Journal of Medicinal Plants and By-products (2021) 2: 133-139

Original Article

Phytochemical Analysis of Essential Oil from the Seed of *Nicotiana rustica* L. and Its Antioxidant and Antimicrobial Activity

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Article History: Received: 20 July 2020/Accepted in revised form: 10 December 2020

Abstract

In recent years, medicinal plants have been used as sources of natural composition and they are used as alternative for synthetic antioxidants and antimicrobial agents in food and pharmaceutical industry, natural therapy and alternative medicine. The present regard is the first report on the assessment of chemical composition, antioxidant and antimicrobial activity of essential oil from the seeds of *Nicotiana rustica* L. Isolated from Iran. Tobacco seeds were collected from the tobacco fields in Borazjan city of Iran. Essential oils of the seed of *N. rustica* were prepared by hydro - distillation using Clevenger type apparatus during 3 hours after that Qualitative and composition of the oil was determined by GC-MS. The antioxidant activity of essential oil has been investigated by using DPPH assay method. For antimicrobial activity and MIC, MBC and MFC of the essential oil Micro broth dilution technique on eight pathogenic bacteria and two fungi has been used. The results obtained showed the presence of 28 components, in the oil with the highest content of Myristicin (32.75%) and ar-Turmerone (5.71%). The antioxidant activity of *N. rustica* oil was IC50 µg/mL = 27.93±0.834. In addition, oil has shown the best antimicrobial activity on *Pseudomonas aeruginosa*, *Bacillus cereus*, *E. coli,* and *Staphylococcus aureus.* The results of antimicrobial assays indicated that the most of examined microorganisms have been affected in low concentration. Also the essential oil demonstrates strong antioxidant activity but antifungal activity was not observed.

Keywords: *Nicotiana Rustica*, Essential Oils, GC/ MS, DPPH, Serial Dilution Method

Introduction

Infectious diseases are caused due to a complex interaction between the pathogen, host and the environment. The discovery of antibiotics and their subsequent use had eradicated the infections that once challenged mankind. However, using antibiotics is going through a crisis due to the development of resistance by pathogens. Moreover, these pathogens have the ability to transmit the resistance gene and thereby create a serious issue in the field of medicine [1] Medicinal plants are rich sources of secondary metabolites, which are potential sources of useful drugs and another useful bio reactive product. Antimicrobial properties of various plant parts like leaves, seeds, and fruits have been well documented for some of the medicinal plants for the past two decades. The ability to synthesis the compound by secondary metabolism possessing antimicrobial potential makes plants an invaluable source of pharmaceutical and therapeutic products [2].

Nicotiana plants are indigenous to the Americas, South West Africa, the south Pasific and Australia. *N. rustica* belongs to the family Solanaceae which also includes some other important crop species such as tomatoes, potatoes peppers, etc. [3]. Because of bioactive compounds of abundant in the tobacco leaves such as polyphenols, proteins and aromatic compounds they have been one of economically valuable plants [4]. Therefore, it is important to investigate and better utilize tobacco plant [5]. Their antioxidant and antimicrobial activities of tobacco leaves and identification of polyphenols were investigated previously [6]. Based on research that has been done flavonoids are one of the naturally occurring phenolic compounds, which occurs in different plant parts like tobacco plant. They are found to have many biological activities including antimicrobial,

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mitochondrial adhesion inhibition, antiulcer, anticancer, and etc. Also flavonoids are particularly beneficial, acting as antioxidants and giving protection against many diseases such as certain forms of cancer and age-related degeneration of cell components [7]. Base on the previous research this study is first report on chemical composition, antioxidant and antimicrobial activities of essential oil from seeds of *N. rustica.*

Material and Methods

Plant Material

Seeds of tobacco species *N. rustica* L. (specimen number in the herbarium of Islamic Azad University, Shiraz Branch is P920322) used in the present research work were collected at summer from the tobacco fields in Borazjan city (Booshehr provinces) of Iran. The seeds were dry and powdered with blender then were soaked in water for one day so they were used directly for essential oil extracting.

Essential Oil Extraction

The essential oil was obtained from dried seeds (100 g) from *N.rustica* that were subjected to hydro-distillation using a Clevenger-type apparatus (w/w) for 3 h. Then the oil was dried by anhydrous sodium sulfate. The isolated oils were stored in tightly closed vials at 4°c until analysis [8].

Essential Oil Analysis

Essential oil was analyzed by Hewlett– Packard GC/MS (model 6890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m' 0.25mm, 0.25 µm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method, using n–alkanes as standard (Van den Dool and Kratz 1963) The compounds were identified by comparison of retention indices (RRI–HP–5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [9].

Microorganisms and Their Growth Conditions

Microbial strains obtained from Persian Type Culture Collection (PTCC) in Iranian Research Organization for Science and Technology that including *Escherichia coli* (PTCC 1154), *Staphylococcus aureus* (PTCC 1189), *Pseudomonas aeruginosa* (PTCC 1571), *Staphylococcus epidermidis* (PTCC 1435), *Bacillus cereus* (PTCC 1816), *Klebsiella pneumonia* (PTCC 1290), *Aspergillus niger* (PTCC 5154), *Penicillium digitatum* (PTCC 5204). All bacterial and fungi strains were cultivated in Nutrient Agar and Potato Dextrose Agar for 48hrs at 37 ℃ (bacteria) and 25 ℃ for 3 days (fungi) respectively following refrigeration storage at 4°C until required for sensitivity testing [10].

Antimicrobial Activity

In vitro antimicrobial activity of the essential oil from seeds of *N.rustica* was measured by microbroth dilution method for determining of minimum inhibitory concentration The tested bacteria and fungi were cultured on Nutrient agar (NA) and Sabouraud dextrose agar medium (SDA) respectively for the period of time required. Then the purified suspensions were prepared with a concentration of 10^8 CFU/mL bacteria and 10^6 CFU/mL fungi on NB and SDB medium [11].

Determination of Minimum Inhibition Concentration (MIC)

Minimum inhibition concentrations (MIC) of *N. rustica* seeds oil against the tested microbial strains were determined using the microbroth dilution method. Briefly, serial two-fold dilutions of *N. rustica* seeds oil (10% W/V) were prepared in 96-well microtiter plate (from 1:2 to 1: 892) Nutrient broth for bacteria and Sabouroud dextrose broth for fungi spices (Merck, Darmstadt, Germany). Control micro-titer plates containing medium and DMSO at the same dilutions were also made. Microbial suspensions were adjusted 1×10^8 CFU/mL for bacteria (OD600=0.1) and 1×10^6 CFU/mL for fungi (by haemocytometer lame). A constant amount of bacteria and fungi were added to all wells and the plates were incubated at 37 ℃ for 24 hours (for bacteria) and 27 ℃ for 48 hours (for fungi). Growth of Each well was compared with control wells. For each test enrofloxacin and gentamycin were used as the control antimicrobial agents. The lowest concentration of an antimicrobial agent in order to inhibit the visible growth of a microorganism is called as the MIC. The experiment was carried out in triplicate [2].

Definition of Minimum Bactericidal and Fungicidal Concentration (MBC) and (MFC)

To the definition, the MBC and MFC of all small wells without turbidity were cultured in NA (for bacteria) and SDA (for fungi) medium. Then the medium at the proper temperature for each microorganism was located inside the incubator. After the required time of bacterial and fungal growth, the lowest concentration of essential oil that 99.9 % of bacteria and fungi have not growth, were considered as bactericidal and fungicidal concentration [12].

Anti-Oxidative Test

DPPH Free Radical Scavenging Activity

The radical scavenging capacities of the essential oil, in different concentrations were estimated. In this method

the decolorization of methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) has been used to examine the hydrogen atom donating ability of the plant extractives. Different aliquots of the sample were dissolved in methanol, incubated with a methanolic solution of DPPH (100µL) in 96-well microplates. After that the mixture has been shaken at room temperature for 30 min. And the absorbance was taken at 517 nm against a blank by using a spectrophotometer (UV-1800) (Milton Roy Company Spectronic 2OD). All reactions were carried out in triplicate. Gallicacid was used as positive controls. The percentage inhibition (I %) for each concentration was calculated by using absorbance (A) values according to the following formula: I %=[(ADPPH-AP)/ADDPH]×100. [13]

Result and Discussion

Ingredients Essential Oils

The chemical compositions of the essential oil from seed of *N. rustica* are presented in (Table. 1). Twenty-eight components were identified in *N. rustica* essential oil that represented 93.87% of the oil. The main components of the oil were, Myristicin (32.75%), ar-Turmerone (5.71%), Apiole (4.57%), n-Hexadecanoic acid (4.22%), Phthalic acid (3.93%) and 1, 4-Eicosadiene (3.72%). The chemical components of the essential oil have been previously reported with Schlotzhauer *et al*, although there were differences in the ratio of their chemicals constituents and identified seventeen compounds in *N. rustica* that nicotine (25.92%) and aromadendrene (11.26%) were the major components [14]. These differences in ingredients essential oils can be ascribed to various agents such as climatic, seasonal, harvesting time plant cultivar and geographical or ontogenesis variations [8].
retention indices in elution order from HP-5 column

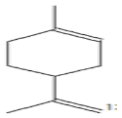
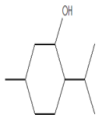
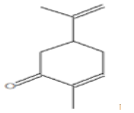
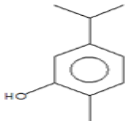
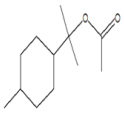
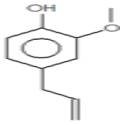
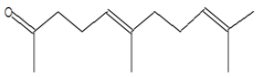
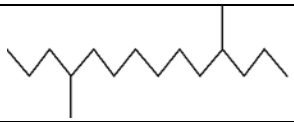
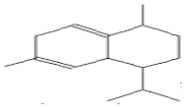
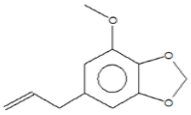
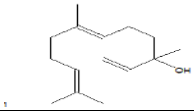
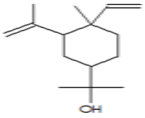
Antimicrobial Activity

The investigations about antimicrobial activity, the action mechanism and potential use of volatile plant oils have received prominence in recent decades in parallel with advances in traditional approaches to protecting the health of humans, animals, and food against the presence of pathogenic and spoilage microorganisms. Thus, research on the antimicrobial activity of plant essential oil against various pathogens have been done worldwide. Our outcomes have significance because they supply information about this issue.
The MIC, MBC and MFC values of the essential oil against all microorganisms experimented are reported in (Table 2). The antimicrobial activity of experimented oil was generally higher against bacteria than fungi. *Pseudomonas aeruginosa*, *Bacillus cereus*, *E. coli* and *Staphylococcus aurous* revealed the highest sensitivity to essential oils, while *Aspergillus niger* and *Penicillium digitatum* didn't reveal any sensitivity. So that, the growth of *Pseudomonas aeruginosa*, *Bacillus cereus*, *E.coli*, and *Staphylococcus aureus* were inhibited by *N. rustica'* seeds essential oil in concentrations of 16 µL/mL, 32 µL/mL and 64 µL/mL respectively. Also, 99.9% of *Pseudomonas aeruginosa*, *Bacillus cereus*, and *E. coli* have not growth in concentrations of 16 µL/mL, 64 µL/mL of *N. rustica*' seeds oil respectively. Some investigations reported that there is a connection between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity [15]. However, the synergistic or antagonistic effect of one compound in the low percentage in the mixture has to be considered [16]. In this study, the antimicrobial activity can relate to the presence of phenolic compounds such as Carvacrol, Menthol, Eugenol, (Z)- Nerolidol, Elemol,Carotol, 1,10-di-epi-Cubenol, and Apiole. Phenolic compounds are one of the most diverse groups of secondary metabolites found in edible plants. In nature, they are involved in plant growth and reproduction, provide resistance from pathogens and predators and protect crops from disease and pre-harvest seed germination [17]. Flavonoids are the most widely occurring polyphenol. Flavonoids are potent antioxidants, free radical scavengers, and metal chelators; they inhibit lipid peroxidation and exhibit various physiological activities like antimicrobial activities [18]. Also, some researchers reported that the polyphenols can affect the growth and metabolism of bacteria, activating or inhibiting the microbial growth according to their constitution and concentration [19].

Antioxidant Activity

In this experiment, the abilities of the essential oil to donate hydrogen atoms or electrons were measured spectrophotometrically. The essential oil from seeds of *N. rustica* reduced yellow-colored product, diphenyl-picrylhydrazine, and decrease the absorbance at 517 nm, possessed antioxidant activity (table 3). The antioxidant activity of *N. rustica'* seeds oil was IC50 = 27.93±0.834 that was close to the Galic acid. The antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants. Moreover, essential oils been also able the scavenging free radicals. Increasing evidence has suggested that some important diseases may result from cellular damage caused by free radicals. [20], in lipid systems, phenolic compounds are effective antioxidants, thus, Carvacrol, Menthol, Eugenol, (Z)-Nerolidol, Elemol, Carotol, 1, 10-di-epi-Cubenol and Apiole molecules are indeed responsible for the antioxidant activity of *N. rustica* essential oils that contain them.

Table 1 Essential oil components of seeds of *N. rustica* L. analyzed by (GC-MS)

No.	compound	RI	Percentage in oil	structure
1	*Limonene*	1029	1.03	
2	*Menthol*	1171	1.90	
3	*Carvone*	1257	2.24	
4	*Carvacrol*	1299	1.18	
5	*α-Terpinyl acetate*	1349	2.42	
6	*Eugenol*	1372	1.05	
7	*n-Tetradecane*	1400	2.09	
8	*Geranyl acetone*	1464	1.72	
9	*4,11-dimetheyl-Tetradecan*	1468	1.33	
10	*Cis-Cadina-1,4-diene*	1495	1.90	
11	*n-Pentadecane*	1500	2.42	
12	*Myristicin*	1518	32.75	
13	*(Z)-Nerolidol*	1532	1.37	
14	*Elemol*	1561	1.65	

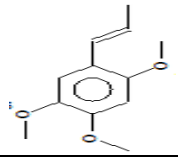
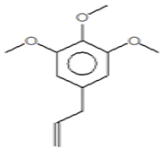
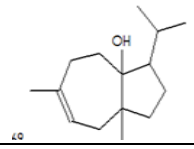
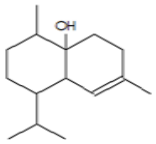
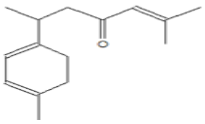
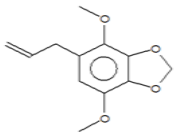
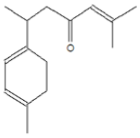
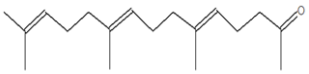
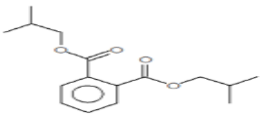
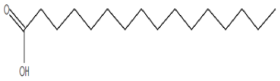
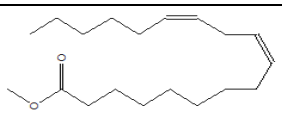
15	*T-Asarone*	1574	0.91	
16	*Elmicin*	1555	3.77	
17	*Carotol*	1594	2.12	
18	*n-Hexadecane*	1600	0.91	
19	*1,10-di-epi-Cubenol*	1619	0.91	
20	*ar-Turmerone*	1669	5.71	
21	*Apiole*	1678	4.57	
22	*Tumerone*	1718	1.78	
23	*1,4-Eicosadiene*	1884	3.72	
24	*(5E, 9Z)- farnesyl acetone*	1861	4.75	
25	*Phthalic acid*	1917	3.93	
26	*n-Hexadecanoic acid*	1959	4.22	
27	*Methyl linoleate*	2085	1.39	
28	*Linolieic acid*	2133	0.13	
Total	-	-	93.87	-

Table 2 Antimicrobial activity of essential oil from seeds of *N. rustica* L.

No	Fungi	Bacteria	MIC[a]	MBC[b]	MFC[c]
1	-	*Echerichia coli*	32µL/mL	ND[d]	ND
2	-	*Pseudomonas aeruginosa*	16 µL/mL	16 µL/mL	ND
3	-	*Klebsiella pneumoniae*	ND	ND	ND
4	-	*Staphylococous aureus*	64 µL/mL	ND	ND
5	-	*Staphylococcus epidermidis*	Not detected	ND	ND
6	-	*Bacillus cereus*	32 µL/mL	ND	ND
7	*Aspergillus niger*		ND	ND	ND
8	*Penicillium digitatum*		ND	ND	ND

a: Minimum inhibition concentration; b: Minimum bactericidal concentration; c: Minimum Fungicidal concentration d: Not detected

Table 3 Anti-oxidant activity of essential oil from seeds of *N. rustica* L. by DPPH assay

Compound	IC_{50} (µg/mL)
Gallic acid	24.70 ± 1.54
N. rustica seeds	27.93 ± 0.83

Conclusion

The present study concluded that the good antimicrobial and antioxidant activity of *N. rustica* essential oil were against *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, and E. coli* respectively. Since there are no reports in the literature on the antimicrobial and antioxidant activity of essential oil from seeds of *N. rustica*, further work needs to be done on this essential oil and extract to isolate active component and treatment diseases or use it as food preserving.

Acknowledgments

The authors are thankful to the College of Science, Agriculture and modern Technology of, Shiraz Branch, Islamic Azad University, Shiraz, Iran for supporting.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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