Journal of
Research in Pharmacy

Research Article
www.jrespharm.com

Chemical Characterizations and Biological Evaluation of Papaya (*Carica papaya* L.) Essential Oils and Fatty Acids

Eda TÜRKER [1], Hamide GÜBBÜK [2] Gözde ÖZTÜRK [3*], Betül DEMİRCİ [3]

[1] Department of Pharmacognosy, Graduate School of Health Sciences, Anadolu University, 26470 Eskişehir, Türkiye.
[2] Department of Horticulture, Faculty of Agriculture, Akdeniz University, 07058, Antalya, Türkiye.
[3] Department of Pharmacognosy, Faculty of Pharmacognosy, Anadolu University, 26470, Eskişehir, Türkiye.
* Corresponding Author. E-mail: g.ozturkau@gmail.com (N.S.); Tel. +90-222-335-05-80.

Received: 20 January 2022 / Revised: 30 May 2022 / Accepted: 30 May 2022

ABSTRACT: The genus *Carica* L. (Caricaceae) is presented by 22 species where *C. papaya* L. is the most widely cultivated and best-known species. It is native in Middle and South America, and consumed for its nutritional values. Also, its medicinal uses such as antimicrobial, antihelmintic, antifungal, hepatoprotective and diuretic among others are known. In this present study, it was aimed to analyse the chemical compositions of *C. papaya* (cv. Sel 42) essential and fatty oils from Antalya cultivars. Essential oils (EOs) from leaf, ripe and unripe fruits of *C. papaya* were obtained by hydrodistillation using a Clevenger type apparatus. The essential oils were subsequently analysed by both Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS). Benzyl isothiocyanate (86.3; 100; 94.4%) was found as the main constituent of the EOs of *C. papaya* leaf, ripe and unripe fruits, respectively. Dried and fresh seeds of *C. papaya* fatty acids (FAs) were extracted with *n*-hexane in a Soxhlet apparatus, and after the methylation the chemical compositions were determined by GC and GC-MS systems. The major FAs of both dried and fresh seeds of *C. papaya* were oleic acid (72; 70%), palmitic acid (15%; 16.4%), linoleic acid (5.3%; 5.1%), respectively. The *C. papaya* EOs and FAs were evaluated also for their *in vitro* antimicrobial properties against different microbial strains by the agar disk well diffusion method. The EOs and FAs showed relatively weak antimicrobial activity against the tested *Escherichia coli* NRRL B-3008, *Bacillus subtilis* NRRL B-4378, and *Candida albicans* ATCC 10231, respectively. Further biological evaluations are ongoing.

KEYWORDS: *Carica papaya* L.; Caricaceae; essential oil; fatty oil; antimicrobial activity.

1. INTRODUCTION

Carica papaya L. (Caricaceae) is a large, arboreal, herbaceous perennial plant with soft, simple stem growing up to 5-10 m tall, with sparsely arranged leaves at the top of the stem, the lower stem is scarred where leaves and fruits are born, widely distributed in tropical and subtropical regions. It is originally from southern Mexico, Central America and the northern part of South America [1,2]. Papaya has spread to different parts of the world, such as Brazil, Mexico, and Hainan Island. Brazil accounts for a large part of the world production. It is followed by Mexico, Nigeria, and India [3,4]. All parts of *C. papaya*, such as leaves, fruits, seeds, flowers, and roots, are used as food and for medicinal purposes. It is a tropical fruit that is widely used for its unique aroma and flavor and is a rich source of vitamins and enzymes. The fruit of *C. papaya* has an aromatic, lemony flavor and a dense, soft flesh. The surface of the fruit has a waxy finish and is thin. However, it is quite rough. The fruits hang directly in clusters on short and thick stems in the leaf axils. While the immature fruits are still green, over time they become quite large and have a different coloration, ranging from yellow to red. This color change during the transition from immature fruit to maturity is an indicator of a decrease in the amount of chlorophyll and an increase in the amount of carotene. The transformation to a red color is also due to the lycopene content. When the fruit is green and firm, it is rich in white milky juice [2,3,5]. In addition, profiles of volatile compounds of *C. papaya* have also been identified. The major volatile components of the fruit are isothiocyanate methylbenzene, D-octalactone, butanoic acid, hexadecanoic acid, benzyl isothiocyanate, and *β*-myrcene [6-8]. The seed of *C. papaya* contains a high percentage of benzyl isothiocyanate [4]. The main volatile compounds of the leaves are benzyl nitrile, *β*-nitroethanol, thiocyanic acid phenyl methyl ester, hexadecen-1-ol, and 9-octadecenamide [9, 10]. Benzyl

How to cite this article: Türker E, Gübbük H, Öztürk G, Demirci B. Chemical Characterizations and Biological Evaluation of Papaya (*Carica papaya* L.) Essential Oils and Fatty Acids. J Res Pharm. 2022; 26(6): 1771-1779.

ISSN: 26-6344
http://dx.doi.org/10.29228/jrp.267

isothiocyanate, oleic acid, palmitic acid, stearic acid, linoleic acid, and linolenic acid are among the important fatty acids present in *C. papaya* [3,4].

The biological activities of *C. papaya* are owed due to high content of vitamin A, B and C, proteolytic enzymes like papain and chymopapain [11]. In addition, it has different *in vitro* biological activities such as antioxidant, antimicrobial, antifertility, antitumor, antiinflammatory [12-14], histaminergic, diuretic, antiamoebic, antihelmintic, antimalarial, hypoglycemic, immunomodulatory activity, antiulcer, wound healing, hepatoprotective, antihypertensive among others [2, 3, 11]. Among the parts of the plant used for medicinal purposes, especially the use of seeds is prominent, which constitute 7% of the fruit weight. Extracts obtained from seeds have been reported to have anticancer, hypolipidemic, anti-inflammatory and antifertility activities. There are many *in vitro*, *in vivo* and clinical studies on *C. papaya* [3, 4, 12-15]. Within the scope of this study, it was aimed to determine the chemical composition of the leaves and fruit essential oils, ripe and unripe fruit seed fatty oils of cultivated *C. papaya* grown in Antalya/Türkiye. In addition, the antimicrobial activities of the essential and fatty oils obtained were investigated.

2. RESULTS AND DISCUSSION

2.1. Chemical Compositions of Essential Oils

The essential oils of *C. papaya* leaves, ripe and unripe fruit obtained by Clevenger apparatus and analyzed by GC and GC-MS, simultaneously. The oil yields of 0.1%, 1.2% and 1.4%, respectively. Benzyl isothiocyanate was determined as the main component for leaves, ripe and unripe fruit essential oils (86.3%, 100%, 94.4%). The other major compound in leaves essential oil was determined as phytol (6.3%). All components of essential oils were given in Table 1. According to these results, it was determined that whether the fruit is ripe or not changes the chemical composition of the essential oil.

Table 1. Chemical components of *Carica papaya* essential oils

RRI[a]	Component	Leaves %[b]	Ripe fruit %	Unripe fruit %	IM
1255	γ-Terpinene	-	-	0.2	RRI, MS
1280	*p*-Cymene	-	-	0.2	RRI, MS
1290	Terpinolene	-	-	0.2	RRI, MS
1740	α-Muurolene	-	-	tr[c]	MS
1773	δ-Cadinene	-	-	0.3	MS
1878	2,5-Dimethoxy-*p*-cymene	-	-	1.9	MS
1957	Benzene acetonitrile	5.4	-	2.4	MS
2084	Benzyl isothiocyanate	86.3	100.0	94.4	RRI, MS, NMR
2622	Phytol	6.3	-	-	RRI, MS
	Total	**98.0**	**100.0**	**99.6**	

[a]RRI: Relative retention indices calculated against *n*-alkanes
[b]%: calculated from FID data
[c]tr: Trace (< 0.1 %)
IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data

Benzyl isothiocyanate (100%) was determined in essential oil as a major component. Benzyl isothiocyanate structure was confirmed by comparing the data obtained according to the analysis results with the literature data of this compound. In previous studies, a volatile compound of benzyl isothiocyanate was found at high rates in the seeds of the *C. papaya*. With the maturation of *C. papaya* fruits, the rate of benzyl isothiocyanate in seeds increases. The presence of such a high rate of benzyl isothiocyanate compounds in the seed is a very rare condition seen in plants [3, 4, 16]. Because of this reason, the essential oil in question contains a single substance at a high rate, spectroscopic data was used to confirm the structure of the benzyl isothiocyanate compound. The mass spectrum of the benzyl isothiocyanate compound is shown in Figure 1. The ^{1}H-NMR and ^{13}C-NMR spectra are shown in Figure 2,3, respectively. It gave multiple proton peaks in the range of 7.00-7.50 ppm in the ^{1}H-NMR spectrum (Figure 4-6). These peaks indicated the presence of the benzene ring. The other specific peak in the structure of benzyl isothiocyanate shown at 4.93 ppm and the carbons in the benzene ring in the ^{13}C-NMR spectrum shown their presence in the range of 128-135 ppm. The carbon attached to the nitrogen atoms peaks at 48.47 ppm. Carbon, which has a connection with sulfur and nitrogen atoms, showed its presence at 129.34 ppm. Proton and carbon NMR values found as a result of the analysis were compared with only proton NMR values in the study of Chem Draw Prof application Barroso et al. [3].

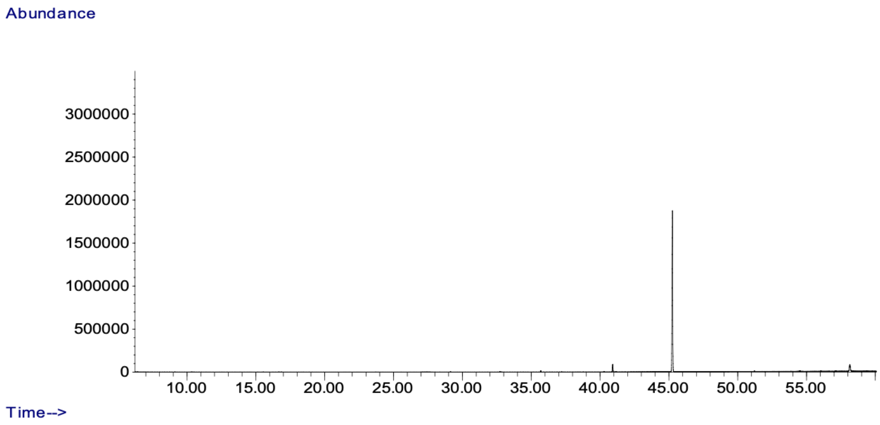

Figure 1. Chromatogram of *Carica papaya* leaves essential oil

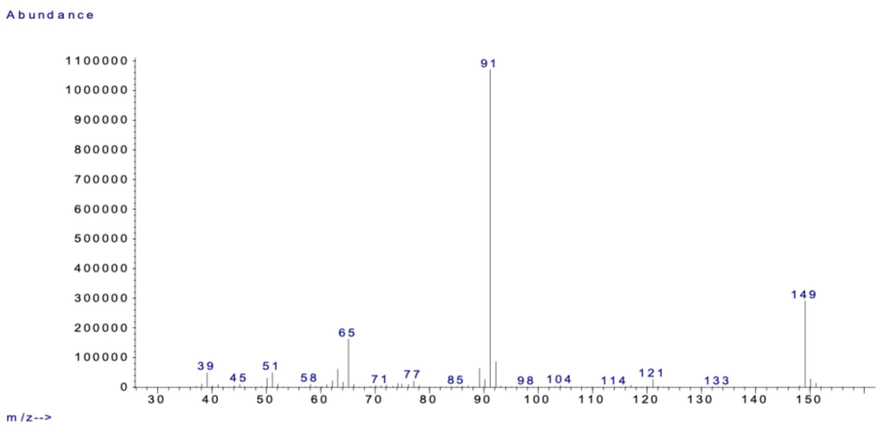

Figure 2. Mass spectrum of Benzyl isothiocyanate

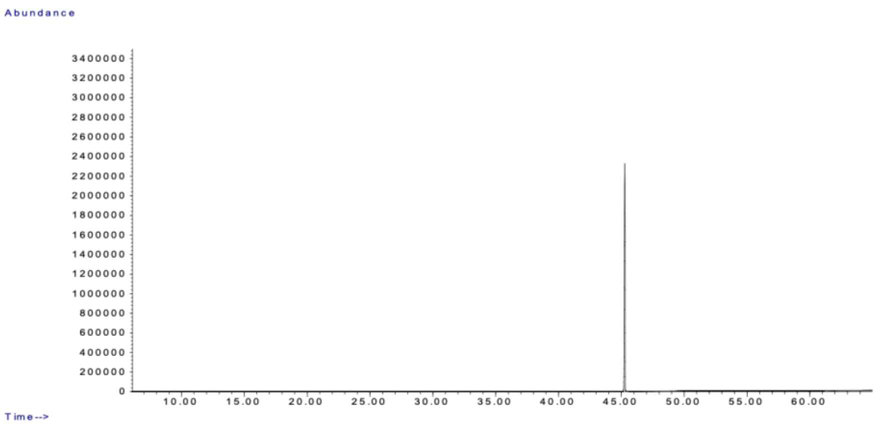

Figure 3. Chromatogram of *Carica papaya* ripe fruit essential oil

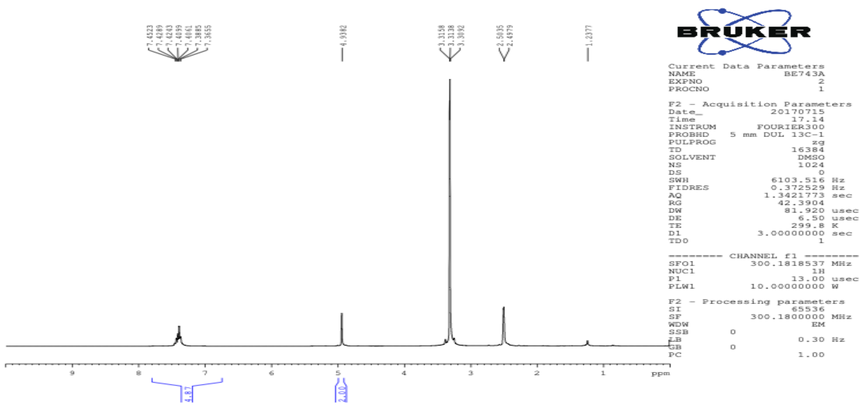

Figure 4. Benzyl isothiocyanate ^{1}H-NMR

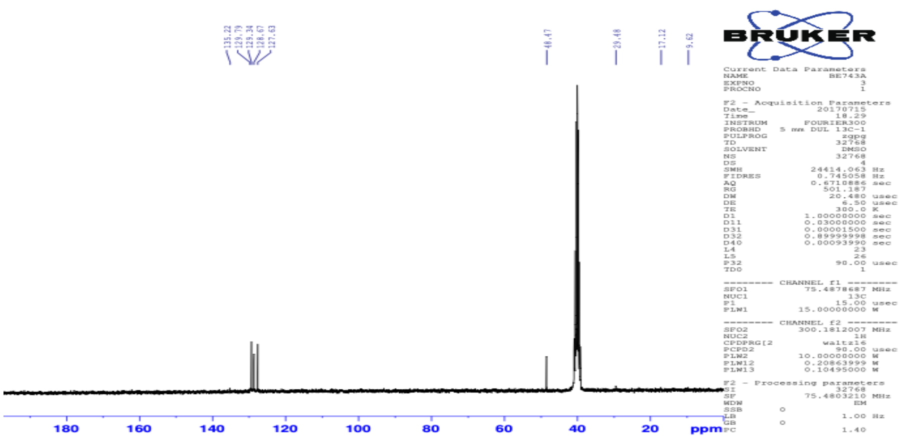

Figure 5. Benzyl isothiocyanate ^{13}C-NMR

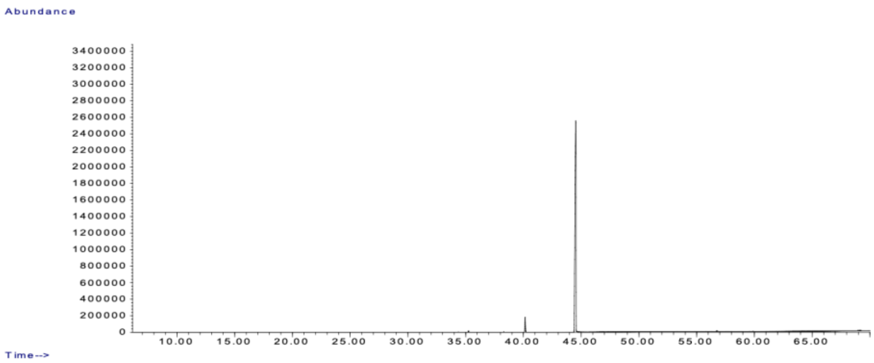

Figure 6. Chromatogram of *Carica papaya* unripe fruit essential oil

2.2. Chemical Compositions of Fatty Acids

Fatty oils were obtained from the dried and fresh seeds of *C. papaya* by Soxhlet apparatus with *n*-hexane. The oil yields of 2.6% and 3.3%, respectively. Analysis was performed after the methylation process. GC and GC-MS results of methylated fatty oils were given in Table 2. The other chromatograms presented in Figure 7and 8.

As a result of the analysis, there is not much difference in terms of fatty acid composition for fatty oils, but only a proportional difference has been detected. Oleic acid (72.0%; 70.0%), palmitic acid (15.0%; 16.4%) and linoleic acid (5.3%; 5.1%) were determined as the main compounds of dried and fresh seed fatty oils, respectively.

Table 2. Fatty acids of *Carica papaya* dried and fresh seeds

Fatty acids		Dried seed (%)[a]	Fresh seed (%)
Myristic (14:0)		tr	0.1
Palmitic (16:0)		15.0	16.4
Palmitoleic (16:1)		0.2	0.3
Margaric (17:0)		tr[b]	0.2
Stearic (18:0)		4.0	4.0
Oleic (18:1)		72.0	70.0
Elaidic (18:1)		2.5	1.5
Linoleic (18:2)		5.3	5.1
Linolenic (18:3)		0.3	0.3
Arachidic (20:0)		0.3	0.5
Eicosadienoic (20:2)		0.2	0.6
Behenic (22:0)		0.2	0.4
	Total	**100**	**99.4**

[a]%: calculated from FID data; [b]tr: Trace (< 0.1 %)

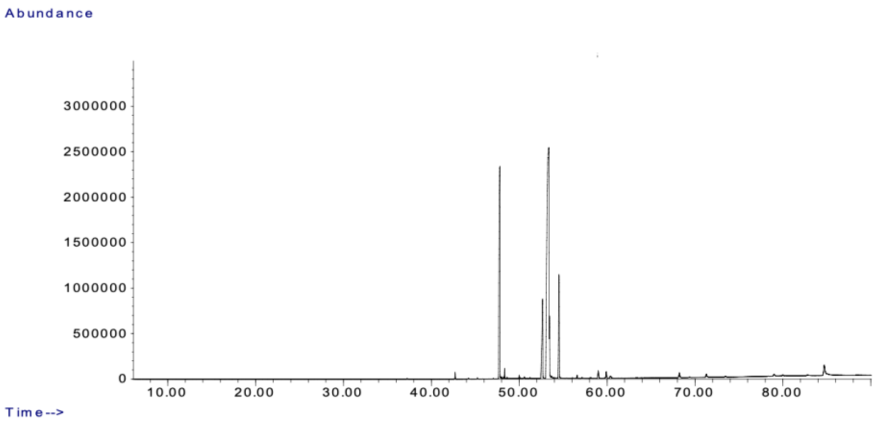

Figure 7. Chromatogram of *Carica papaya* dried seed fatty acids

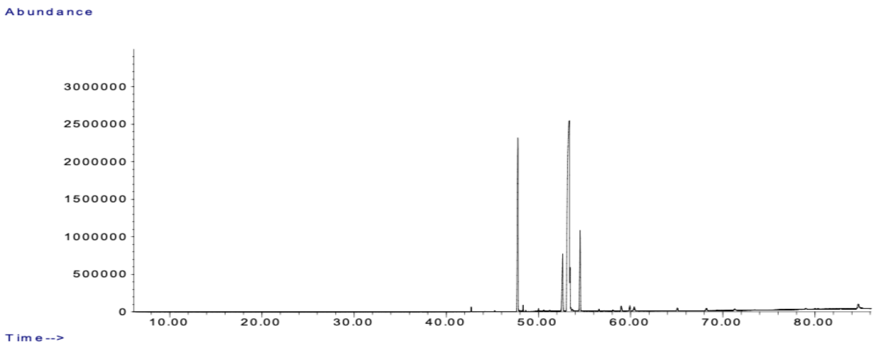

Figure 8. Chromatogram of *Carica papaya* fresh seed fatty acids

In previous studies, Soxhlet apparatus, solvent extraction and CO_2 supercritical fluid methods were used to obtain fatty oil from the seeds of *C. papaya* [3, 17, 18]. The methanol:diethyl ether (70:30) solvent system was preferred in the Soxhlet apparatus. Benzyl isothiocyanate compound was found as the main compound in the extractions made using supercritical fluid methods [3, 19]. Oleic acid, palmitic acid and stearic acid were determined as the main compounds in the extractions with hexane and in the studies performed by Soxhlet apparatus [16-18]. Oleic acid, one of the fatty acids found most in fatty oil, showed thermal oxidative properties, because it is a monounsaturated fatty acid in its structure. Oil with monounsaturated fatty acid content is generally used in skin care products due to its emollient properties, while it is also used in bath oils, hair preparations and make-up materials [18, 21].

2.3. Antimicrobial activity

Antimicrobial activity of *C. papaya* essential and fatty oils was tested against Gram negative *Escherichia coli* NRRL B-3008, Gram positive *Bacillus subtilis* NRRL-B4378 and *Candida albicans* ATCC 10231 by Agar disc diffusion method. The standards, amphotericin B for fungi, ampicillin and ciprofloxacin for bacteria, used as positive controls. According to the results, no inhibition zones were determined in the concentrations tried in essential and fatty oils. However, highest antimicrobial activity ampicillin and amphotericin B with 5 mm zone diameter against *B. subtilis* and *C. albicans*, respectively. Ciprofloxacin showed moderate activity against *E. coli* (10 mm).

According to the He et al. (2017) the highest antifungal activity was found seed essential oil of *C. papaya* against *C. parapsilosis* ATCC 22019 strain (33.2±0.8 and 4.0 μg/mL) [4]. In addition, in previous antimicrobial activity studies, different extracts of the flower, root, fruit and seeds of the *C. papaya* were studied. Antimicrobial activities of extracts prepared with polar solvents were found to be more effective than essential oils [22-25].

C. papaya, which is a very valuable plant, has the potential to be an important source in areas such as the food and cosmetics industry, thanks to the oleic acid contained by its fresh and dry seed fatty oil. Benzyl isothiocyanate, which is found as the main compound in the essential oils obtained from the *C. papaya*, shows that the plant can be used for the purpose of obtaining active substance in the pharmaceutical industry as well. To the best of our knowledge, benzyl isothiocyanate is found in essential oil obtained from leaves of *C. papaya* for the first time in this study.

3. CONCLUSION

The increasing interest in tropical fruits, which also have biological activities with their rich and valuable chemical content, also has commercial potential in various fields. The initial results of *C. papaya* from Turkish cultivars from Antalya suggest further detailed studies of both chemical variation and biological activities. In addition, benzyl isothiocyanate was detected for the first time in the essential oil of *C. papaya* in this work.

4. MATERIALS AND METHODS

4.1. Plant materials

Leaves, ripe and unripe fruits of *C. papaya* were collected grown in subtropical condition (Antalya province of Türkiye). Essential oils (EOs) from leaf, ripe and unripe fruits of *C. papaya* were obtained by hydrodistillation using a Clevenger type apparatus for 3 h. Dried and fresh seeds of *C. papaya* fatty acids (FAs) were extracted with *n*-hexane (Sigma-Aldrich) (250 mL-8 h) in a Soxhlet apparatus, and after the methylation the chemical compositions were determined by GC and GC-MS systems.

4.2. GC and GC-MS analyses

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses processes were performed with reference to Demirci et al. (2015) [26].

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) and in-house ''Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils [26].

4.3. ^{1}H-NMR and ^{13}C-NMR

Major component of *C. papaya* essential oil was analyzed in DMSO-d6 solvent against tetramethylsilane (TMS) standard using Bruker DPX 300 NMR spectrometer for proton NMR and Bruker DPX 57 spectrometer for carbon NMR [27].

4.4. Antimicrobial activity

The antimicrobial activities of the essential and fatty oils were evaluated by agar disc diffusion assay according to a modified Clinical and Laboratory Standards Institute (CLSI) CLSI M02-A11 and CLSI M44-A2 method [28, 29]. Concentrations of test samples were 10 mg/mL in dimethyl sulfoxide (DMSO, Sigma-Aldrich). Antimicrobial activity of samples evaluated against *Escherichia coli* NRRL B-3008, *Bacillus subtilis* NRRL B-4378, *Candida albicans* ATCC 10231 strains. 100 µL of microorganisms with a turbidometric setting of 0.5 McFarland were inoculated into Petri plates containing Muller Hilton Agar (Sigma-Aldrich), for bacteria and Potato Dextrose Agar (Sigma-Aldrich) for fungi. After the medium surface dried, 20 µL test samples were added to the blocks opened on petri dishes with a 6 mm diameter sterile agar drill. Zones of inhibition (IZ) formed after 24 hours of 37°C incubation were measured. Ampicillin (20 µg/mL) for *B. subtilis*, ciprofloxacin (10 µg/mL) for *E. coli*, and Amphotericin B (20 µg/mL) for *C. albicans* were used as reference materials. The experiment was carried out in duplicate [28, 29].

Acknowledgements: Part of this work was presented at the ISOPS (2018) - International Symposium on Pharmaceutical Sciences 2018, Ankara, Türkiye, 26-29 June 2018, which was part of the MSc Project of E.T.

Author contributions: Concept – E.T., B.D.; Design – B.D.; Supervision – B.D..; Resources – E.T., H.G.; Materials – E.T., H.G.; Data Collection and Processing – E.T., G.Ö, B.D.; Analysis and Interpretation – E.T., B.D., G.Ö.; Literature

Search – G.Ö., B.D.; Writing – G.Ö., B.D.; Critical Reviews – E.T., H.G., G.Ö., B.D.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Bamisaye FA, Ajani EO, Minari JB. Prospects of ethnobotanical uses of pawpaw (*Carica papaya*). J Med Plants Stud. 2013; 1(4): 171-177 [CrossRef]

[2] Anitha B, Raghu N, Gopenath TS, Karthikeyan M, Gnanasekaran A, Chandrashekrappa GK, Basalingappa KM. Medicinal uses of *Carica papaya*. J Nat Ayurvedic Med. 2018; 2(6): 2-11. [CrossRef]

[3] Barroso PT, de Carvalho PP, Rocha TB, Pessoa FL, Azeanddo DA, Mendes MF. Evaluation of the composition of *Carica papaya* L. seed oil extracted with supercritical CO_2. Biotechnol Rep. 2016; 11: 110-116. [CrossRef]

[4] He X, Ma Y, Yi G, Wu, J, Zhou L, Guo H. Chemical composition and antifungal activity of *Carica papaya* Linn. seed essential oil against *Candida* spp. Lett Appl Microbiol. 2017; 64(5): 350-354. [CrossRef]

[5] Moy JH, Papayas, second ed., American press, USA 2003.

[6] Ulrich D, Wijaya CH. Volatile patterns of different papaya (*Carica papaya* L.) varieties. J Appl Bot Food Qual. 2010; 83: 128-132. [CrossRef]

[7] Pino, JA. Odour-active compounds in papaya fruit cv. Red Maradol. Food Chem. 2014; 146: 120-126. [CrossRef]

[8] Kelebek H, Selli S, Gubbuk H, Gunes E. Comparative evaluation of volatiles, phenolics, sugars, organic acids and antioxidant properties of Sel-42 and Tainung papaya varieties. Food Chem. 2015; 173: 912-919. [CrossRef]

[9] Melariri P, Campbell W, Etusim P, Smith P. Antiplasmodial properties and bioassay-guided fractionation of ethyl acetate extracts from *Carica papaya* leaves. J Parasitol Res. 2011; 2011: 1-7. [CrossRef]

[10] Gao Z, Zheng L, Wu W, Fu Y. Analysis of essential oils from leaf of *Carica papaya* with GC-MS. Journal of Fruit Science, 2010; 27(2): 307-311.

[11] Vij T, Prashar Y. A review on medicinal properties of *Carica papaya* Linn. Asian Pac J Trop Dis. 2015; 5(1): 1-6. [CrossRef]

[12] Singh O, Ali M. Phytochemical and antifungal profiles of the seeds of *Carica papaya* L. Indian J Pharm Sci. 2011; 4: 447–451. [CrossRef]

[13] Li ZY, Wang Y, Shen WT, Zhou P. Content determination of benzyl glucosinolate and anticancer activity of its hydrolysis product in *Carica papaya* L. Asian Pac J Trop Med. 2012; 3: 231–233. [CrossRef]

[14] Julaeha E, Permatasari Y, Mayanti T, Diantini A. Antifertility compound from the seeds of *Carica papaya*. Procedia Chem. 2015; 17: 66–69. [CrossRef]

[15] Asghar N, Naqvi SAR, Hussain Z, Rasool N, Khan ZA, Shahzad SA, Sherazi TA, Janjua MRSA, Nagra SA, Zia-Ul-Haq M, Jaafar HZ. Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. Chem Cent J. 2016; 10: 1–11. [CrossRef]

[16] Lee WJ, Lee, MH, Su NW. Characteristics of papaya seed oils obtained by extrusion–expelling processes. J Sci Food Agric. 2011; 91(13): 2348–2354. [CrossRef]

[17] Viana GM. Avaliação do isotiocianato de benzila natural como inseticida e precursor sintético de tiouréias e derivados. Rio de Janeiro Federal University, Brasil 2009.

[18] Samaram S, Mirhosseini H, Tan CP, Ghazali HM. Ultrasound-assisted extraction (UAE) and solvent extraction of papaya seed oil: yield, fatty acid composition and triacylglycerol profile. Molecules. 2013; 18(10): 12474-12487. [CrossRef]

[19] Gomes BL, Fabi JP, Purgatto E. Cold storage affects the volatile profile and expression of a putative linalool synthase of papaya fruit. Food Res Int. 2016; 89: 654-660. [CrossRef]

[20] Puangsri T, Abdulkarim SM, Ghazali HM. Properties of *Carica papaya* L. (papaya) seed oil following extractions using solvent and aqueous enzymatic methods. J Food Lipids. 2005; 12(1): 62-76. [CrossRef]

[21] Malacrida CR, Kmura M, Jorge N. Characterization of a high oleic oil extracted from papaya (*Carica papaya* L.) seeds. Cienc Tecnol Aliment. 2011; 31(4): 929-934. [CrossRef]

[22] Zakaria ZA, Mat Jais AM, Sulaiman MR, Mohamed Isa SSP, Riffin S. The *in vitro* antibacterial activity of methanol and ethanol extracts of *Carica papaya* flowers and Mangifera indica leaves. J Pharmacol Toxicol. 2006; 1: 278-283. [CrossRef]

[23] Doughari JH, Elmahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. Afr J Microbiol Res. 2007; 1(3): 037-041. [CrossRef]

[24] Oladimeji HO, Nia R, Kalu N, Attih EE. *In vitro* biological activities of *Carica papaya*. Res J Med Plant. 2007; 1: 92-99. [CrossRef]

[25] Eke ON, Augustin AU, Ibrahim HF. Qualitative analysis of phytochemicals and antibacterial screening of extracts of *Carica papaya* fruits and seeds. Int J Modern Chem. 2014; 6(1): 48-56. [CrossRef]

[26] Ozturk, G, Demirci B, Çelik M, Başer KHC. Chemical Characterization of Bunium allioides and B. brachyactis. Chem Nat Compd. 2020; 56: 1146–1147. [CrossRef]

[27] Levent S, Kaya Çavuşoğlu B, Sağlık B, Osmaniye D, Acar Çevik U, Atlı Ö, Özkay Y, Kaplancıklı Z. Synthesis of oxadiazole-thiadiazole hybrids and their anticandidal activity. Molecules. 2017; 22(11): 1-13. [CrossRef]

[28] CLSI. Method for Antifungal Disk Susceptibility Tests; Susceptibility Testing of Yeasts; Approved Standard, M44-A2, Clinical and Laboratory Standards Institute, 17, 2009.

[29] CLSI. Method for Antimicrobial Disk Susceptibility Tests; Approved Standard, M02-A1, Clinical and Laboratory Standards Institute, 11, 2012.