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ORIGINAL ARTICLE

Elaeagnus angustifolia L. Whole Fruit Ethanolic Extract: Phytochemical Composition and Antimicrobial Effects

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KEYWORDS

Elaeagnus angustifolia; Antimicrobial; Phytochemical; Extract

ABSTRACT: In this research chemical composition and antimicrobial effects of *Elaeagnus angustifolia* L. (*E. angustifolia* L.) whole fruit ethanolic extract was investigated on common pathogenic microorganisms (Staphylococcus *aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Candida albicans)*. The phytochemical composition of *E. angustifolia* was screened by Gas Chromatography-Mass Spectrometry (GC/MS). The minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of the extract were determined using the broth dilution technique. According to the results, 13 major compounds such as flavonoid, aldehyde, alcoholic and fatty acids were identified by GC/MS. In addition, the extract could inhibit the growth of all examined pathogenic strains. The MIC was 3.75 to 1.87 mg ml^{-1} while maximum activity was found against *Staphylococcus aureus.* Besides, the MBC was ranging from 7.5 to 3.75 mg ml^{-1}. Since *E. angustifolia* whole fruit ethanolic extract contains phytochemical compounds and has antimicrobial potential it can be recommended as a natural active agent for application in the food industry.

INTRODUCTION

Elaeagnus angustifolia L. (E. angustifolia L.) one of a the native trees known as silver berry, oleaster, Persian olive, or wild olive which is widespread in northern Europe and sia [1-5]. In Iran, the fruits of *E.angustifolia L.* are not only commonly consumed as a food in the shape of dried fruit, grounded whole fruit powder, or pulp of fruit but also it is traditionally used as an herbal remedy painkiller for rheumatoid arthritis [6, 7]. Different components belonging

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to phytochemical classes have been identified in the whole fruit of *E. angustifolia L.* The major constituents are including proteins, amino acids, polysaccharides, and inorganic elements also β-carboline alkaloids, flavonoids, phenolic acids, steroids, terpenes, and miscellaneous compounds.[7-9].

Different parts/products of this plant such as fruits, flowers, leaves, and extract have been utilized as herbal agents in folk medicine. There is some evidence of its usage for the treatment of jaundice, urinary diseases, gastric disorders, diarrhea, asthma, flatulence, and inflammation of rheumatoid arthritis nausea, vomiting, jaundice, and abdominal distention Furthermore, numerous *in vivo*, *in vitro* and human studies have been reported a vast range of pharmacological and biological activities related to this medicinal plant. Du reported the potential immunological activities of *E. angustifolia* L. Pulp as natural medicine, and Ebrahimi found positive effects in supplemented patients with osteoarthritis of the knee with *E. angustifolia* L. medulla powder[10, 11]. Moreover, functional properties like anti-inflammatory, anti-edema, anti-nociceptive, and muscle relaxants were observed from extracts and essential oil of different parts of *E. angustifolia* L.[12-15].

The aspect of the antimicrobial effect, many studies have been conducted on *E. angustifolia* L. extract against pathogenic microorganisms such as *Yersinia entrocolitica*, *staphylococcus aureus* (mastitis bacteria), *Yersinia enterocolitica*, s*treptococcus pyogenes*, *Klebsiella pneumonia*, *E. coli*, *Alternaria solani*, *Botrytis cinerea*, *Aspergillus fumigates*, *Aspergillus flavus* and *Aspergillus niger*[14, 16-19]. However, the extraction method and choice of solvent had a significant impact on the release of an active agents from the plant matrix [20-22]. The aim of this study is the evaluation of ethanolic extract whole fruit of *E. angustifolia* L. phytochemical components and antimicrobial effects against selected pathogenic strains.

MATERIALS AND METHODS

Material

The fruits of *E. angustifolia L.* were collected from Damghan city (Semnan province, Iran). The fruits were cleaned and washed with distilled water then were dried in an oven with air circulation at 40 °C for 48 h. The whole fruit was milled using a laboratory hammer mill and then sieved to an average particle size of 0.5 mm. the obtained powder was packed in an airtight glass jar and kept at 4°C for the next analysis. All chemical agents and media were purchased from Sigma (Steinheim, Germany).

Moisture and pH content

The AOAC method No. 934.06 (1990) was used to determine the moisture content of the powdered *E. angustifolia* L. whole fruits. The pH was determined using a calibrated pH meter.

Protein and Fat content

Protein content was determined according to the Kjeldahl method considering 6.25 as the conversion coefficient of nitrogen to crude protein. The fat content was measured by the Soxhlet method using n-Hexan as a solvent in an automated soxhlet set (B- 811, Buchi Switzerland)[20].

Fatty acid composition

The fatty acid composition was identified using gas chromatography (6890 N, Agilent, US) equipped with a BPX70 capillary column (120 m×0.25 mm×0.25 μm) a flame ionization detector. The used analysis conditions included the initial temperature (16°C), the final temperature (220°C), the heating rate (18°C/min), the detector temperature (250°C), the injector temperature (230°C), and nitrogen as carrier gas (42.12 psi) according to Ce1f-96 Method of AOAC [23].

Extraction

The extract of *E. angustifolia* L. was obtained by maceration method using ethanol at the volume of 70% v/v for 48h in a shaker incubator. The obtained solution was filtered using Whatman no. 1 and stored in at 4 °C for further analysis[6].

GC-MS analysis

The phytochemical composition of *E. angustifolia* L. whole fruits extract was detected using gas chromatography-mass spectroscopy (Agilent 7890equipped with HP-5MS capillary column) by comparison of properties of each peak (Figure 1) with reference library (about 400000 spectrums) according to Wieiy 7 databases. The sample was injected at 1 µl and the rate of Helium gas was adjusted to 1 mL min^{-1} at 280°C [24].

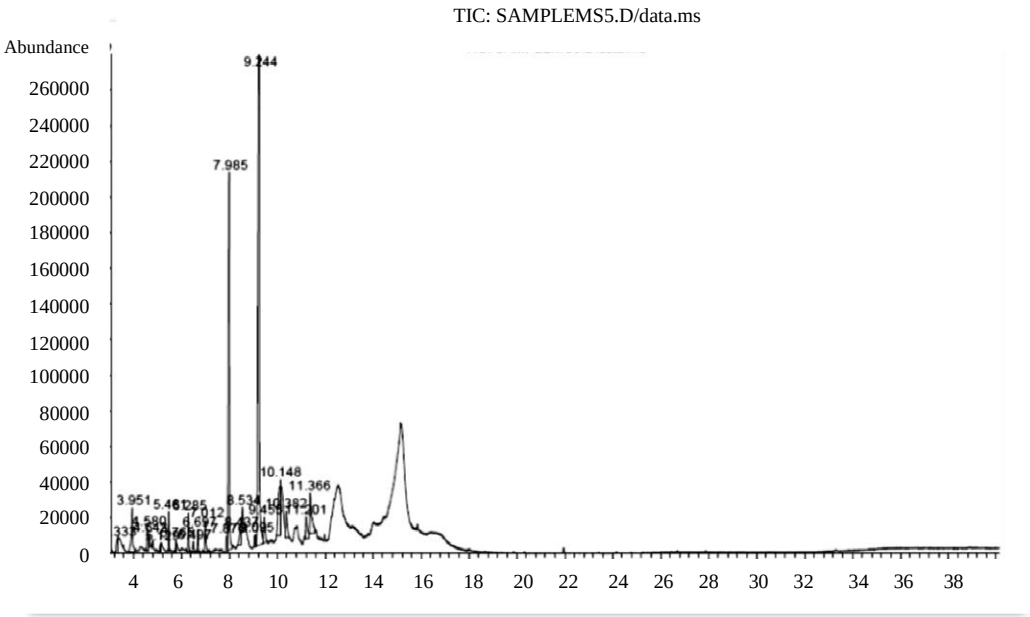

Figure 1. The chromatogram of *E. angustifolia* L. whole fruit extract was obtained using GC-MS.

Bacterial strains

Two Gram-negative bacteria (*Enterobacter aerogenes* PTCC 1221 and *Escherichia coli*PTCC 1276), one Gram-positive bacteria (*Staphylococcus aureus* PTCC 1112), and one fungus strain (*Candida albicans* PTCC 5027) were used in this study.

MIC, MBC/MFC determination

The minimum inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) of the extract against studied strains were determined using the broth macro-dilution method.

A single colony of bacteria strains was transferred on Mueller Hinton broth (MHB) and *Candida albicans* in sabouraud dextrose broth (SDB) was incubated at 37 °C for 24 h. The cells were separated from the medium by centrifugation (5000×g and 15 min) and then were suspended in fresh MHB to adjust the turbidity at 1×10^6 CFU mL^{-1} (0.5 McFarland). The serial dilution of *E. angustifolia* L. extracts at the concentration of 7.5, 3.25, 1.62, 0.81, 0.4, 0.2, and 0.1 mg/ml was prepared and used in test tubes containing 4 ml of sterile nutrient broth. After that 0.5 ml of the obtained solution was added to 9.5 ml MHB for bacteria and SDB for fungi and finally incubated (37°C for 24 h). The inoculated medium containing microorganism strains without extract was regarded as control positive. Also, a tube containing medium was used to control sterile conditions. The growth of strains was monitored regarding the turbidity of the medium and the presence of pellets in the tubes. The lowest concentration of extracts in which the growth of microorganisms is prevented was recorded as MIC. For MBC/MFC, 100 µl of each tube without color change were sub-cultured in Petri dishes with MHA and incubated at 37ºC for 24 h. The minimal concentration of extracts which showed bactericidal/fungicidal effects on species was indicated as MBC/MFC[14].

RESULTS AND DISCUSSION

Chemical characterizations of E. angustifolia L. whole fruit

The chemical characterization of *E. angustifolia* L. extract is shown in Table 1. As can be seen carbohydrate is constituted the main part of the extract and after that fiber, protein, and fat are the most constituents respectively. In a study by Akbolat, the content of protein of oleaster (*Elaegnus angostifolia* L.) fruit was reported 12.3%[25]. According to the fatty acid profile of *E. angustifolia* L. whole fruit (Table 1), it was observed that stearic acid (26.42%) and oleic acid (26.10) had the highest contents. Also, the concentration of essential fatty acids including linoleic (7.46%), linolenic (2.78%), and arachidonic (3.03%) was noticeable.

Table 1. Chemical specification of *Elaeagnus angustifolia L* fruits.

Chemical specification	Content (%)	Fatty acid		Content (gr%)
Fat	1.25	**Lauric acid**	**C12**	0.83
Protein	5.63	**Myristic**	**C14:0**	1.04
Moisture	4.21	**Myristoleic**	**C14:1**	0.78
Acidity (citric acid)	1.24	**Palmitic**	**C16:0**	11.34
Carbohydrate	74.42	**Palmitoleic**	**C16:1**	0.10
Fiber	11.45	**Stearic**	**C18:0**	26.42
Vitamin C (mg/100g)	140	**Oleic**	**C18:1**	26.10
Ash	1.8	**Linoleic**	**C18:2**	7.46
PH	5.14	**Linolenic**	**C18:3**	2.78
		Arachidic	**C20:0**	3.03
		Arachidonic	**C20:1**	1.30
		Behenic	**C22:0**	2.08
		Lignoceric	**C24:0**	1.39

Phytochemical composition of the extract

The phytochemical composition of the *E. angustifolia* L. extract is shown in Table 2. Besides, there were thirteen major components in which the main components were included: *2-Furancarboxaldehyde, 5-(hydroxymethyl)* (46.26%) *and 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6- methyl (*18.74%). All identified compounds generally were categorized as aldehydes, alcohols, carboxylic acids, and heterocyclic, flavonoid, epoxy, benzyl, and fatty acid compounds. Our results are in agreement with previous studies regarding different type of flavonoid compounds such as catechin and epicatechin observed in *E. angustifolia* extracts[13, 15]. Among all flavonoid compounds, there were quercetin aglycones (like rutin which is quercetin 3-rutinoside) that were identified in the fruits[26]. Contrary to the findings of Bendaikha, we did not find phytosterols as major components[15]. In another study, major compounds from *E. angustifolia* L. extract from a different region of Iran were reported as 0.21 % (w/w) kaempferol [27]. Moreover, in the crude extract of *E. angustifolia* L. alkaloids, flavonoids, saponins and tannins were also reported as major components[26]. However, it seems the variation of plant origin, extraction methods, and used solvents had influenced results.

Table 2. Major phytochemical compounds of *E. angustifolia* L. whole fruit ethanolic extract identified using GC-MS

Compounds	relative peak area%	structure	Function
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	18.74	Flavonoid compound	Antimicrobial, Anti-inflammatory, Antioxidant agent
2H-Pyran-2,6(3H)-dione	0.52	Aromatic carboxylic acid	Antibacterial agents, Antiallergic agents, Food preservation
2,5-Dimethyl-4-hydroxy-3(2H)-furan	2.68	Heterocyclic aromatic organic compounds	Antimicrobial, DNA repair for cancer in research
2-Furancarboxaldehyde, 5-methyl-	0.58	Aldehyde compound	Antiasthmatics, Expectorants, and Antitussive agents
Benzeneacetaldehyde	1.38	Aldehyde compound	Antimicrobial, Food preservation
2(3H)-Furanone, 5-heptyldihydro-	0.65	Fatty acid and their ester	Antimicrobial and Antifungal properties at low pH
2-Furancarboxaldehyde	0.95	Aldehyde compound	Anticancer Drug in research
2-Furanmethanol	2.98	Alcoholic compound	Antimicrobial, Antifungal
styrene oxide	1.38	Epoxy compound	-
Benzenemethanol	2	benzyl compound	Flavoring Agent
2-Furancarboxaldehyde, 5-(hydroxymethyl)	46.26	Alcoholic compound	Antimicrobial, Antifungal
Ethanamine, N-ethyl-N-nitroso-	0.68	nitroso compound	-
2,3-Dihydro-3,5-dihydroxy-6-methyl	0.81	Flavonoid compound	Antimicrobial, Anti-inflammatory, Antioxidant agent

Antimicrobial activity of the extract

Results showed that the extract could inhibit the growth of all tested strains. Furthermore, the comparison of the antibacterial effect of the extracts indicated that it was generally more effective on Gram-negative bacteria, compared to Gram-positive ones. Also, the extract was equally effective in inhibiting the growth of *S. aureus* and *E. fecalis.*

From this finding, it seems that the possible target site of this extract may be the structures except for the cell wall. The extract might affect the outer membrane of Gram-negative bacteria or protein synthesis mechanisms. In any case, it can be a proper choice to utilize against infections caused by Gram-negative bacteria. Furthermore, in some cases, they showed an effective dose lower than 1.9 mg which can be a significant finding. A similar pattern was obtained in research by *Sabir,* in which an aqueous extract of *Elaeagnus umbellata* strongly inhibited the growth of *E. coli* whereas, it exhibited a very small zone of inhibition against *B. subtilis* [28].

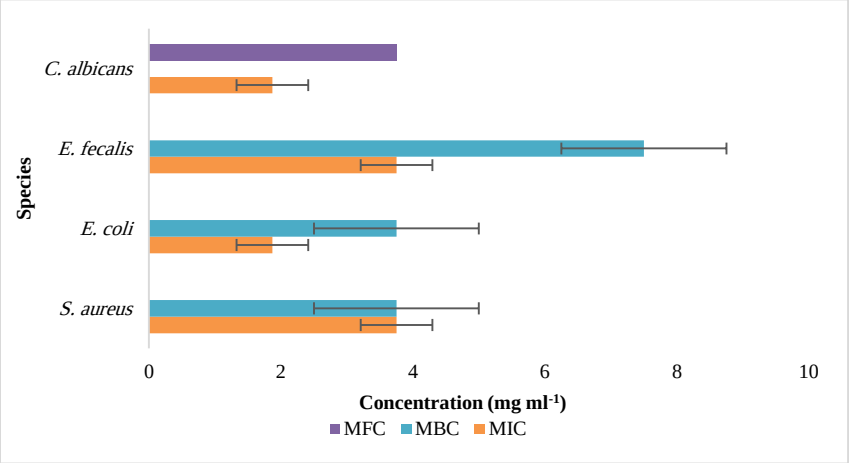

Figure 2. Antibacterial activity of plant extracts of *Elaeagnus angustifolia L.*

Most of the antimicrobial effects of *E. angustifolia* L. are related to their components and secondary metabolites like phenolic compounds [29, 30]. Phytochemical studies showed that this plant contains components like saponin, triterpenoids, glycosides, anthraquinones, steroids, and flavonoids that inhibit the growth of the tested bacterial strains [31, 32]. The presence of this component inhibits the growth of tested bacterial strains. Flavonoids and phenolic compounds have antibacterial and antifungal activities.

It has been shown in various studies that the polarity of antibacterial compounds is crucial for their activity[33]. These polar compounds are well extracted by ethanol in the present study. In other words, our obtained data using GC-MS can properly verify the observed antimicrobial results. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes. However, the phenolic compounds could destruct the cell membrane and destroy its permeability consequently release of intracellular constituents. Herein the functions of the membrane such as electron transport, nutrient uptake, protein, nucleic acid synthesis, and enzyme activity may interfere consequently. Bioactive compounds may be able to inhibit the bacteria as invasive targets. Furthermore, the phenomenon of intracellular content release can stimulate by many antibacterial substances. The concentration of phenolic could affect the level of release from *E. coli* and *Staphylococcus aureus*[34]. In Rizvi et al. study, the *Cassia* species had significant activity against Gram-positive as a result of some flavonoids and polysaccharides [32]. Similar studies in this field also reported considerable antimicrobial activity for extracts of *C. fistula* leaves [17], and methanolic extracts of *C. fistula*(against Gram-positive bacteria more than Gram-negative) [35]. However, different antimicrobial compounds may have different antimicrobial mechanisms.

CONCLUSIONS

The survey of antibacterial effects of an extract of *E. angustifolia* L. whole fruit indicated moderate to strong antibacterial properties. The experimental results of MIC, MBC, and MFC showed significant potential inhibitory effects against fungi, and Gram-positive and Gram-negative bacteria. Analysis of the phytochemical composition of the extract verified this finding, according to some flavonoid compounds which act as an antibacterial against a wide range of bacteria. From these findings the E. *angustifolia* L. extract can be used in the treatment of infectious diseases caused by pathogen microorganisms. Besides, it is recommended to apply it as a natural antimicrobial alternative substitute for chemical agents.

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ETHICAL CONSIDERATION

The study project had approved by the research ethics committee of Semnan University of Medical Sciences (approval ID: IR.SEMUMS.REC.1396.91).

Conflict of interest

All authors have declared they don't have any conflict of interest.

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