Chemical Composition, Antimicrobial and Antioxidant Activity of *Echinophora platyloba* DC

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Abstract: The existence of artificial additives in food seems to be unhealthy from the consumers' point of view, and it is why scientists are looking for some solutions to reduce the unhealthy varieties of additives in manufactured products. This article presents a revision of studies published in recent years on this topic and looks at possible future trends in the sector on one of the indigenous plant activities, which is used as a food seasoning in Iran. *Echinophora platyloba* DC has a high potency to act as an antimicrobial and antioxidant at the same time. In 3 sections this article explains the subject as follows. The first part includes determination of chemical constituents of isolated fresh and dry aerial parts by different extraction methods. They were analyzed by Gas Chromatography. In the second part antibacterial and antifungal activity of both plant extracts and its essential oil were evaluated against gram positives and gram negatives followed by fungus. Finally in the last part, antioxidant properties of the plant are mentioned. The essential oil and the crude extracts from *E. platyloba* species are of active candidates which can be used as antioxidant, antimicrobial agents so they still would be the novel ones for further researches.

Keywords: Echinophora platyloba DC, natural preservative, essential oil, plant extract, antibacterial, antifungal.

1. INTRODUCTION

Almost all the vegetable plants available in the world have great potential sources for discovery as well as production of new products benefit to mankind [1]. Scientists are sought to find new antimicrobial and antioxidants ingredients from variety of natural sources such as microorganisms, animals, soil and plants, among these resources development of products derived from plants are of particular interest because of their safety, their wide acceptance by consumers and their uses for potential multi-purpose functional uses [1-6]. It is been reported that, plant's derivatives such as spices have been used for their preservative properties since antiquity [7]. Of the known EOs of plants, only oil of *turpentine* was mentioned by Greek and Roman historians [2, 8]. In general, herbs are known by non-woody plants, and are leafy materials in their fresh state [9]. The word herb comes from the Latin “herba”, meaning grass, green stalks or blades of plants, and is generally used as dried products [9]. The herb *Khosharize* or *Tightooragh* (*Echinophora platyloba*) one species of the genus *Echinophora* [10] is of unique plants in Iran which is traditionally used for flavoring of cheese and yogurt, rather than preventing tomato paste and pickles from mold [6]. Some studies showed that besides its pharmacological role in medicine, it has showed other aspect to be noticed like it is currently the subject in primary dysmenorrhea [11, 12]. Recently *in vitro* analysis of inhibition of growth and induction of apoptosis in Fibrosarcoma Cell Lines by *Echinophora platyloba* DC was studied [13]. The genus *Echinophora* (Umbelliferae, subfamily Apioideae, tribe Echinophoreae), is represented in the flora of Iran by four species including two endemics species: *E. platyloba* DC. and *E. cinerea* [14]. There are studies which have determined chemical constituents of *E. platyloba* followed by different extraction methods. The hypothesis of these serial studies was based on the plant’s specific characteristic as food preserver which might have been due to its antimicrobial properties [15]. A study in Iran showed that, *E. platyloba* indeed exhibits a potent antifungal and a weak antibacterial activity [16]. Its inhibitory action against *C. albicans* was the highest followed by *T. schenlaini* and *T. verucosum* and some degrees of synergy was recorded in combination of Amphotericin B plus *E. platyloba* 5% ethanolic extract covering *C. albicans* [16, 17]. The synergistic combined mixture in these *in vitro* studies needs further *in vivo* studies to evaluate its actual effects [15]. The ethanolic and aqueous extracts of *E. platyloba* D.C. were found to be effective against all the strains of the tested bacteria [15]. *Alcaligenes faecalis* was the most sensitive bacterium against aqueous extract and *Listeria monocytogenes* was the most sensitive against ethanolic extract [18]. In food processing, chemical reactions such as lipid oxidation not only causes a loss in nutritional and gustative quality of foods but also generates oxidized products such as free radicals which lead to various undesirable chemical reactions [19]. To avoid or delay this autoxidation process, conventional artificial

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antioxidants such as butylated hydroxy anisole (BHA) butylated hydroxy toluene (BHT), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) have been used for more than five decades. However, these synthetic antioxidants have been suspected to or promote negative health effects [19-22]. Therefore, there is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources [23] Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improving the quality and nutritional value of food, for this reason, there is a growing interest in studies of natural additives as potential antioxidants In Iran [24]. Returning to *Khosharize*, in a study ten components have been identified, of which the major constituents were found to be trans-b -ocimene (67.9%) [25]. Regarding to more than 67 percent of trans-b-ocimene in *Khosharize* results, its anti-oxidation potency is noticeable.

2. MATERIAL AND METHODS

2.1. Sample Collection

Echinophora platyloba D.C is a wild plant which usually grows in west and north western parts of Iran [26]. In studies related to *Echinophora platyloba* DC, taxonomy verification of plant materials were done by using data of the biological part assisting from the book of Dr. Vali-allah Mozafarian [11, 14, 26]. The collected aerial parts (stem and leaves) of the plants were shade dried in ambient temperature of 25°C, were then mixed and grinded to make the powder more homogeneous [18]. Powders were kept in tight containers protected from oxygen [13, 27].

2.2. Preparation Methods of the Plant Extract and Essential Oil Extraction

2.2.1. Aqueous and Ethanolic Extracts

Aqueous extracts were obtained in room temperature by maceration in a ratio about 1:5 (100g to 500 cc) of powder in distilled water for 48hrs [15, 28]. Alcoholic extracts of plant samples were made by soaking in 80% denatured ethanol using the same ratio [28, 29]. Then, the macerated plant material was extracted with 70% ethanol solvent using percolator apparatus [15, 30]. The plant extract was removed from percolator, filtered through Whatman filter paper (NO.4) and dried under reduced pressure at 37°C with rotator evaporator [15].

2.2.2. Hydro Distillation of Essential Oil

Distillation as a method of producing EOs was first used in the East (Egypt, India and Persia) [8], more than 2000 years ago and was improved in the 9th century by the Arabs [31]. The first experimental measurement of the bactericidal properties of the vapors of EO is said to have been carried out by De la Croix in 1881[2, 31]. The hydrodistilled essential oil composition of 25 grams air-dried aerial parts of *Echinophora platyloba* DC from Iran was obtained by glass Clevenger type and its essential oil was trapped and collected with organic solvents [33-35]. The oil was dried over anhydrous sodium sulphate (Na_2SO_4) and kept in refrigerator at 4°C in sealed glass vials prior to analysis [18, 35]. Essential oil content was expressed as volume per weight (V / W) based on the dry weight of plant material [18].

2.2.3. Microwave-Assisted Hydro Distillation Extraction

All the reported applications have shown that microwave-assisted extraction is an alternative to conventional techniques for such matrices [36]. The main benefits are decrease of extraction time and solvents used [36]. This extraction method which is by Microsynth (microwave extractor equipped with Clevenger-type apparatus) was used only once for *E. platyloba* (Figure **1** shows the apparatus) [6, 37]. Powdered sample in flask with water were mixed using a stirrer. The extraction was carried out at different extraction conditions [6]. The microwave vessel was irradiated at a 20 to 45 min and irradiation power (400 to 700 W), trapped in organic solvents, afterwards the essential oil was collected, dried under anhydrous sodium sulphate and stored at 0°C until analyzes [6, 38].

Figure 1: Adapted microwave distillation apparatus [6].

2.2.4. Solid Phase Microextraction

Solid phase microextraction (SPME) was first introduced in 1989 [39] it has now become a technique for extraction which is been extensively used [40-42]. Compared with hydrodistillation (HD), HS-SPME, provides the advantages of a small amount of sample, timesaving, simplicity and cheapness [43, 44]. For HS-SPME analysis, 2.0 g of plant was transferred to a 10 mL vial, sealed with a rubber septum and immersed in an oil bath at fixed temperature, 70°C [43, 45]. Prior to use, SPME fiber was conditioned in the hot GC injection port at 250 ◦C for 30 min in order to remove contaminants and blank run must be performed to confirm the absence of contamination peaks [43, 45]. Subsequently, the fiber was with drawn into the needle then introduced into the GC injection port and held there for 2 min at 260°C to completely desorb the volatile flavor compounds and then analyzed by GC–MS [43].

2.3. Analyzing Chemical Compositions

2.3.1. Gas Chromatography (GC)-Mass Spectrometry Analysis

Investigation of *Echinophora Platyloba* DC antioxidant and antimicrobial activities is based on GC-MS results of its essential oil and extracts. The analysis of the oil was carried out using GC Agilent technologies Avondale model equipped with a detector like FID and HB-5 fused capillary silica columns with 3 diameters (30 m, 0.25 mm, 25 µm film thicknesses) [18, 27]. The samples, dissolved in ethanol were injected (20 µL**)** in the split less mode into helium carrier gas with a constant flow rate of 1.1 ml/min with injector and detector temperature of 250 and 280°C [18, 27, 39, 43]. Column temperature was held in about 50°C for 2min and then programmed until 160°C, it was finally increased to about 280°C, mass selective detector (MSD) was directly coupled to gas chromatography [18, 27, 39, 43].

2.3.2. Identification and Quantification of the Compounds

The identification of the volatile oil components was based on computer matching against the library spectra NIST and WILEY library built up using pure substances and components of known constituents, MS literature data and evaluation of fragmentation patterns of compounds, this was confirmed by their gas chromatography calculation of Kovats retention indexes (RI) from retention times of n-alkanes (C6–C24) [16, 18, 25, 43, 45, 46]. The percentage of the essential oil composition was computed from gas chromatography, the total peak area (TIC) was obtained by software of the apparatus without using correction factors [6, 43].

2.4. Antimicrobial Activities

2.4.1. Minimum Inhibitory Concentration (MIC) of Plant Extracts and Essential Oils

Minimal Inhibitory Concentrations (MIC) of the essential oils against the tested microorganisms was determined by the broth micro dilution method which is recommended by Natural Committee for Clinical Laboratory Standard-NCCLS [47]. Bacteria were cultured on nutrient agar and potato dextrose broth was used for yeast and fungi at their proper growth temperature [28]. The tubes were shaken well, as extracts and essential oils could be dispersed throughout the medium. The growth of microorganisms was observed as turbidity determined by measure the optical density at 600 nm by spectrophotometer which lowest concentration or the highest dilution (showing no visible growth) is referred as minimum inhibitory concentration [48, 49].

2.4.2. Estimation of Antimicrobial Activity of Essential Oils and Plant Extracts by Disc Diffusion Method

Microorganisms which were homogenized in Mueller-Hinton Broth of standardized inoculum (0.5 Mac-Farland equivalents to 1.5 ×108 CFU/ml) were poured into sterile Petri dishes, were permitted to solidify in room temperature and sealed with laboratory films [12, 49, 50]. Blank discs (DMSO) were used as negative control. Ciprofloxacin, ketoconazole and amphotericin B were used in the test as positive control [15, 17].

2.5. Antioxidant Activities

2.5.1. Estimation of Total Phenolics

The amount of total phenolic contents in the obtained extracts was estimated by Folin ciocalteu method with some modifications [28]. Different concentrations of extracts were mixed with diluted Folin-ciocalteu reagent (McDonald) and saturated sodium salt solution and after 30 min at 30°C the absorbance was measured by spectrophotometer [28]. Total phenolics were calculated using a standard gallic acid curve and results were expressed as mg gallic acid equivalent/g of extract [28].

2.5.2. Determination of Total Flavonoids

Flavonoids are polyphenolic compounds widely distributed in dietary fruits and vegetables [51]. The

average daily intake in the occidental diet is about 23 mg, of which quercetin (3, 3, 4, 5, 7-pentahydroxyflavone) is one of the most abundant representing 60 to 75% of the average polyphenol ingestion [51, 52]. For determination of these components colorimetric methods were used. In study of *E. platyloba*, aluminum chloride colorimetric was used and methanol extracts were mixed with aluminum chloride, potassium acetate and distilled water, they were kept in room temperature for half an hour [28]. The absorbance of the reaction mixture was measured by UV–visible spectrophotometer (USA) at 415 nm, and the calibration curve was prepared by preparing quercetin solutions in methanol [28].

2.5.3. Evaluation of Antioxidant Potential of Extracts

2.5.3.1. DPPH Radical Scavenging Assay

This method which is occurred by reduction of methanolic solution of DPPH (1, 1-diphenyl-2-picryl-hydrazyl) by free radical scavengers does not need any special preparation and reactions [53-55]. The change in the absorbance of the samples was measured using spectrophotometer. Antioxidant capacities of the extracts were compared with those of butylated hydroxy toluene (BHT) and blank [28].

Total antioxidant activity (TAA %) (Formula 1) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation:

$$TAA\ \% = (A_{control} - A_{sample}/A_{control}) \times 100 \quad (1)$$

Where TAA is the total antioxidant activity, $A_{control}$ is the absorbance of the control reaction (containing all reagents except the tested compound) and A_{sample} is the absorbance of the tested compound. The control contained 2 mL of DPPH solution and 2 mL of methanol [55].

All samples were run in triplicate and radical scavenging activity was reported as mean ± SD [28, 55].

3. RESULTS AND DISCUSSION

3.1. Chemical Compositions of *Echinophora platyloba*using Different Extraction Methods

3.1.1. Aqueous and Alcoholic Extraction

As it is shown in Table **1** chemical compounds of *E. platyloba* alcoholic extract in this study are as follows: Ocimene (28.66%) the most abundant component [27]. The other major components were beta-cis-ocimene

Table 1: Chemical Composition (%) of *Echinophora Platyloba D.C* Methanolic Extract Analyzed by GC/MS [27]

Compound	KI	Composition
Methanol	315	8.50
Alpha-pinene	948	7.42
Beta-terpinene	1071	1.49
Beta-myrcene	993	3.22
O-cymene	1028	28.66
Beta.-cis-Ocimene	1040	9.77
Isopinocarveol	1198	1.06
Beta-Linalool	1109	4.99
13-Tetradece-11-yn-1-ol	1663	1.08
2-Nonenal	1161	1.31
Naphthalene	1157	3.47
5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]-heptan-2-ol	1294	1.42
Acetic acid	1009	1.84
Trans-Z-alpha-bisabolene expoxide	1746	1.34
5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-1	1442	1.00
Gamma-decalactone	1431	5.20
Formic acid	1576	1.24
Spathulenol	1699	2.92
Trans-Farnesol	2021	3.17
2-Butloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	3942	1.60
1.3-Ethyl-5-(2'ethylbutyl)octadecane	3430	1.00
Sum	**-**	**91.68**

(9.77%), methanol (8.50%), alpha-pinene (7.42%), gamma-decalactone (5.20%) and beta-linalool (4.99%) [27].

3.1.2. Hydro Distillation

Study 1

Twenty-nine components were characterized representing 95.3% of the oil. (E) β-ocimene (49.9%) was the main constituent of the oil, followed by γ-decalactone (8.4%), α-pinene (6.0%) and linalool (5.6%) [45].

Study 2

In this study composition of aerial parts of *E. platyloba* DC essential oil obtained by hydrodistillation which was analyzed by GC/MS showed ten components, of which the major constituents were found to be trans-b -ocimene (67.9%), 2-furanone (6.2%), myrcene (6.0%), linalool (3.1%) and cis-b -ocimene (2.3%) [25].

Study 3

Twenty-nine constituents representing 97.43% of the total oil have been identified in this research. The main constituents of the oil were found to be (Z)-β-ocimene (26.71%), ∆-3-carene (16.16%), Limonene (6.59 %). Other representative compounds were identified as Cis-3-hexylbenzoate (4.57%), Spathunenol (4.57%), Myristicin (4.48 %), Myrcene (4.31%), 4-decanolide (4.2%) and a-Pinene (4.03 %) [55].

Study 4

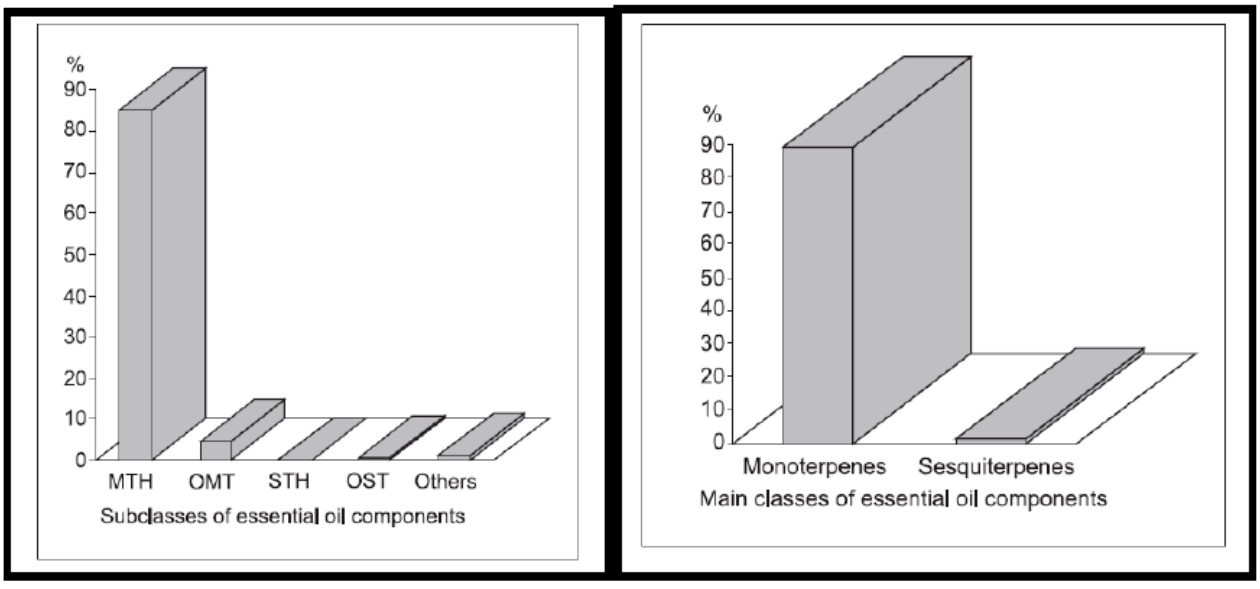
The major compound of essential oil in this study was ocimene (26.51%), followed by 2, 3 Dimethyl-1,3-cyclohexadiene (9.87%), alphapinene (7.96%), gamma-dodecalactone (5.84%) and nerolidol (5.66%) [27].

Study 5

The most abundant components were found to be Monoterpenes and of Monoterpene hydrocarbons (Z)-β-ocimene with more than 89 and about 40 percentage comprising 91.9% of total oil [18]. As it is shown in the Table **2** it is followed by minor proportions of some C9 to C17 compounds (1.1%) and sesquiterpenes (0.9%)with four components – trans-caryophyllene (0.1%), cis-nerolidol (0.2%), spathulenol (0.5%) and caryophyllene oxide (0.1%) (Figures **2a**, **b**) [18].

Ocimene is followed with other monoterpene hydrocarbons (MTH) such as α-phellandrene (24.2%), p-cymene (7.4%), β-phellandrene (6.3%), α-pinene(3.4%) and myrcene (1.6%) as the main ones (Table **2** and Figure **2a**) [18]. (Z)-β-ocimene and α-phellandrene (Sum 63.1%) comprised about 70% of the total essential oil identified components (Table **2**) [18].

Other monoterpenes like oxygenated monoterpenes (OMT) (4.8%) were the second subclass of monoterpenoidal compounds, with γ-decalactone (1.7%) and linalool (1.2%) as their representatives (Figure **2b**). In total, 11 alcohol monoterpenes were



a **b**

Figure 2: a: Major subclasses of *E. platyloba* DC. EO. **b**: Main classes of *E. platyloba* DC. EO [18].

Table 2: Constituents of *E. platyloba* essential oil [18]

No.	Compound	RI	%
1	n-Nonane	0900	0.3
2	α-Thujene	0930	0.3
3	α-Pinene	0939	3.4
4	Sabinene	0975	0.7
5	β-Pinene	0979	0.3
6	Myrcene	0991	1.6
7	α-Phellandrene	1003	24.2
8	α-Terpinene	1071	0.5
9	p-Cymene	1025	7.4
10	β-Phellandrene	1030	6.3
11	(z)-β-oclmene	1037	38.9
12	γ-Terpinene	1060	0.1
13	Terpinolene	1089	0.5
14	Linalool	1097	1.2
15	Allo-ocimene	1132	0.8
16	Cis-b-Terpineole	114	0.1
17	Terpinene-4-ol	1177	0.2
18	p-Cymene-8-ol	1183	0.1
19	α-Terpineole	1189	0.5
20	Myrtenol	1196	0.4
21	Cis-piperitol	1196	0.1
22	Thymol	1290	0.1
23	Carvacrol	1299	0.4
24	α-Terpinyl acetate	1349	0.2
25	Methyl eugenol	1404	0.2
26	Trans-caryophyllene	1419	0.1
27	γ-Decalactone	1467	1.7
28	Cis-Nerolidol	1533	0.2
29	Cis-3-Hexenyl benzoate	1567	0.4
30	Spathulenol	1578	0.5
31	Caryophyllene	1583	0.1
32	γ-Dodecalatone	1678	0.2
33	(E)-Nerolidol acetate	1717	0.1
34	**Total**		**91.9**

identified in the essential oil of *E. platyloba* DC from which carvacrol (0.4%) and thymol (0.1%) were the characteristic compounds showing the possible chemotaxonomic similarities of *E. platyloba* DC with *Thymus* species plants (Table **2**) [18].

Study 6

Table **3** shows the percentages of the main components present in the essential oil isolated from the aerial parts of *E. platyloba*. The components are listed in order of their elution time on the HP-5MS column. *E. platyloba* volatile oils are generally variable

Table 3: Constituents of *E. platyloba* Essential Oil [57]

Constituents	%	RT	KI
α-Pinene	0.54	4.85	942
Myrcene	2.90	6.10	986
Phellandrene	9.98	6.49	1002
p-Cymene	0.25	6.99	1020
β-Phellandrene	0.86	7.11	1025
β-Ocimene	0.73	7.31	1027
trans-β-Ocimene	82.70	7.81	1038
α-Terpinolene	0.41	8.78	1074
Linalool	1.10	9.15	1092
2-Furanone	0.63	20.81	1466

mixtures of principally terpenoids, specifically acyclic hydrocarbon monoterpenes ocimene, myrcene, monocyclic; phelanderen, cymene, terpenolene, bicyclic pinene and an alcohol acyclic monoterpene; linalool [57]. Trans-b-ocimene (82.70%) was the main constituent of the oil used in this study and the major components were phellanderene (9.98%), myrcene (2.9%), linalool (1.10%), 2-furanone (0.63%), and pinene (0.54%) [57].

Study 7

The major compound of essential oil as shown in the Table **4**, was ocimene (26.51%), followed by 2,3 Dimethyl-1,3-cyclohexadiene (9.87%), alphapinene (7.96%), gamma-dodecalactone (5.84%) and nerolidol (5.66%) [27].

3.1.3. Microwave-Assisted Hydro Distillation Method

As it shown in the following Table **5** from 96.4 % of total compounds, γ-decalactone and (Z)-β-ocimene showed the highest amount of area percentage 43.96% and 21.56%. Myrcene has got the lowest by 0.22 % of area [6].

3.1.4. Solid-Phase Microextraction Method

The chemical volatile components in the *E. platyloba* were identified by mass spectra library and retention indices [43]. Fifty-three components were identified, and are listed in Table **6**, where compounds are listed in order of their elution from the DB-5 column. E-β-ocimene (47.63%) was found to be the major constituent of the volatile compounds extracted by HS-SPME method followed by R-D-decalactone (13.28%), α-pinene (7.43%), nonane (6.71%), and α-phellandrene (4.45%) [43].

3.2. Antimicrobial Effect on Microorganisms

All studied microorganisms, which were used for determination of *Echinophora platyloba* DC inhibitory activities are as follows: gram positives: *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus* and *Bacillus subtilis*, followed by the Gram negatives: *Campylobacter jejuni, Campylobacter coli, Serratia marcescens, Escherichia coli O157:H7, Alcaligenes faecalis, Providencia rettgeri, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella enteritidis, Salmonella thyphimurium, Yersinia enterocolitica* and fungus including *Trichophyton schenlaini, Trichophyton verucosum, Aspergillus niger, Aspergillus flavus, Penicillium expansoum* beside *Candida albicans*.

Study 1

A study of *E. platyloba* in Iran by Avijgan *et al.* in 2012 showed that, this plant indeed exhibits a potent antifungal and a weak antibacterial activity. They concluded that, inhibitory action against *C. albicans* was the highest followed by *T. schenlaini* and *T. verucosum* and some degrees of synergy was recorded in combination of Amphotericin B plus *E. platyloba,* while 5% ethanolic extract covering *C. albicans* [15].

Study 2

Sharafati *et al.,* 2012 reported that, the ethanolic and aqueous extracts of *E. platyloba* D.C. were found to be effective against all strains of the tested bacteria [28]. *Alcaligenes faecalis* was the most sensitive bacterium against aqueous extract and *Listeria monocytogenes* was the most sensitive bacterium against ethanolic extract [28].

Table 4: Chemical Composition (%) of *E. Platyloba* D.C Essential Oil Analyzed by GC/MS [27]

Compound	KI	Composition
Hexanal	806	1.25
2,3-Dimethyl-cyclohexa-1,3-diene	863	9.87
Alpha-pinene	948	7.69
Ocimene	958	26.51
Beta-linalool	1082	1.80
Benzopyran	1342	1.18
Cyclohexene, 2-ethenyl-1,3,3-trimethyl	1105	1.44
2,5-Octadecadiynoic acid, methyl ester	2112	2.30
Caryophyllene	1494	2.48
Dihydropseudoionone	1420	1.48
Gamma-dodecanolactone	1582	5.84
4-(2,2-Dimethyl-6-methylencyclohexylidene)-3-methylbutan-2-1	1475	1.13
Nerolidol	1564	5.66
All-trans-farnesol	1710	3.30
Gamma-dodecalactone	1582	3.28
Heptacosane	2705	2.10
Nonacosane	2904	4.11
Cis-Z-alpha-bisabolene epoxide	1531	1.11
2-[4-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	2561	2.89
Sum	**-**	**85.42**

Table 5: The Chemical Compositions of the Essential Oil of *E. Platyloba* Aerial Parts Extracted by Microwave- Assisted Hydro Distillation (MAH) Method (650 W, 35 min) [6]

Entry	Compounds	Retention Index (RI)[a]	Area (%) MAH
1	α-Pinene	936	0.37
2	β-Pinene	978	0.77
3	β-myrcene	989	0.22
4	Limonene	1025	0.51
5	(Z)-β-ocimene	1034	4.23
6	(E)-β-ocimene	1046	21.56
7	γ-Terpinene	1061	0.33
8	Linalool	1095	0.54
9	Allo ocimene	1131	1.96
10	Cis-verbenol	1133	1.75
11	p-Cymene-8-ol	1185	0.5
12	α-Terpineol	1189	1.71
13	Cis-3-hexenyl 2-methyl butanoate	1227	1.41
14	Carvlacro	1278	0.38
15	Bornyl acetate	1291	1.35
16	Methyl eugenol	1404	3.01
17	Trans-caryophyllene	1418	0.83
18	γ-Decalactone	1466	43.96
19	2-furanone	1471	1.24
20	Cis-Nerolidol	1533	0.54
21	Cis-3-Hexenyl benzoate	1568	2.1
22	Spathulenol	1577	1.9
23	Caryophyllene oxide	1583	2.86
24	g-Dodecalactone	1678	2.27
25	**Total percentage**		**96.4**

Table 6: Chemical Constituents of *E. platyloba* Oil [43]

	Compounds	RI[a]	HD[b]	HS-SPME[c]	RSD[d]
1	α-Thujene	925	0.15	0.08	4.9
2	α-Pinene	932	8.53	7.43	7.1
3	Camphene	946	0.06	0.03	8.3
4	Nonane	967	6.02	6.71	5.0
5	Sabinene	971	0.42	0.81	8.6
6	β-Pinene	973	0.25	0.21	5.6
7	Myrcene	990	1.31	0.95	4.6
8	α-Phellandrene	1003	5.02	4.45	4.5
9	p-Cymene	1022	2.98	1.06	5.2
10	β-Phellanderne	1027	2.21	2.02	6.1
11	cis-Ocimene	1040	1.33	1.72	6.4
12	E-β-Ocimene	1057	37.09	47.63	4.6
13	γ-Terpinene	1060	1.01	1.52	7.5
14	Z-Hex-3-enyl methoxy formate	1073	0.25	0.18	4.9
15	1-Nonane-3-one	1076	0.39	0.45	8.6
16	α-Terpinolene	1088	0.37	0.25	9.3
17	Linalool	1098	1.52	1.57	7.8
18	cis-β-Dihydroterpineol	1130	0.18	0.19	4.4
19	Cis-Verbenol	1142	0.21	0.41	6.1
20	Z-2-Nonenal	1147	0.20	0.15	5.8
21	iso-Borneol	1157	0.81	0.46	5.3
22	Nonanol	1160	0.26	0.09	7.3
23	Safranal	1163	0.16	0.07	6.7
24	Terpinen-4-ol	1174	0.23	0.11	8.5
25	Borneol	1180	0.06	0.05	7.4
26	α-Terpineol	1188	0.24	0.09	9.0
27	cis-Piperitol	1194	0.11	0.06	7.6
28	neo-Isomenthol	1198	0.25	0.08	9.2
29	Verbenone	1207	0.43	0.13	8.6
30	Linalool-oxid	1212	0.07	0.03	4.9
31	Trans-carveol	1214	0.05	0.07	8.1
32	Carvone	1224	0.05	0.06	9.2
33	Methyl	1234	0.12	0.08	5.8
34	cis-Chrysanthenyl acetate	1241	1.01	0.03	5.7
35	DEC-2-Enal	1250	0.06	0.09	6.8
36	Phenyl acetic acid	1262	0.04	0.06	7.7
37	Carvacrol	1298	0.18	0.15	4.9
38	α-Terpinyl acetate	1345	0.08	0.05	8.7
39	β-Bourbonene	1379	0.09	0.06	6.9
40	β-Elemene	1386	0.11	0.15	4.7

(Table 6). Continued.

	Compounds	RI[a]	HD[b]	HS-SPME[c]	RSD[d]
41	cis-Jasmone	1397	2.13	0.26	6.5
42	Eugenol	1400	0.04	0.13	7.9
43	β-Caryophyllene	1412	0.29	0.38	7.4
44	α-Humulene	1445	0.07	0.06	6.7
45	R-D-decalactone	1469	15.08	13.28	5.6
46	Bicylogermacrene	1490	0.12	0.11	7.1
47	E-β-Farnesene	1498	0.07	0.05	9.0
48	β-Bisabolene	1514	0.17	0.15	8.2
49	Z-β-Farnesene	1528	0.42	0.33	6.8
50	Nerolidol	1549	0.16	0.05	7.3
51	3-Hexen-1-ol,benzoate	1554	0.53	0.32	4.8
52	Spathulenol	1560	3.43	2.15	7.6
53	Germacrene D-4-ol	1572	0.05	0.06	4.8

[a]Retention indeses using a HP-5MS column.
[b]Relative area (peak area relative to total peak area) for hydrodistillation method.
[c]Relative area (peak area relative to total peak area) for HS-SPME method.
[d]RSD values for HS-SPME method (relative peak area).

Study 3

The ethanol extract of *Echinophora platyloba* showed promising antifungal activities against *C. albicans* [50].

Study 4

This study declares a wide spectrum of microorganisms affected by *E. platyloba*, it demonstrated that all the tested strains had a degree of sensitivity to the plant and it has antifungal properties. Results indicated that Gram positive *B. subtilis, S. aureus*, and the important food pathogen *L. monocytogenes* were the most sensitive strains under investigation with the strongest inhibition zone (18.8 ± 0.8 mm). The volatile oils of *E. platyloba* appeared to be equally effective against all the tested Gram positive microorganisms. Moderate activities were observed against Gram negative strains; *E. coli* and *S. enteritidis*. Gram positive bacteria are more sensitive than Gram negative bacteria the antibacterial properties of plant volatile oils verified this study result [57]. *C. albicans* was the most susceptible microorganism to the essential oil of *E. platyloba*, and the most resistant was *P. aeruginosa* [57].

Study 5

The essential oil indicated strong antimicrobial activity against the tested bacteria, whereas the methanol extract almost remained inactive against gramnegative bacteria. The most sensitive bacteria to essential oil and extract of *E. Platyloba D.C* were *L. monocytogenes* and *Staphylococcus aureus* [27].

Study 6

This preliminary study revealed a great promise that ethanolic extract of *Echinophora platyloba* has anti *Candida albicans* effect and may be used in the production of drug and clinical assay [16].

Study 7

This study showed that, *S. aureus* and *P. aeroginosa* growth can be inhibited and even it can be zero by using higher concentrations of *E. platyloba* ethanolic extract. It was reported that there were none of indicated considerable growth prevention corona for three species of fungi *Candida albicans, Aspergilus flavus* and *Aspergilus niger* [26].

3.3. Antioxidant Activity of Echinophora platyloba DC

There is only little information about this plant's antioxidant activity. A study indicated that, the highest values of antioxidant capacity and phenolic compounds were observed in aqueous extracts of *E. platyloba* D.C ($P<0.05$) [28]. However the antioxidant capacity of two extracts was lower than BHT as the reference antioxidant. The total contents of phenolic, flavonoid and flavonol in mg per gram of ethanolic and aqueous extracts are shown in Table **7** [28].

Antioxidant capacity of BHT, aqueous and ethanolic extracts of *E. platyloba* are compared in the following Figure **3**.

Table 7: Total Contents of Phenolic, Flavonoid and Flavonol in *E. platyloba* Extracts [28]

Extract	Phenolic	Flavonoid (mg/g)	Flavonol (mg/g)
Ethanolic	138±3.25	127±1.63	132±3.87
Aqueous	230±3.26	92±3.26	88±3.3

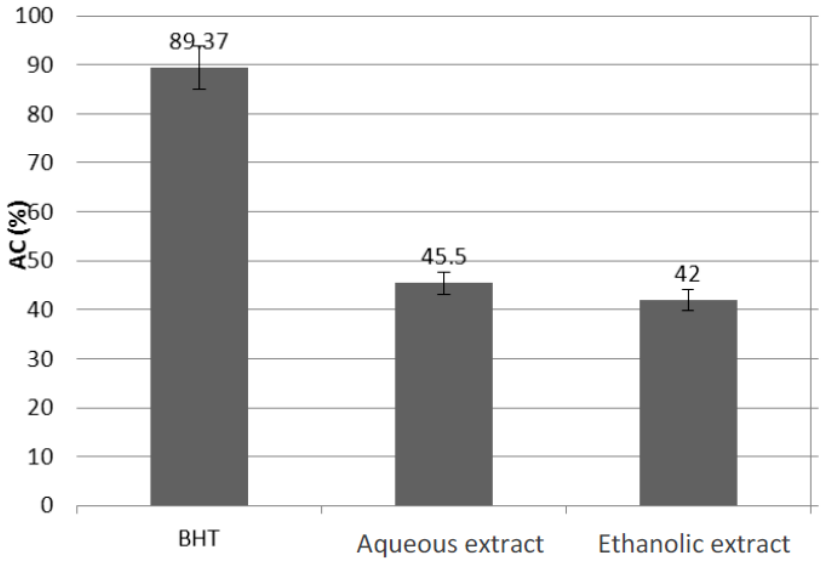


Figure 3: Antioxidant capacity of BHT, aqueous and ethanolic extracts of *E. platyloba* [28].

4. CONCLUSION

According to the results of *Echinophora platyloba* DC studies, the aerial parts (stem and leaves) extracts and essential oil of these plants clearly demonstrate a significant antimicrobial and antioxidant effect. Therefore it can be used as a good alternative to artificial and conventional additives for food preservation.

This plant content and chemical constituent considerably differ by extraction method, geographical, growth stages, climate and season changes, and this effect on the plants' activities. Four different extraction methods were used to isolate *E. platyloba* extracts and oil and varieties of chemical content were observed. For instance, alcoholic extract showed total amount of Ocimene by 28.66% and 9.77 the beta-cis ocimene. Where in essential oils, β-ocimene contents were as follows: 49.9, 67.9, 26.60, 26.71, 40, 82.70, and 26.51% and in methods Microwave-assisted HD and HS-SPME it was 43.96 and 47.63 %. This is why further methods are to be allocated for extraction of *E. platyloba*. Moreover growth stages also effects on essential oil content of *E. platyloba*, which showed remarkable increase at rosette stage (Ghani *et al.,* 2009). The proportion of (Z)-β-Ocimene, as a major component at rosette, drastically decreased in floral budding and then slightly increased at full flowering stage. In contrast, (E) - β-ocimene was not detected at the rosette stage but was the major component at the subsequent harvest time and then decreased at full flowering stage [58]. In all studies monoterpenes were the most chemical constituents.

Echinophora platyloba DC inhibitory activity was studied against following gram positives: *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus* and *Bacillus subtilis*. Followed by the Gram negatives: *Campylobacter jejuni, Campylobacter coli, Serratia marcescens, Escherichia coli O157:H7, Alcaligenes faecalis, Providencia rettgeri, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella enteritidis, Salmonella thyphimurium, Yersinia enterocolitica* and fungus including *Trichophyton schenlaini, Trichophyton verucosum, Aspergillus niger, Aspergillus flavus, Penicillium expansoum* beside *Candida albicans*. Avijgan *et al.* study show this plant indeed exhibits a potent antifungal and a weak antibacterial activity, where Sharafati indicates ethanolic and aqueous extracts of *E. platyloba* D.C. were found to be effective against all the strains of tested bacteria, *Alcaligenes faecalis* was the most sensitive bacterium against aqueous extract

and *Listeria monocytogenes* was the most sensitive bacterium against ethanolic extract. Furthermore a study showed *S. aureus* and *P. aeroginosa* growth can be inhibited and even it can be zero in higher concentrations by *E. platyloba* ethanolic extract were it was reported that there were none of indicated considerable growth prevention corona for three species of fungi *Candida albicans, Aspergilus flavus* and *Aspergilus niger.* But in most studies, essential oils and their components were more effective against Gram positive than against Gram negative organisms. Some studies showed this plant has an promising antifungal effect and some degrees of synergy was recorded in combination of Amphotericin B plus *E. platyloba* 5% ethanolic extract covering *C. albicans.* Studies show there is a lack of eugenol and thymol in *E. platyloba* compounds and its antimicrobial activity may be related to its terpenoids [57].

E. platyloba aqueous extract show the highest antioxidant activity and phenolic compounds compared to its ethanolic extracts. Higher total content of phenolic compounds were isolated in aqueous extracts, where flavonoid and flavonol showed a higher response in ethanolic extracts. By these, further studies are needed *in vitro* which might clarify *E. platyloba's* antimicrobial and antioxidative activities and definitely in food models, as there has not been any survey in food systems.

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