

Evaluation of the Antimicrobial Effects of Essential Oil of *Reseda Lutea* L. on Pathogenic Bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*

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Abstract

Background and aim

Medicinal plants due to natural source, low risks and side effects, accessibility and lower prices than synthetic drugs has much utilization among people. The purpose of this study was to evaluate the antimicrobial effects of essential oil on *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*.

Methods

After collecting plant and confirmation of its scientific name, essential oil of *Reseda lutea* extracted using steam distillation by Clevenger method, after drying in shade and antimicrobial effects of essential oil on bacteria was discussed by the well diffusion method. Some amount of essence was injected to gas chromatography instrument linked to mass spectrometer (GC/MS) to identify the amount and type of essential oil compounds.

Results

The results showed that the essential oil extracted from plant has germicidal effects. Meanwhile, the greatest influence is on the strains *Escherichia coli* and the least effect is on strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. In this plant 40 components were identified.

Conclusion

The results of the present study indicate the weeds can be seen in medicinal plants and also herbal Essences can be used as alternative for esthetic drugs to treat infections of course, all of these effects should be checked *in vivo* and *in vitro* conditions, carefully.

Keywords: Antibacterial effects; Essential oil; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Escherichia coli*

Introduction

Staphylococcus genus grows easily in many of the cultures and it is considered as metabolically active. The two *staphylococcus* species, namely *Staphylococcus aureus* and *Staphylococcus epidermidis*, have been turned in to one of the critical health care problems worldwide due to their high pathogenic potential as well as their increasingly high rate of resistance development to antimicrobial medications. *Staphylococcus aureus* produces beta-lactamase which renders it resistant to penicillin. Methicillin resistance is indicative of being resistant to all types of penicillin counteracting the effects of Penicillinase and Cephalosporins [1,2]. The infections resulting from these bacteria, in spite of having antibiotic medication, leave severe symptoms behind. Therefore, taking proactive measures in regard of the emergence of the infections stemming from such bacteria and finding the outbreak epicenters in the hospitals are necessary.

Staphylococcus aureus and *epidermidis* cause a vast spectrum of diseases including endocarditis, osteomyelitis, pneumonia

and toxic shock syndrome. The aforementioned cases and the daily increasing rate of staphylococci resistance to the other antibiotics such as erythromycin, tetracycline and even the genera with relative resistance to Vancomycin (VISA) or vancomycin-resistance genera formed the basis of performing continuous efforts to seek for new antimicrobial medications [2]. On the other hand, *Escherichia Coli*, as one of the most prevalent bacterial factors, has been separated from human infections. The bacteria's resistance to the medications has a significant importance especially regarding the patients who are to stay in hospitals. *Escherichia coli* is one of the opportunistic pathogens in the hospitals and it incorporates a wide array of pathogenic factors due to the acquisition of plasmids slowing down the beta-lactamase; moreover, they are resistant to beta-lactamase antibiotics, so treating the infections resulting from *Escherichia coli* is very cumbersome [3]. Microbial resistance in *Escherichia coli* has been reported from all around the world and the pace with which such bacteria develops resistance has caused a lot of concerns in developing and developed countries [4,5].

Undoubtedly, resorting to herbal medicines is the most ancient solution exercised by the mankind in treating the ailments and there has always been a close and tight connection between the human beings and the plants within the entire mankind civilizations' course of development. However, there are numerous plant species that are yet to be studied and a great majority of them has been left unidentified and we are still so far away from claiming to have discovered all the new and invaluable plant resources. In this way, the plants are realized as a source of potentially useful chemical compounds a fraction of which has been exploited so far. These potentially useful chemical substances can be applied not only in the form of medications but also as unprecedented pattern marking a turning point for the production of drug analogues; furthermore, they can be of great help in the direction of gaining a more subtle and better insight and understanding of the biological phenomena [6-8]. One of the most important therapeutical challenges is fighting the infectious diseases due to their high rates of outbreak and dispersion. After the penicillins were identified in 1940s and the expansion that took place in taking advantage of such medicines in curing the diseases, we were bearing witness to the emergence of a new antibiotic for the treatment of infections on a daily basis. The result was the expanded use of natural and synthetic antibiotics in treating the clinical infections. The irrational use of antimicrobial drugs led to the development of medication resistance against different antibiotics by a great many of the bacteria. The same issue was one of the reasons why the plants were increasingly taken advantage of as containing less risky natural materials which are, simultaneously, less expensive and available for treating the bacterial infections in comparison to the synthetic antibiotics. Also, these medicinal herbs have a far greater agreeability and are more popularly consumed by the people [10-12]. These factors caused an increase in the new wave of worldwide research and study as well as introducing various plants featuring antibacterial effects during the recent years [13]. The plants belonging to this genus are from the family Asteraceae and the genus *Reseda* includes species with

one to several years' growth type; and, they are herbaceous with entire or toothed leaves. The leaves are usually seen in a jagged form in their brinks. The number of sepals reaches to 4 to 7 and the quantity of petals reaches to a number ranging from 4 to 10. The fruit has a large number of kidney-like seeds. The color of the flowers, their scent and the shape of the fruit plays an important role in the correct identification of this genus [14]. Yellow *Reseda* (*Reseda lutea L.*) is an annual winter plant and there are also plants that last several years. It is an upright standing plant and it reaches to a height of 30 to 100 cm and it propagates via producing

g seeds [15]. This genus is widely spread across Asia and Europe and it is usually found as a valuable weed on alfalfa, clover, vegetables, beet and potato farms as well as orchards, vineyard, also along the roads and barren lands [14]. Yellow *Reseda* grows more densely in Tehran, Karaj, Qom, Qazvin and Azerbaijan [15].

Materials and Methods

Sampling and the investigation of the antimicrobial characteristics

The plant samples were gathered from the natural areas at the periphery of Marand County, Eysh-Abad Village, in East Azerbaijan Province in two periods of year, one in the late May and the other in the early 2016. After the samples were gathered and transferred to the location, they were cleaned and dried in a large and appropriate space, protected from direct sunlight. After the plants were found completely dried in visual examination, the aerial stem and leave organs were isolated from the roots and underwent a preparatory process before grinding.

After powdering the samples, 100 g of herbal powder are mixed as 1:8 with distilled water and ethanol 80%. The mixtures are kept for 48h at lab temperature and are mixed by a glass rod each hour. The mentioned mixtures are filtered by four-layer sterile gas and funnel. To separate the impurities in extract, it is centrifuged at 2500 rpm for 20 m at temperature 4°C then the filtered extract is transferred to distilled system in vacuum to remove the solvents and finally, a strong extract is obtained. The extract is divided by microbial filters 0.45 micron sterile and in micro tubes 1.5 ml sterile and is kept at temperature -80 [7].

The milled samples were added to the Clevenger Device for five hours so as to extract their essence. Then the essence underwent a dehydration process by making use of sodium sulphate; afterwards, it was dissolved into n-hexane solvent and finally it was used for the antibacterial investigations.

The bacteria used in the present study included *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* isolated from the strains found in several patients. First of all, a suspension equivalent to 0.5 McFarland's standard turbidity was prepared and after the bacteria under study in the present research was dispersed over Mueller-Hinton agar surface, microholes, 5 mm in diameter, were immediately caused in the aforesaid culture surface and 100 microliters and

50 microliters of the essence of concern to this study were poured into the microholes followed by admixing the same amounts of n-hexane solvent as control agent. The plates were placed in 37°C for 24 hours in an incubator [16,17].

Analyzing essence by the use of gc/ms device (gas chromatography connected to mass spectrometer)

After a thermal programming system was procured, a sample of essence was injected to GC/MS device (American Agilent Type). The ingredients' identification is carried out by taking advantage of the device information bank and comparison of their mass spectrum.

The results of the experiments were statistically analyzed by means of SPSS software.

Results

The diversity of the climatic and geographical conditions in Iran is the underlying cause for our country's diverse and rich

resources of plant species. Some of these plants possess medicinal traits such as antibacterial features. The studies performed in the current research regarding the antibacterial effects of the essence of the plant of concern herein indicated that:

The yellow Reseda (*Reseda Lutea L.*) exerts a curbing effect on all of the studied bacteria and the highest effect was reported for *Escherichia coli*. The results pertaining to the Reseda essence antimicrobial effect investigation has been provided in (Table 1). It was found that n-hexane solvent, as controlling agent, has no curbing effect on the bacteria under study. The results are very fascinating and considerable according to the clarification of the relationship between the natural material (essences) and the antimicrobial activity of these materials and it can be stated that weeds can be applied as active biological sources against pathogenic factors.

Table 1: antibacterial effect of Reseda Lutea L. essence on microorganisms isolated from the patients strains (Mean \pm Standard deviation).

Control	Concentrations (micro liter)		Gram +/-	
n- hexane	(Inhibition zone diameter to millimeters)			Isolates
	Reseda lutea L.			
	100	50		
-	0.16 \pm 13.5	0.47 \pm 10.66	+	<i>Staphylococcus aureus</i>
-	0.18 \pm 12.6	0.16 \pm 8.5	+	<i>Staphylococcus epidermidis</i>
-	0.26 \pm 14.5	0.47 \pm 11.66	-	<i>Escherichia coli</i>

Chemical ingredients of the *Reseda Lutea L.*'s aerial parts essence was evaluated under laboratory conditions. The essence was extracted by taking advantage of condensation by water vapor by means of a Clevenger Device and it was injected to the GC device and GC/MS after purification (extracting the essence

from the plant and dehydration). The amounts and the types of the essence ingredients were determined. Forty ingredients, consisting a 90.29% of the total identified compounds, were identified. The percentage of the materials existent in the essence has been presented in Table 2).

Table 2: Materials existent in the essence has been presented in a tabular form.

Percent	Compound	row	Percent	Compound	row
0.22	4-undecene	21	1.16	Heptane	1
2.24	Decandioic acid, Didecyl ester	22	0.01	Furan	2
1.3	Heneicosane	23	2.7	Octane	3
0.98	Pentatriacontane	24	9.43	Decane	4
0.67	1-Heptadecanol	25	3.86	Tridecane	5
0.87	Pentane	26	5.57	Pentadecane	6
2.61	Rhodinal	27	0.82	1-Eicosanol	7
3.89	Heptadecane	28	1.94	Nonane	8
6.16	Hexadecane	29	0.92	Tetracontane	9

2.49	1-Nonadecene	30	1.17	Cyclohexanone	10
0.24	1-octadecanol	31	1.51	1-Dotriacontanol	11
1.79	1-Decanol	32	0.79	p-Menthan-3-one	12
0.94	1-Heptadecene	33	8.41	Dodecane	13
1.73	Heptacosane	34	6.34	Undecane	14
3.7	Octadecane	35	0.37	Naphthalene	15
2.74	Tetradecane	36	0.59	Nonadecane	16
0.77	1-pentadecene	37	0.93	Stereoisomer	17
1.85	Docosane	38	0.71	1-Hexadecanol	18
0.82	1-Hexadecene	39	3.29	Diocadecyl ester	19
2.1	3-Buten-2-one	40	0.66	3-Eicosene	20
Total 90.29					

Discussion and Conclusion

According to the increase in the bacteria's resistance to the different types of antibiotics, a lot of efforts have been made to acquire and make use of the compounds extant in the plants as well as figuring out the feasibility of applying them for curing various diseases. From thousands of years ago, plants have played a very important role in keeping human beings healthy and improving their quality of life. Medicinal herbs possess useful attributes among which the antibacterial, anti-parasite, anti-fungus and antioxidant features can be pointed out [18].

In 2012, Chubineh carried out a research titled "the survey of the volatile compounds existing in *Reseda lutea* and *Centaurea Depressa* species and evaluation of the allelopathic potentials in these plants"; in addition, Gas Chromatography (GC) device connected to Mass Spectrometer (GC/MS) was used to separate and identify the essence ingredients. The results found in this latter research conform to the results obtained herein [19].

Abdulameer Abdullah Al-Mussawi (2014) studied the antibacterial activity of a species called *Reseda lutea*. It was found that ethanol extract of this plant had a weak antibacterial effect on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* [20]. Mobaiyen et al. [18] determined the inhibitory effect of two species weed plant on gram-positive and gram-negative bacteria, especially on *Escherichia coli* [21].

Generally, the results of the investigations under laboratory circumstances indicated that the essence extracted from the medicinal herb studied in the current research, named yellow Reseda, possesses antimicrobial activities against *Staphylococcus aureus* and *Staphylococcus epidermidis* as well as against *Escherichia coli*. So, it can be applied against the abovementioned bacteria with the least likely side effects after sufficient amount of investigations performed on laboratory animals.

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