *P. aeruginosa* and fluconazole resistant *C. albicans.* Only the combination of *Myrtus communis* extract with voriconazole had synergistic activity against fluconazole resistant *A. fumigatus.* Our results agree with those previous studies [27], where *Myrtus communis* extracts proved inhibitory activities against many bacterial species including *E. coli*, MRSA, *P. mirabilis* and *K. pneumoniae*. **Aabed et al.** [28] indicated that E. coli was the most sensitive microbe to *Myrtus communis* leaf extract. Moreover, antagonistic effect was detected when combining *Myrtus communis* with ciprofloxacin against *E. coli* and *P. aeruginosa.*

Ruta graveolens extracts showed no activity against Corynebacterium *diphtheriae*, *Escherichia coli* and *Candida albicans*. However, they exhibited inhibitory effects and selectivity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes* and *Bacillus subtilis*. the ethyl acetate extract inhibits the growth of *Staphylococcus epidermidis* [11]. In this study, the ethanolic extracts of *Ruta graveolens* had only strong antibacterial activity against quinolone resistant *S. typhi*, meanwhile strong and moderate antifungal activity against fluconazole resistant *A. fumigatus* and *C. albicans*, respectively. Still, synergetic activity was detected only when *Ruta graveolens* extract interacted with voriconazole against fluconazole resistant *A. fumigatus.* **Donadu et al.** [29] revealed antifungal activity of *Ruta graveolens* essential oil against *C. tropicalis* and *C. albicans* with synergetic effect when interacted with amphotericin B against *C. albicans* and *C. tropicalis*. Meanwhile, **Reddy et al.** [30] showed antibacterial activity of *Ruta graveolens* against gram-positive and gram-negative bacteria, in addition to antifungal activity against *Candida albicans* and *A. fumigates* which was the most susceptible fungus to the *Ruta graveolens* essential oil.

Vitex agnus-castus has only shown strong activity against ESBL-producing *E. coli* and a weak activity against quinolone resistant *S. typhi*, with antagonistic activity when combined with antibiotics. Several reports studied the antimicrobial effectiveness of the different parts of *Vitex agnus-castus* [13]. The *Vitex agnus-castus* leave extracts demonstrated an inhibitory effect against Gram positive bacteria [31] and Gram negative bacteria [14] including the drug resistant Gram negative strains [13].

The findings of this study revealed antibacterial activity of the ethanolic extract of *Aberia caffra* Hook. f. & Harv. leaves only against Gram negative antibiotic resistant bacteria (strong activity towards ESBL-producing *E. coli* and weak activity towards both ESBL and carbapenemase-producing *P. mirabilis* and *K. pneumoniae*), still antagonism was detected when the extract interacted with ciprofloxacin or colistin. Earlier analysis of extracts from different parts of *Aberia caffra* for potential antimicrobial activity showed that the seeds were the most active part against *S. aureus* [32], whereas extracts from fruit pulp had antifungal activity against *Saccharomyces cerevisiae*, *Candida utilis* and *Candida albicans* [3]. However, extracts from the leaves of *Aberia caffra* exhibited neither antibacterial nor antifungal activity against any of the tested organisms (11 bacterial strains: *E. coli, Aerobacter aerogenes, P. aeruginosa, S. typhosa, Mycobacterium phlei, S. aureus, Micrococcus species, Sarcina species, Bacillus subtilis, Actinomyces species, Clostridium butylicum*; 3 fungal strains: *Saccharomyces cerevisiae, Candida utilis* and *C. albicans*) [3].

Neem (*Azadirachta indica*) is a popular medicinal plant famous for its therapeutic uses [33]. Neem have showed good *in vitro* antibacterial activity [34]. In the current study, ethanolic extracts of Neem leaves showed strong antibacterial activity only against ESBL-producing *E. coli*, however, with antagonistic activity when interacted with ciprofloxacin. Other studies reported Neem

antibacterial activity against *E. coli* at several concentrations [4]. Furthermore, **Fujdar et al.** [35] noted antimicrobial activity of Neem against ESBL-producing *E. coli, K. pneumoniae, Citrobacter* spp., *Enterobacter* spp. *and Proteus* spp. as well as metallobetalactamase producing *A. baumannii* and *P. aeruginosa.* Neem leaves extracts had inhibitory effect against fungi including *Aspergillus* spp., *Microsporum gypseum* and *Candida albicans* [36]. Phytochemical studies have attributed the following phytochemicals responsible for the reported antibacterial; limonoids such as mahmoodin and tetranortriterpenoids like azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin and 17-hydroxyazadiradione, as well as the protolimonoid, naheedin [4].

Our results showed that the ethanolic extract of *Olea europaea* L. had strong activity only against quinolone resistant *A. baumannii*, yet with antagonistic activity when combined with colistin. Inconsistently, previous studies detected antifungal [37] and antibacterial activity against other organisms including *E. coli, K. pneumoniae, P. aeruginosa and S. aureus* [38]. Leaves extract of *Olea europaea* inhibited effectively growth of gram positive bacteria but less effective against gram negative bacteria. In addition, the phenolic compounds of *Olea europaea* were able to inhibit the growth of flagella and the motility of *L. monocytogenes* [10].

The results of this study showed that the ethanolic extract of *Ficus nitida* L. had weak antibacterial activity only against gram positive cocci (MRSA and glycopeptide and macrolide resistant *E. fecalis*]. **Salem et al.** [6] reported moderate antibacterial activity of *Ficus nitida* leaves. The combination of *Ficus nitida* phenolic extract with tetracycline showed significant enhancement in its antibacterial activity against both Gram positive and Gram negative bacteria [39]. However, the combination of *Ficus nitida* L. extract with ciprofloxacin in our study revealed antagonistic effect against MRSA and glycopeptide and macrolide resistant *E. fecalis.*

Lanatana camara L. has broad antibacterial activity [8]. Triterpenes from *L. camara* were found to be active against *S. aureus* and *S. typhi*. By comparison, chloramphenicol for *S. aureus*, and tetracycline for *S. typhi* in addition to a number of furan naphthoquinones have been shown to possess antimicrobial activity against Gram-positive bacteria and fungi [7]. The current study showed moderate activity of *Lanatana camara* L. leaf extract against ESBL and carbapenemase-producing *K. pneumoniae* while weak activity against other bacteria [glycopeptide and macrolide resistant CNS and *E. fecalis*, in addition to ESBL and carbapenemase-producing *P. mirabilis*). However, when interacted with ciprofloxacin or colistin, antagonism was detected. Previous reports described activity of *Lanatana camara* L. extracts against similar organisms [7].

Although the antimicrobial activity of the plant extracts tested in this study has been already described for other bacterial and fungal species [8, 27], controversial efficacy can be attributed to the variation in the extraction method and microbial species tested. Yet, little is known about their effect against drug resistant organisms. Our results showed the tested extracts had activity against the drug resistant bacteria and fungi with MICs ranging from 0.012 to100 mg/mL. **Aligiannis et al.** [40] classified plant material based on MIC values into those with strong inhibition [≤0.5 mg/mL); moderate inhibition [between 0.6 to 1.5 mg/mL); and weak inhibition [>1.6 mg/mL).

Strengths and limitations of the study

This work is among the few studies that evaluated the activity of several plant leaf extracts against drug resistant clinical isolates, however, few limitations existed. Interaction of plant extracts with antimicrobials was merely evaluated at the MIC values of both. Synergistic activity might have been detected if higher concentrations of plant extracts or other antimicrobials were used. Additionally, only the crude ethanolic extracts of the plant leaves were tested.

Conclusion

The results of the present study are promising. All tested plant leaf extracts have showed great potential as antibacterial and/or antifungal agents against at least one drug resistant microorganism. *Ruta graveolens* L. and *Myrtus communis* L. were the only extracts showing synergistic effect in association with voriconazole against fluconazole resistant *A. fumigatus.* Isolation of the active components and further preclinical and clinical studies are needed to evaluate the efficacy and toxicity of these products.

Conflict of interest

The authors report no conflicts of interest in this work.

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