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Phytochemical, Antioxidant and Antibacterial Potential of *Ducrosia anethifolia* in Northern Border Region of Saudi Arabia

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Authors' contributions

This work was carried out in collaboration among all authors. Author ERE designed the study, collected plant material, performed chemical analysis and antioxidant testing. Author EMA carried out the bacteriological screening. Author MHS performed the analysis of GC-MS and participated in discussion of results. Author SA supervised the entire study and participated with author ERE in plant collection. All authors read and approved the final manuscript.

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ABSTRACT

Ducrosia anethifolia (*D. anethifolia*) is a drought-tolerant plant widely distributed over Arar valley at the Northern region of Saudi Arabia. The aerial parts of this plant were investigated for its phytochemical constituents, antioxidant and antibacterial potential. GC-MS analysis of the ethyl

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acetate fraction of methanol extract revealed the presence of some major compounds such as 8-Ethoxypsoralen (6.5%), Prangenin (6.26%), Isoaromadendrene epoxide (7.5%), Aromadendrene oxide (0.96%) and Ferulic acid methyl ester (0.46%). FRAP and DPPH method were used to test the antioxidant capacity of ethyl acetate fraction of *D. anethifolia*, the results revealed the presence of high reduction capacity (EC_{50} equals 0.63±0.03g/L), compared with the reducing capacity of the standard ascorbic acid and quercetin which were 0.091±0.002 g/L and 0.026±0.002 g/L, respectively. Moreover, the results of the DPPH test showed that the extract presented a remarkable antioxidant capacity with an IC_{50} of 0.38±0.02 g/L, This considerable antioxidant capacity is attributed to its richness of some bioactive phytochemical compounds. The antibacterial potential was evaluated by disc-diffusion test, the plant extract was tested on nine different bacterial strains. Results exhibited that, only Gram-positive bacteria recorded good to moderate susceptibility, namely *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* clinical isolate and *Staphylococcus aureus* ATCC 25923, which recorded 14.5, 14.0, 9.5- and 7.5-mm zone of inhibition, respectively. In conclusion, the aerial parts of *D. anethifolia* are rich in some important phytochemical molecules and could be used in the formulation of antioxidant drugs. Whereas, its efficacy against some Gram-positive bacteria only should be studied in-depth. Further studies are also recommended to these phytochemical molecules against various physiological disorders and diseases.

Keywords: Phytochemical; secondary metabolites; GC-MS; antibacterial; antioxidant.

1. INTRODUCTION

Plants were used as medicines since times immemorial. The oldest written evidence of the usage of herbal medicine has been found on a clay slab dates back to Sumerian civilization in Iraq back to 5,000 years ago. This clay slab containing about 12 recipes of drugs prepared from up to 250 medicinal herbs such as mandrake, poppy and henbane [1]. Interestingly, many plants growing in the arid zones are known of their therapeutic efficacy and have been utilized for thousands of years; these arid plants produce high contents of certain phytochemical compounds enable it to survive under harsh conditions, biotic and abiotic stresses such as alkaloids, tannins, essential oils, glycosides and many more [2]. On the other side, the world witnessed growing employment of herbal medicine for the primary healthcare and recently there are up to 80% of the world population employ medicinal plants as a drug, a supplement or to maintain good health and prevention from diseases [3].

Physiological and microbial diseases are amongst major global health issues. Hence, herbal medicine plays an important role in improving human health. Recent scientific reports showed that there were tremendous medicinal plants having promising antioxidant, anticancer, antibacterial, antifungal, antiviral activities and many more [4]. Furthermore, plants are a substantial source for antioxidant compounds, these molecules protect the human cells from the free radicals which lead to cell damage, they also protect the body from various biotic stresses such as ageing, neurological diseases, physiological diseases and cancer [5]. Moreover, medicinal plants are a promising source for new antimicrobial drugs and there is a growing interest in innovating new antibacterial, antifungal and antiviral drugs from medicinal plants in the near future to control the growing global phenomenon of antibiotics-resistant pathogens [6].

Ducrosia anethifolia (DC.) Boiss (*D. anethifolia*), belonging to Apiaceae family, is a medicinal plant endemic to Iran, where it is known as 'Moshgak' or 'Roshgak'. It is a herbaceous and biennial plant with a height range of 10–30 cm. The stems are glabrous and branched mostly from the base. The leaves are ovate-oblong, 2–6 cm long, branched, with the petiole length of 5–18 cm. The edges of the petals are jagged and slightly shaggy and the compound umbel inflorescence has white flowers [7].

In literature, the crude extract of *D. anethifolia* exhibited numerous therapeutic benefits such as antidiabetic, anti-microbial, anti-radical scavenging, anti-inflammatory, anti-cancer, anti-locomotor and anxiolytic effects [8], however, scientific studies on this plant species are scant. The current study aimed to evaluate the effects of drastic condition of Arar valley northern region of Saudi Arabia on the behavior of *D. anethifolia* in accumulation of antioxidant compound and relation of these compound with the antimicrobial activity of this plant.

2. MATERIALS AND METHODS

2.1 The Study Area

The target area is the Northern region of Saudi Arabia (30º55'13" N , 41º1 3" E) , Wadi Arar is one of main wadis in northeast of Saudi Arabia, this region is famous with Mediterranean desertic climatic, the area are characteristic by hot, arid average annual temperature 21.5 C, and average annual rainfall 20.2 mm which fall during winter months. The extreme rainfall from January to May with more than 90 mm. (http://www.globalbioclimatics.org) [9].

2.2 Plant Material

The fresh aerial part of *D. anethifolia* was collected in spring 2016 from Arar valley, northern region of Saudi Arabia, the sample was identified in the biology department and the authentic sample was deposited.

2.3 Plant Extraction

Aerial parts of plants were air-dried at room temperature in the shade and ground to powder. The powders were subjected to extraction by with 80% methanol by Soxhlet apparatus for 4 hours and the extract was then dried under reduced pressure by rot vapor at 40ºC. The dried residue was suspended in water and then partitioned successively in turn with ether and ethyl acetate. The ethyl acetate fraction was dried over anhydrous sodium sulfate and stored in sealed vials at the temperature of 4-6ºC in dark for further analysis.

2.4 Analysis of the Ethyl Acetate Extract

The compounds were analyzed using a Thermo GC-Trace ultra system (Thermo Co. USA), they were separated on 30 m X 0.25 mm X 0.25 µm Elite-5MS column (Thermo Scientific GC Column). The column temperature was increased from 40ºC to 220ºC at a rate of 4ºC/ min; injector temperature is 250ºC; injection volume is 1 µl; helium carrier gas flow rate is 20 ml/min; transfer temperature is 280ºC. MS parameters were as follows: EI mode, with ionization voltage 70 ev, ion source temperature, 180ºC; scan range, 50-600 Da. The peaks were tentatively identified based on library search using NIST and Wiley Registry 8 Edition [10].

2.5 Antioxidant Testing

2.5.1 Fe (III) to Fe (II) reduction capacity

One mL of each concentration was mixed with 2.5 mL of potassium hexacyanoferrate (III) $K_3Fe(CN)_6$ solution and 2.5 mL of phosphate buffer (0.2 mol/L, pH 7.0) and incubated at 50ºC for 30 min. After, we added 2.5 mL of trichloroacetic acid (10%) to the mixture. Then, 2.5 mL of this solution was homogenized with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm and the concentration of the samples at which the absorbance of 0.5 (EC_{50}) was determined. Ascorbic acid and Quercertin were used as a positive control for comparison [11].

2.5.2 DPPH radical scavenging capacity

In this study, 0.5 mL of each sample concentration was homogenized with the same volume of DPPH methanolic solution (0.04 g/L). The mixture was shaken vigorously and allowed standing for 30 min in darkness at a temperature of 25ºC; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer [11]. The percentage of inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \times 100$$

Quercetin and ascorbic acid were used as a positive control and the concentration that providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted.

2.6 Antibacterial Testing

2.6.1 Microorganisms

Bacteria used in this study were varied bacterial species from Gram-negative and Gram-positive bacteria, these bacteria were; Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876 and *Staphylococccus aureus* clinical isolate), Gram-negative bacteria (*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC 27736 and clinical isolates of *Pseudomonas aeruginosa* and *Acinitobacter baumannii*). Referenced bacterial strains were taken from the microbiology lab, Qassim University. Clinical isolates were generously

provided from Al-Rass General Hospital, Saudi Arabia.

2.6.2 Disc diffusion test

The antibacterial test used in this investigation was the Kirby-Bauer disc diffusion test [12], with minor modification to be suitable for the plant crude extract. The plant extract of the aerial parts of *D. anethifolia* was reconstituted in 10% DMSO (Di-methyl-sulphoxide) to make 500 mg/ml. Microbial strains were sub-cultured in nutrient broth, samples from the broth cultures were pipette and diluted with sterile normal saline to make a suspension equivalent to the turbidity of the 0.5 McFarland standard. A sterile cotton swab was dipped in the adjusted suspension and smeared over previously prepared plates containing 20 ml Mueller Hinton agar. Then, sterile paper discs, 6 mm in diameter were cut from No.1 Whatman filter paper and were immersed in the reconstituted extract (the paper disc is able to trap about 15 µl) and loaded over the smeared plates. Another paper disc of erythromycin (15 µg) was also used as positive control. A disc saturated with 10% DMSO was also added (negative control), the pre-experimental experiment showed that 10% DMSO has no effect on the growth of bacterial cells. All plates were incubated at 35-37ºC for up to 24 hours. Then, the diameter of inhibition zones (in mm) was measured using a ruler, recorded and the mean was calculated from two repetitions.

2.7 Statistical Analysis

Quantitative data were analysed using SPSS software (Statistical Package for the Social Sciences), version 14. If applicable, results were expressed as means with a standard error of means or standard deviation.

3. RESULTS AND DISCUSSION

3.1 Chemical Compositions

The ethyl acetate fractions of the aerial parts of *D. anethifolia* were subjected to GC/MS analysis and reveal the presence of many compound. 8-Ethoxypsoralen (6.5%), Coumarin-6-ol-3,4-dihydro-4,4,7,8-tetramethyl (0.11%), Isoaromadendrene epoxide (7.5%), Aromadendrene oxide (0.96%), Ferulic acid methyl ester, (0.46%) Pterin-6-carboxylic acid, Vitamin A palmitate (0.09%) and Ursodeoxycholic acid (1.39) as shown in Table 1 and Fig. 1. The presence of high percentage of coumarin compounds reflects the importance of the plant as an anti-inflammatory agent where methoxsalen and Prangenin, belongs to a class of organic natural compound (furanocoumarins) which used as anti-inflammatory compound. It can also be injected and used topically [13]. Isoaromadendrene epoxide, α-Santanol acetate and aromadendrene oxide belong to sesquiterpene natural compound [14]. Moreover, the crude extract of *D. anethifolia* and most plants of family apiaceae exhibited numerous therapeutic benefits such as antidiabetic, anti-microbial, anti-radical scavenging, anti-inflammatory, and anxiolytic effects [15].

3.2 Antioxidant Activity

In the present study, the FRAP method was used to test the antioxidant capacity of the *D. anethifolia*, plant extract by reducing the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). The results obtained showed that the extract presents the higher reduction capacity with an EC_{50} of 0.63 ±0.03 g/l. However, the reducing capacity of ascorbic acid and quercetin were 0.091±0.002 g/l and 0.026 ±0.002 g/L respectively (Table 2). In the other hand, 50% of the DPPH radical (IC_{50}) was measured and the results showed that ethyl acetate extract of plant *D. anethifolia* present the higher antioxidant capacity with an IC_{50} of 0.38± 0.02 g/L, however, the antioxidant capacity of ascorbic acid and quercetin were 0.033±0.001g/L and 0.017±0.001g/L respectively (Table 2) the result of antioxidant activity of plant extract was positively correlated with the concentration of sample and reaction time [16], this indicated that antioxidant correlated with chemical composition of extract which responsible for the reaction. The chemical composition of the extract may have an impact on its antioxidant due to the presence of some chemical compound has high antioxidant activity as, ferulic acid methyl ester, vitamin A palmitate and carotene. The ester forms of ferulic acid should act as potent antioxidants in plants and also in plant-derived foods [17].

3.3 Antibacterial Activity

In general, the antibacterial activity of the aerial parts of *D. anethifolia* showed good to moderate antibacterial activity against the Gram-positive bacterial strains which ranged from 14.0 to 7.5 mm Zone of inhibition. Whereas, no antibacterial activity of the plant extract detected against the Gram-negative strains (Table 3). In details,

bacteria that showed moderate susceptibility compared with the tested antibiotic were; *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876, *Staphylococccus aureus* clinical isolate and *Staphylococcus aureus* ATCC 25923, Which recorded 14.5, 14.0, 9.5- and 7.5-mm zone of inhibition, respectively. It is believed that, the inhibition zone from 14 mm and above using disc diffusion test, is considered as good antibacterial activity [18]. Accordingly, *Staphylococcus epidermidis* and *Bacillus cereus* showed high susceptibility to the plant extract of the aerial parts of *D. anethifolia* (14.5±0.5). On the other side, the gram-negative bacteria, namely *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC 27736 and clinical isolates of *Pseudomonas aeruginosa* and *Acinitobacter baumannii* presented no susceptibility toward the plant extract. Plenty of previously published articles cited that, in herbal medicine research, the Gram-positive bacteria were mostly much susceptible towards the plant extracts rather than the Gram-negatives. This can be explained in light of the fact that the lipopolysaccharide layer and periplasmic space that surround the Gram-negative bacterium protects the cell membrane from the destructive effects of the plant extract [19]. Little is known regarding the antibacterial potential of *D. anethifolia*. Amongst them, few studies were conducted only on the essential oils of this plants. As an example, Janssen, et al. [20], demonstrated that the essential oil of *D. anethifolia* and the main oxygen-containing aliphatic components showed a remarkable antibacterial activity against only Gram-positive bacteria, in addition to yeasts and some dermatophytes. Finally, the antibacterial activity against the Gram-positive strains is attributed to the nature of the phytochemical compounds

Table 1. GC-MS analysis of ethyl acetate fraction of *D. anethifolia*

Compound Name	RT	SI	Area %
Ferulic acid methyl ester	4.04	633	0.46
Curan-17-oic acid, 19,20-dihydroxy, methyl ester	4.43	629	0.01
Pterin-6-carboxylic acid	4.73	652	0.15
8-hydroxymenthol	5.62	664	0.14
Desulphosinigrin t-Butyl-{2-[3-(2,2-dimethyl-6-methylene-cyclo	8.81	606	0.01
Morphinan-4,5-epoxy-3,6-di-ol, 6-[7-nitrobenzofurazan-4-yl]amino	15.24	622	0.02
Milbemycin B, 6,28-anhydro-15-chloro-25-isopropyl-13-dehydro -5-O-demethyl-4-methyl	16.36	411	0.01
Erucic acid	18.23	620	0.14
cis-Adamantane-2-carboxylic acid, 4-hydroxy	19	590	0.25
Coumarin-6-ol, 3,4-dihydro-4,4,7,8-tetramethyl	19.12	568	0.11
psi.,.psi.-Carotene	21.6	456	0.02
Ethyl iso-allocholate	22.45	689	0.02
Digitoxin	22.45	681	0.60
Picrotoxinin	22.61	481	0.04
Geranyl isovalerate	24.1	520	0.05
α- Carotene	24.96	398	0.02
Gibberellic acid	25.73	641	0.06
Vitamin A palmitate	25.85	630	0.09
à-D-Glucopyranoside	30.43	719	0.03
Pseudosolasodine diacetate	33.02	738	1.50
Isoaromadendrene epoxide	34.99	697	7.49
Ethyl iso-allocholate	35.79	761	0.17
(-)-isolongifolol, methyl ether	36.18	641	0.93
Aromadendrene oxide	36.37	609	2.94
Deoxyspergualin	36.79	733	2.08
Sccucinic anhydride	38.8	774	3.37
α-Santanol acetate	39.13	608	0.36
Prangenin	40.88	740	6.26
8 -Ethoxypsoralen	40.88	573	6. 50
Docosahexaenoic acid, 1,2,3-propanetriyl ester	44.06	714	0.12
Dasycarpidan-1-methanol, acetate	46.51	699	0.03
Ursodeoxycholic acid	46.89	620	1.39

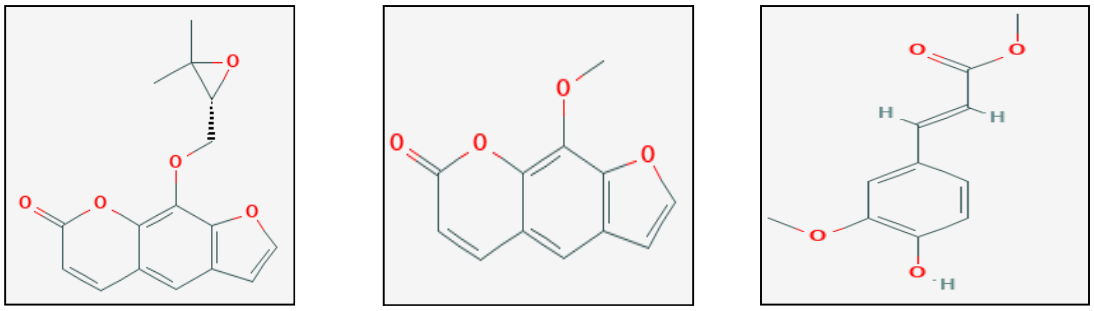
Table 2. The antioxidant capacity of the tested Extract of the aerial parts of *D. anethifolia*

Test	*D. anethifolia*	Ascorbic acid	Quercetin
IC_{50} (g/L)	0.38±0.02	0.033±0.001	0.017±0.001
EC_{50} (g/L)	0.63±0.03	0.091±0.002	0.026±0.002

Table 3. The antibacterial activity of the aerial parts of *D. anethifolia*

Tested compounds	Mean zone of inhibition (mm)*								
	Sa1	Sa2	Se	Bc	Ec	Pa	Ab	Kp1	Kp2
D. anethifolia	9.5±0.5	7.5±0.5	14.5±0.5	14.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Erythromicin	18.5±0.5	23.5±0.5	30.5±0.5	28.0±2.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

**6.0 mm one of inhibition = no activity, Sa1= Staphylococccus aureus clinical isolate, Sa2=Staphylococcus aureus ATCC 25923, Se=Staphylococcus epidermidis ATCC 49461, Bc=Bacillus cereus ATCC 10876, Ec=Escherichia coli ATCC 35218, Pa=Pseudomonas aeruginosa clinical isolate, Ab=Acinitobacter baumannii clinical isolate, Kp1=Klebsiella pneumoniae ATCC 27736, Kp2=Klebsiella pneumoniae ATCC 700603*



Prengenine **Methoxsalen** **Ferulic acid methyl ester**

Fig. 1. Structure of identified compounds

present in the extract of the aerial parts of *D. anethifolia*. Accordingly, further investigations are required in order to isolate, purify and identify the antibacterial agent from the plant extract, which could show a higher effect on bacteria as a pure compound.

4. CONCLUSION

It has been concluded that desert plant growing in arid zones very rich in phytochemical molecules that enable these plants to survive and withstand against the biotic and abiotic stresses under extreme conditions of drought and high temperature. Accumulation of considerable amounts of phytochemicals in desert plants makes them unique in herbal medicine, as they have been a source of remedies since Pharaonic civilization. GC-MS analysis of ethyl acetate fraction of *D. anethifolia* revealed the presence of interesting phytochemical compounds that showed antioxidant activity and antibacterial efficacy against Gram-positive bacteria. Compounds reported in the current study are recommended for further investigations in order to evaluate and utilize the potential bioactive effects on some serious physiological disorders and diseases such as diabetes and cancer. Finally, such scientific reports should attract the pharmaceutical companies to invest in the desert plants which are still neglected so far. Hopefully, the desert medicinal plants might be proven fruitfully in the field of drug discoveries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Petrovska BB. Historical review of medicinal plants usage. Pharmacogn. Rev. 2012;6(11):1-5.
2. Rizvi MA, Saeed A. Medicinal plants of arid zones utilization, cultivation and conservation. Hamdard Medicus. 2013; 56(4):53-80.
3. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology. 2014;4: 177.
4. Veeresham C. Natural products derived from plants as a source of drugs. Journal of Advanced Pharmaceutical Technology & Research. 2012;3(4):200–201.
5. Ayoub Z, Mehta A, Mishra SK, Ahirwal L. Medicinal plants as natural antioxidants: A review. Journal of Botanical Society University of Saugor. 2017;48:1-16.
6. Abdallah EM. Plants: An alternative source for antimicrobials. Journal of Applied Pharmaceutical Science. 2011;1(6):16-20.
7. Arbabi M, Badi HN, Labbafi M, Mehrafarin A, Saboki E. Morphophysiological and Phytochemical Variability in Some Wild Populations of Ducrosia anethifolia from

Iran. Chem. Biodivers. 2018;15(12): e1800301.
8. Venugopala KN, Rashmi V, Odhav B. Review on natural coumarin lead compounds for their pharmacological activity. BioMed Research International; 2013. Available:http://dx.doi.org/10.1155/2013/963248
9. Osman AK, Al-Ghamdi F, Bawadekji A. Floristic diversity and vegetation analysis of Wadi Arar: A typical desert Wadi of the Northern Border region of Saudi Arabia. Saudi J Biol Sci. 2014;21(6):554–565.
10. Muhammad D S, Mohammad I. Antioxidant activity, Phytochemical analysis and total polyphenolic content of Essential oil extract and methanol fraction from Commelina nudiflora, International Journal of Pharmacy and Pharmaceutical Sciences. 2018;10:8-13.
11. Elsharkawy ER, Ed-dra A, Alghanem S, Abdallah EM. Comparative Studies of Chemical Composition, Antimicrobial and Antioxidant Activity of Essential Oil of Some Species from Genus Artemisia, Journal of Natural Remedies. 2018;18(1): 11-20.
12. Mottaghipisheh J, Nové M, Spengler G, Kúsz N, Hohmann J, Csupor D. Antiproliferative and cytotoxic activities of furocoumarins of Ducrosia anethifolia, Pharmaceutical Biology. 2018;56(1):658-664.
13. Mi Z, Jing H, Ya-fang D, Bao-cai L, Chen-xing Z. Chemical composition and antioxidant activity of the essential oil from the flowers of Artemisia austroyunnanensis. Journal of Chemical and Pharmaceutical Research. 2014;6(7): 1583-1587.
14. Masuda T. Systematic studies based on the relation between antioxidant activity and chemical structure of food phenolics, in Annual report of study for food Skylark Food Science Institute, Tokyo; 2000.
15. Christov R, Bankova V, Hegazi AG, Abd El-Hady FK, Popov S. Chemical composition of Egyptian propolis. Z. Naturforsch. 1998;53:197-200.
16. Abdallah EM, Elsharkawy ER, Ed-dra A. Biological activities of methanolic leaf extract of Ziziphus mauritiana. Bioscience Biotechnology Research Communication. 2016;9(4):605–614.
17. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, document M100-S12, Wayne, PA, USA; 2002.
18. Philip K, Malek SNA, Sani W, Shin SK, Kumar S, Lai HS, Serm LG, Rahman SNSA. Antimicrobial activity of some medicinal plants from Malaysia. American Journal of Applied Sciences. 2009;6:1047-1058.
19. Koohsari H, Ghaemi EA, Sadegh Sheshpoli M, Jahedi M, Zahiri M, The investigation of antibacterial activity of selected native plants from North of Iran. Journal of Medicine and Life. 2015;8(2): 38-42.
20. Janssen AM, Scheffer JJ, Baerheim Svendsen A, Aynehchi Y. The essential oil of Ducrosia anethifolia (DC.) Boiss. Chemical composition and antimicrobial activity. Pharm Weekbl Sci. 1984;6(4):157-160.

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