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Ethnopharmacology, Antibacterial and Antioxidant Activity of Dittrichia graveolens (L.) W. Greuter. Which Has Been Used as Remedies Antirheumatic, Anti-inflammation and Antiinfection against Leishmaniasis in the Traditional Medicine of Gorgan, Iran

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Abstract

Objective: This study was survey to investigate of ethnopharmacology, antibacterial and antioxidant capacity of Dittrichia graveolens (L.) W. Greuter extract in in vitro from waste ground region of Gorgan, Iran, which has been used in traditional as a strong anti-inflammation, antirheumatism, antitumor, antipathogene, and antiinfection.

Materials and Methods: Ethnopharmacological data were obtained among well-known indigenous herbal practitioner (70 ages) in Gorgan, Iran. Aerial parts of plant in blooming were collected from Gorgan waste ground (80 m) in October 2013. Methanol and acetone extracts were obtained by maceration, antioxidant activity were evaluated spectrophotometrically by 1,1-diphenyl-2-picryl hydrazyl (DPPH), total antioxidant capacity and reducing power to compare of butylated hydroxytoluene and butylated hydroxyanisole antioxidant standard and antibacterial activity were determined by disc diffusion and minimal inhibitory concentrations (MICs) method against tree Gram-positive and negative pathogenic bacteria.

Results: D. graveolens (L.) W. Greuteris is usually wild grow in Golestan Province and has been used in traditional medicine as a strong anti-inflammation, antirheumatism, antitumor, antipathogene and antiinfection specially in treat of leishmaniosis metanolic extract of plant has strong antioxidant activity against free radical scavenging specially in DPPH methode than aceton extract with IC50 (6.2 ± 0.13 µg/ml) and Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis and Bacillus cereus with inhibition zone 35, 30, 26, 21 mm were the most sensitive bacteria, with MIC ranging from 12.6 to 112 μ g/ml, respectively. Escherichia coli and Salmonella typhimurium have moderate sensitivity and other bacteria were resistant to the plant extract.

Conclusion: Results demonstrate that the methanolic extract of D. graveolens can become good potential antioxidant and antibacterial activity for controlling certain Gram-positive and negative bacteria, which produces many infectious diseases.

Keywords: Antioxidant, Antibacterial, Dittrichia graveolens (L.) W. Greuter, Ethnopharmacology, Gorgan, Inflammation, Rheumatism Leishmaniasis

Introduction

Aromatic herbs have been used as remedies for many infectious diseases, because they have a rich valuable natural source of secondary metabolites

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and have been shown antibacterial, antipathogene, anti-inflammation, antifungal, antiviral, and antioxidant properties (1-3).

According to the WHO in 2008, due to the increasing prevalence of multi drug resistant strains of bacteria, urgency to the search for new natural antioxidant and anti-infection drugs from natural plants and their ethno pharmacology to the identification of natural source, which containing both antioxidant and antibacterial activity is will be necessary (4).

Dittrichia graveolens (L.) W. Greuter. belongs to the Asteraceae family, is an annual aromatic herb, and is locally known as "Atre paizii". Its wild variety grows in temperate climates, along roadsides, on waste grounds, and in humid soil in Mediterranean regions of Iran, Pakistan, Afghanistan, and South West of Asia (5). It has been used in Asian and European traditional medicine as anti-infective, anti-inflammatory, anti-pathogenic, and sedative medication to treat UTI, hemorrhoid, wounds, and leishmaniosis (6).

It has been reported about phytochemical and antioxidant of D. graveolens (7-9). Although, few study of antibacterial and antioxidant activities have been reported of the extracts of D. graveolens from North of Iran (Golestan Province).

In many phytochemical survey, they were reported that the flavonoids, phenols and terpenoids such as: bornyl acetate, borneol, β-caryophylene and camphene were the most secondary metabolites of D. graveolens extract with have strong antioxidant and antibacterial activity (9,10-12).

Antibacterial activities and essential oil composition of D. graveolens were studies and they are available in the literature Iran, Lebnese and Greece (9), but although, few study about of ethnopharmacology, antibacterial and antioxidant activities of D. graveolens has been reported.

Golestan Province in North of Iran is blessed with a rich source of aromatic plants, and throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of many various infection diseases which have not been previously investigated for their chemical constituents and biological potentials and hence in this research the Etnopharmacology, antibacterial and antioxidant activity of D. graveolens (L.) W. Greuter in Golestan Province (Gorgan, Iran) were studied.

Materials and Methods

In many field observation, aerial parts of the plant in blooming were collected from Gorgan waste ground in Golestan province in December 2013. The voucher specimen was identified and deposited at the Herbarium of Research Center of Medicine Plants (HRCMP) of Islamic Azad University of Gorgan Branch and was preserved on (No. HRCMP: 189).

For interviews, we selected one elderly rural practitioners (especially bonesetters-70 year) and the traditional data have been interviewed about local name of plants, part used and its medicine effect to treat of their current diseases, then all obtained data from questionnaires were compared with the findings in vivo and in vitro experiments in other similar reports.

One gram of plant parts with 100 ml (methanol 80%) were extracted by maceration. Extracts were filtered through Whatman No. 1 filter paper. The filtrates obtained from extracts were evaporated on dry rotary evaporator at 40° C and were stored at 4 ˚C (10).

Chemicals:2,2'-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St., Louis, USA). butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and methanol were purchased from Merck Co. (Germany).

This assay is based on Arabshahi-Delouee method. First, The dried extract (12.5-1000 µg) in 1 ml of the corresponding solvent was combined with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 $g.l^{-1}$), after the mixture was incubated at 50 ˚C for 30 min. Then, 2.5 ml of trichloroacetic acid (100 $g.l^{-1}$) were added and the mixture centrifuged at 1650 g for 10 min. Then, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1 $g.l^{-1}$), and the samples absorbance was measured at 700 nm (11).

The ability of the extracts for free radical scavenging was assessed by the method suggested by (3). Briefly, 1 ml of a 1 mm methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 12.5-1000 µg of dried extract). The mixture was then vortexes vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

DPPH scavenging activity (%) = [(A control−A sample)/A control] × 100

This experimental procedure was adapted from Arabshahi-Delouee method, which is based on the reduction of Mo (VI) to Mo (V) by the sample and observation of a green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1 ml of sample solution, containing 12.5-1000 µg of dried extract in corresponding solvent, was combined in a tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mm sodium phosphate, and 4 mm ammonium molybdate). They were incubated in a thermal block at 95 ˚C for 90 min. Then, we got cold the samples and measured their absorbance at 695 nm. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent was used for the sample, and was incubated under the same conditions as the rest of the samples (10).

The bacterial strains were obtained from the Microbiology Laboratory, Golestan University of Medical Sciences. The extract of D. graveolens were

individually tested against two strains of Gram-positive and Gram-negative bacteria: Shigella dysenteriae (PTCC1188), Pseudomonas aeruginosa (PTCC1430), Escherichia coli (PTCC1399), Staphylococcus aureus (PTCC1431), Bacillus cereus (PTCC1015), Salmonella typhimurium (ATCC1596), Staphylococcus epidermidis (PTCC1114), Enterococcus faecalis (PTCC1393) and Klebsiella pneumoniae (PTCC1291).

At a first screening, the extracts were tested against the above mentioned bacteria. Minimal inhibitory concentrations (MICs) were determined by the agar serial dilution method at concentration ranging from 0.93 to 60 µg/ml. Two fold serial dilutions were made from essential oil in molten Mueller Hinton agar (Pronadisa-Madrid) cooled to 45-50°C in a water bath. The essential oil was dispersed in mixture using dimethyl sulfoxide (DMSO). The amount of 0.01 ml of every bacterial suspension, equivalent to McFarland tube No. 0.5 (108 CFU/ml), inoculated on the agar of every well. The culture plates were then incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration at which no visible growth was observed (3). The Mueller Hinton agar were contained DMSO without essential oil was used a negative control while gentamicin was used as positive control.

For all assays, data were expressed as means ± standard error and differences at $P < 0.05$ were considered as statistically significant.

Results

The antioxidant activity of plant extracts were showed in table 1, which the methanolic extract of plant especially in DPPH method with IC50 = 6.2 ± 0.13 µg/ml had higher content of IC50 to free radical scavenging to compare of BHT and BHA control standard ($P < 0.01$). Staphylococcus aureus, Staphylococcus epidermis, E. faecalis and B. cereus with inhibition zone 35, 30, 26, 21 mm were the most sensitive bacteria, with minimum inhibitory concentrations (MIC) ranging from 12.6 to 112 µg/ml, respectively. E. coli and Salmonella typhimurium have moderate sensitivity and other bacteria were resistant to the plant extract (Table 2).

Ethno pharmacological survey showed that aerial parts of D. graveolens with locally name "Atre Paizii" is one of the most wild aromatic herb, which have been used in traditional medicines of rural gorgan as an aromatic, anti-inflammation, antiinfection, antipathogene, insecticide, antifangus, antiulcer and sedative to treat of rheumatism, back pain, infection ulcer and wounds with combination with other herbs such as below:

Antiulcer and ant-infection: consumption the dusin per day (the mixed of Nigella sativa in honey) in plus of little of Ferula asafetida gum.

Anti-inflammation to treat of rheumatic pain and leishmaniasis: External uses of Artemisia annua in plus of perovskia abrotanoides and D. graveolens.

Discussion

D. graveolens showed very remarkable in vitro antioxidant activity at concentrations, especially by DPPH assay in methanolic extract, and due to before research would be confirmed that there is positive correlation between of extract to their high quantity of polyphenols, and flavonoids (5). These data are in accordance with others that had shown the high total polyphenols, flavonoids and tannins content increases antioxidant activity (11).

Table 1. Comparison of antioxidant activity in different extracts of D. graveolens extracts from three methods in IC50 content (µg/ml)

Activity parts	Antioxidant		
	IC50RP	IC50 TAC	IC50DPPH
Methanol extract	27.85 ± 0.80	15.13 ± 0.1	6.20 ± 0.13
Acetone extract	45.17 ± 0.45	36.16 ± 0.6	8.09 ±0.50

TAC: Total antioxidant capacity; DPPH: 2,2'-diphenyl-1-picrylhydrazyl; D. graveolens: Dittrichia graveolens

Table 2. The average in vitro antibacterial activity and minimum inhibitory concentrations (MIC) values of essential oils from flowering aerial parts of D. graveolens L. (in Disc diffusion method) from two extracts of plant in Gorgan region

Micro organisms	Methanolic extract			Acetone extract	
	Inhibition zone (mm) ±SD	MIC (µg/ml)	Gentamycin	Inhibition zone (mm) ±SD	MIC (µg/ml)
S. aureus	35.2 ± 0.6	14.454		17.2 ± 0.30	87.35
S. epidermidis	30.4 ± 0.4	32.16	18	13.2 ± 0.90	41.70
B. cereus	21.1 ± 0.8	112.412		12.4 ± 0.12	105.30
E. faecalis	26.2 ± 0.8	84.02112		10.5 ± 0.10	164.30
E. coli	13.1 ± 0.3	94.3214		-	-
P. aeruginosa	12.1 ± 0.5	148.6223		-	-
K. pneumonia	10.1 ± 0.6	218.08278		-	-
S. typhymorium	12.1 ± 0.8	112.0	78.3	-	-
S. dysentria	9.4 ± 0.2	92.5	116.0	-	-

MIC: Minimum inhibitory concentrations; S. aureus: Staphylococcus aureus; S. epidermidis: Staphylococcus epidermidis; B. cereus: Bacillus cereus; E. faecalis: Enterococcus faecalis; E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa; K. pneumonia: Klebsiella pneumonia; S. typhimurium: Salmonella typhimurium; S. dysenteriae: Shigella dysenteriae; D. graveolens: Dittrichia graveolens

The results of the antibacterial activities of plant extract in table 2 shows that the methanol extracts of D. graveolens had more antibacterial activity against tested bacteria than acetone plant extract, especially on Gram-positive bacteria: S. aureus, S. epidermidis, E. faecalis and B. cereus, respectively (diameter of inhibition ranging from 21 to 35 mm) and MIC = 14-112 µg/ml, which are given in table 2 and showed the highest antioxidant activity in free radical scavenging with IC50 = 6.2 ± 0.13 μ g/ml.

The Gram-negative strains tested: E. coli, Salmonella typhimurium and Pseudomonas aeruginosa were the moderate bacteria respectively (diameter of inhibition ranging from 12 to 13 mm) and Shigella dysenteriae and Klebsiella pneumoniae were resistant to the effect of both plant extracts.

As shown in tables 1 and 2, the methanolic extract of D. graveolens was more antioxidant activity and better effective against the Gram-positive and negative bacteria.

In previous study, the extract of D. graveolens also shows moderately antibacterial activity that can be related to its phenolic and flavonoid compounds (6). Several studies proved that the important phytoconstituents like flavonoids (6,9), polyphenols, tannins, sesquiterpenes (10), in D. graveolens extracts are effective antimicrobial substances against a wide range of microorganisms.

Thus, there is a correlation closely associated with the antibacterial and antioxidant activity of the plant extracts with their phenolic and flavonoid constituents of the plant (6). Also, these researches proved this correlation with tannins and polyphenols. So further, this study is clearly in agreement with another reports and they are in agreement with positive correlation of secondary metabolites of plant extract to its high antioxidant and antibacterial activity, especially against S. aureus, S. epidermisis, B. cereus and E. faecalis.

Our results and before reports were suggested that Gram-positive bacteria are generally more sensitive to the D. graveolens extracts than Gram-negative bacteria and, although the moderately inhibitory activities of this plant against negative bacteria can be explained probably that this plant contents some particular anti Gram-negative substances.

The antibacterial activity of this plant is not reported from North of Iran, furthermore, our study showed activity of D. graveolens against both Gram-positive and Gram-negative bacteria. In contrast with other reports antioxidant and antibacterial activity of this plant is indicative of the presence of some secondary antioxidant compounds (flavonoid and phenols (6,12). In Similar research, 1,8-Cineol (54.89%), P-cymen (16.2%), beta-pinene (6.94%) and borneol (5.44%) were the most anti-Candida and antibacterial activity of the volatile oil of the aerial parts of D. gravolence against different isolates of Candida albicans was studied in vitro with MIC = 30.675 mg/ml (13).

Conclusion

These data due to confirming of plant traditional medicine of Gorgan region to treat of infection and inflammation, contributes that the methanolic extracts of D. graveolens had good antioxidant and antibacterial activity especially on Gram-positive bacteria.

The S. aureus and P. aeruginosa have been implicated in cases of boils, sores and wounds are considered as a main pathogen of causing hospitalized patients infections and also the good effective of essential oil growth inhibition against E. coli justifies its use in the control of diarrhea and dysentery in humans (14).

So we offered that further investigations are needed to focus on isolation, separation and purification of the important secondary metabolites of this plant according to its medicinal effects such as anti-infection, anti-inflammation and sedative in in vitro and in vivo model, especially in fungus and leishmaniasis and evaluate the safety of this plant. In addition, these investigations confirm the traditional medicine of this plant and demonstrate that the plant extract could become potentials for controlling certain important Gram-positive and negative bacteria, which produces many current infectious diseases.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

We declare that we have no conflict of interests.

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