Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

STORAGE EFFECT ON THE GC-MS PROFILING AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OILS FROM LEAVES OF *ANNONA SQUAMOSA* L.

Sara Hammoud, Ali Jaber*, Ghassan Ibrahim, Edmomd Cheble
Laboratory for Research and Development of Medicines and Natural Products, RDMPN, Faculty of Pharmacy, Lebanese University, Beirut, Lebanon.

Article Info:

Article History:
Received: 4 April 2022
Reviewed: 10 May 2022
Accepted: 12 June 2022
Published: 15 July 2022

Cite this article:
Sara H, Ali J, Ghassan I, Edmomd C. Storage effect on the GC-MS profiling and antioxidant activities of essential oils from leaves of *Annona squamosa* L. Universal Journal of Pharmaceutical Research 2022; 7(3):51-57. *https://doi.org/10.22270/ujpr.v7i3.785*

***Address for Correspondence:**
Ali Jaber, Laboratory for Research and Development of Medicines and Natural Products, RDMPN, Faculty of Pharmacy, Lebanese University, Beirut, Lebanon. Tel-009613451884; E-mail: *ali.jaber.2@ul.edu.lb*

Abstract

Aim and objective: Medicinal plants, their biological activities, and their phytochemical contents are important for finding safe and potent new compounds for therapeutic use. In order to investigate the chemical contents and to evaluate the storage effect on the antioxidant activity of Lebanese *Annona squamosa* (AS) leaf essential oil, the current study was undertaken.
Methods: Shade-dried leaves of AS were taken from Batroun (Lebanon), and the essential oil (EO) was extracted by hydrodistillation. The gas chromatography-mass spectrometry (GC-MS) technique was used to analyze the composition of the EO. Concerning the antioxidant activity, two different methods namely radical scavenging activity (DPPH test) and ferric reducing antioxidant power (FRAP) were used.
Results: A total of 21 compounds were identified. The majority of the identified compounds belong to sesquiterpenoids. β-elemene (11.39 -14.14%) and β-carophyllene (10.15-15.56%) were the most abundant components. On the other hand, the storage of the plant materials containing the EOs or the EOs themselves leads to a loss in the volatile compounds, which is reflected in the bioactivity as shown in the results of the antioxidants assays. The EOs demonstrated antioxidant activities with IC50 lower than 9 $\mu g.mL^{-1}$. DPPH test and FRAP test exhibited a strong positive correlation (r=0.99).
Conclusion: The obtained results suggest that EO extracts from AS have an antioxidant to protect people. Thus, the EO of fresh samples of AS can have interesting applications in versatile areas such as the pharmaceutical and food industries.
Keywords: *Annona squamosa*, Antioxidant, DPPH, Essential oil, FRAP, GC-MS.

INTRODUCTION

Medicinal plants are part of the history of all continents. Through the centuries, knowledge about plants has been organized, documented, and passed down across generations[1]. Herbal medicine is now used daily as prevention rather than therapy to protect our health. They are resources of phytochemicals such as flavonoids, polyphenols, alkaloids, tannins, terpenoids, coumarins, and others. *Annona squamosa* (AS) edible fruit plants belonging to the Annonaceae family is commonly known as the sugar apple, custard apple, and sweetsop[2]. Research on this plant showed several medicinal properties such as cardiotonic, antimicrobial, insecticidal, and anti-cancerous activities[3]. Including, but not limited to, Chen *et al*., isolated new diterpenes and tested their cytotoxic activities[4]. One of the five diterpenes evaluated showed potent cytotoxicity with an IC_{50} value of under 20 µM.

AS spread in many countries among them Lebanon, due to its geographical location, and its Mediterranean climate where it adapts well. It is a small, well-branched tree that grows at altitudes of 0 to 2,000 m and does well in hot, dry climates. The plant has been reported to possess a wide variety of pharmacological activities[5]. Essential oils (EO), volatile compounds extracted from plants, are complex compounds with strong odors, made up of various plant metabolites[6]. EOs are believed to have many different biological activities[7], it have been used for their positive effects on humans since ancient times, as attested by early writings[8]. Furthermore, the composition of EOs in the same plant species is affected by several parameters, such as harvest time, extraction method, and protection[9-11]. Numerous investigations were done to

study the chemical components of *A. squamosa* Essential oils (ASEOs), terpenes and sesquiterpenes were the major reported classes[2,12,13]. ASEOs have shown a wide range of biological properties[13-16]. EO from AS bark showed significant antimicrobial activity against two gram-positive bacteria species namely *Bacillus subtilis* (non-pathogenic bacterium) and *S. aureus* (opportunistic pathogen)[12].With IC50 values less than 20 g.mL^{-1}, the ASEO showed strong trypanocidal and antimalarial effects. Furthermore, significant ultrastructural changes, particularly in the cell membrane and mitochondria, block the growth of amastigotes and ultimately lead to necrotic parasite death[13]. Furthermore, EO from AS pericarps exhibited significant anti-hepatoma activities with IC50 lower than 55 µg.mL^{-1} against SMMC-7721 hepatoma cell line[14].

Earlier studies were devoted to studying the leaves and seeds of Lebanese AS[5,17]. However, there is no report concerning the essential oils. The present study aimed to extract and identified the volatile organic components from dry leaves of AS using hydro-distillation and GC/MS, to study the changes in EO composition during storage of leaves in paper bags or the storage of EO samples, and measure the effect on the antioxidant activity.

MATERIALS AND METHODS

Plant materials: Collection, Identification, and Preparation

The leaves of AS were collected from the producer in Batroun, in northern Lebanon (80-100 m AMSL). The plant has been identified and confirmations were done via the Flora of the presidency of Madras, by Gamble J.S.[18]. The voucher specimen (No. 1806) of the plant material is maintained in the laboratory.

AS leaves were collected in February and beginning of March 2019 from *Annona* trees. The leaves were shade dried for 3 weeks and then pulverized into fine powder. The powder was divided into two batches, the first being stored at room temperature in dark for further use in the next year, and the second part was undergone hydro-distillation extraction to yield the first EO sample (S1). The latter, in turn, was stored at -18°C, in stoppered glass vessels containing some air for one year yielding the second EO sample (S2) (March 2020). Finally, the third sample (S3) is the EO obtained after fresh extraction of the conserved leaves.

Extraction of essential oils of *A. squamosa* (ASEO)

Foremost, 50 g of AS powder were added into a 1000 mL round bottom flask containing 500 mL of distilled water. The hydro-distillation was performed in a Clevenger-type distillation apparatus designed according to British Pharmacopoeia specifications[19]. After 3 hours of distillation, the ASEO was collected in the receiver arm. For further use, the oils were sealed and maintained in amber glass vials at 4°C.

Analyses of volatile organic compounds

One microliter of ASEO sample was diluted (1:100) with hexane and injected into the gas chromatography-mass spectrometry (GC-MS) system. GC SHIMADZU QP2010 system was used to analyze the volatile compounds in the *N. tabacum* extract (without derivatization). DB-5MS (5% Diphenyl/95% Dimethylpolysiloxan) capillary column having (30 m length, 0.25 i.d., film thickness 0.28 µm) and helium as carrier gas (1 mL/min, constant flow) was used for compound separation. The oven temperature was programmed from 65°C (2 min initial time) increased to 300°C at 10°C/min (isothermal for the final time). The actual temperature in the MS source reached 230ºC, and the MS was operated in the electron impact mode at 70EV ion source energy. The injector temperature was 250°C, while the injection volume was 1 µL and a total run of one hour is performed, with a mass detector scan range *m/z*=50–550. Data receipt and processing were performed using Shimadzu GC-MS solution software. The detected compounds were tentatively identified, by MS spectral correlations using NIST08 (National Institute of Standards and Technologies, Mass Spectra Libraries), as well as published data.

In vitro antioxidant activities

The antioxidant activity was measured using two methods namely DPPH free radical scavenging assay and reducing power assay.

DPPH assay

2, 2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) method for determining antioxidant activity isa spectrophotometric method based on the hydrogen atom transfer and single electron transfer mechanisms. These assays included radical scavenging activity, based on the antioxidant reduction of the violet DPPH radical via a hydrogen atom transfer mechanism, resulting in the color change (Sirivibulkovit *et al.*,)[20]. The violet DPPH radical is measured by a UV-Vis spectrophotometer at approximately 515 to 520 nm. Several solutions of increasing concentrations varying from 0.81 ng.mL^{-1} to 10.81 ng.mL^{-1} of ascorbic acid were prepared in test tubes. About 1 mL of the ASEO solution of different concentrations (5 to 25 µg.mL^{-1}) was taken in test tubes, then 1 mL of the DPPH methanolic solution (81.15 µM) was added. Simultaneously, a control was generated by mixing 1 mL of DPPH solution with 1 mL methanol. After 30 min of incubation in dark at room temperature, the decrease in absorbance of each mixture (due to quenching of DPPH free radicals) was determined at 517 nm against a blank (methanol) using a UV-VIS spectrophotometer.

Based on graphic values of the percentage of DPPH inhibition vs EO concentrations, the half-maximal inhibitory concentration (IC_{50}) (the concentration of the sample needed to inhibit 50% of the DPPH) of each sample was estimated. The antioxidant activities of all the samples were compared to the antioxidant activity of ascorbic acid, i.e., ascorbic acid was used as a reference standard.

Reducing power assay

The reducing power assay method is designed based on the reduction potential of the components by reacting with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}). The latter product mixed with ferric chloride forms a ferric–ferrous complex having an absorption maximum of 700 nm[21].

The reducing ability of ASEO was determined according to a method reported by Oyaizu[22]. The aliquots of different concentrations (10 to 100 µg.mL^{-1}) of the standard/test sample (methanolic solutions) were mixed with 2.5 mL of (pH 6.6) phosphate buffer + 2.5 mL of (1%) potassium ferricyanide. Subsequently to a cooling step, the mixture was placed in a water bath at 50°C for 20 minutes. About 2.5 mL (10%) trichloroacetic acid aliquots were added to the mixture, which was then centrifuged for 10 minutes at 3000 rpm. The upper layer of solution of 2.5 mL was mixed with 2.5 mL distilled water and a freshly prepared 0.5 mL of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in a UV spectrometer. The solutions were prepared on the day of the experiment and well protected from sunlight. Ascorbic acid at various concentrations (5 to 40 µg.mL^{-1}) was used as standard.

The sample was prepared using a similar procedure but by replacing the EO with an equal volume of methanol. The absorbance values were plotted against the concentration, and a linear regression analysis was carried out. The higher absorbance of the reaction mixture indicates a greater reducing power. All data were recorded as mean ± SD for three replicates.

Statistical analysis

The experimental runs and the analyses were carried out in triplicate. The experimental results derived in the study were expressed as the mean ± standard deviation (SD). The correlation coefficients were calculated with Pearson's test using Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Linear regression analysis was used to calculate the IC_{50} values. Independent samples *t*-tests were used to analyze the obtained data. Statistical significance was considered where $p<0.05$.

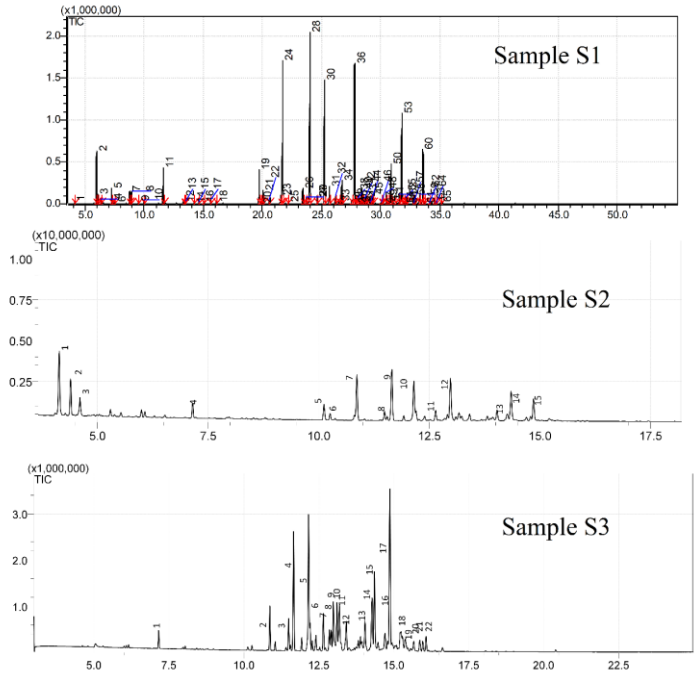

Figure 1: GC-MS of different ASEOs.

RESULTS AND DISCUSSION

Compound identification using GC-MS

The essential oil obtained by hydro-distillation from the leaves of *A. squamosa* was yellowish-green and the yield was 0.1% (v/w), based on dry weights. The chemical constituents of ASEO samples were analyzed by GC-MS (Figure 1). This led to the identification of different compounds that were determined by referring to previously published articles and referring to the suggestions of the NSIT Library[23]. The chemical composition of the ASEO is shown in Table 1.

The components are listed in order of their elution on the DB-5MS column. The results showed that the EO of the three samples was mainly composed of the sesquiterpenoids. By comparing samples, S2 to S3, some of the sesquiterpenoids that were present in S3 were not found in S1. Besides, by comparing sample S2 with the results from the previous year (S1), the compounds that were present in S1 disappeared from S2. A total of 19 (61.2%), 11 (51.07%), and 10 (43.33%) compounds were identified in the sample S1, S2, and S3 respectively. ASEO was predominantly composed of sesquiterpenes (50.78%), and the remainders are monoterpenes. Bicyclic sesquiterpenes comprised 19.66% of the sample. The three major constituents that were discovered were δ-elemene (11%), carophyllene (10.15%), and β-elemene (14.14%). While β-elemene is absent, the two other compounds are in agreement with the results of Al-Nemari *et al.*,[24,]. EOs from the leaves of numerous *Annonaceae* genera, including *Annona*, have been discovered to include spathulenol and caryophyllene oxide, which could be used as chemotaxonomic identifiers for these genera[25,26]. After storage, the percentage of α-humulene increases from 0.6 to 2.57% in agreement with the work of Mockutë[10].

In contrast, although the amounts of compounds with caryophyllene (β-caryophyllene + caryophyllene oxide) were nearly the same in fresh and stored EOs, caryophyllene oxide decreased from 8.15% (S1) and disappear in S2. On the other hand, the proportion of constituents with a low molecular weight (monoterpenes) significantly decrease, while the conditional percentage of larger molecular weight molecules having three isoprene units (sesquiterpenes) increased as a result of the above decrease. It's worth noting that the content in α-pinene, linalool, and thymol in S2 increases per the results of Baritaux *et al.*[9]. Other researchers have found lower amounts of monoterpenes and higher levels of certain sesquiterpenes in dill and ginger[27,28].

EOs derived from fruit and seeds of annonacea species are mainly consti-tuted of monoterpene hydrocarbons[29], while sesquiterpenes predominate EOs of leaves as hydrocarbons forms and in bark and roots as oxygenated forms.

Table 1: Major compounds identified in the ASEOs obtained by hydro distillation using GCMS.

Compound	% Area			Formula	Classification
	S1	S2	S3		
α-pinene	2.71	3.7	-	$C_{10}H_{16}$	Bicyclic monoterpene
β-Pinene	0.9	-	-	$C_{10}H_{16}$	Bicyclic monoterpene
β-Myrcene	0.18	-	-	$C_{10}H_{16}$	Monoterpene
O-Cymene	0.75	-	-	$C_{10}H_{14}$	Monoterpene
D-limonene	0.74	-	-	$C_{10}H_{16}$	Bicyclic monoterpene
3-Carene	0.24	-	-	$C_{10}H_{16}$	Bicyclic Monoterpene
Linalool	2.29	3.2	-	$C_{10}H_{18}O$	Monoterpene
Thymol	2.6	2.8	-	$C_{10}H_{14}O$	Monoterpene
δ-Elemene	11	12.1	3.81	$C_{15}H_{24}$	Monocyclic sesquiterpene
α- Cubenene	0.13	-	0.67	$C_{15}H_{24}$	Sesquiterpenoid
Copaene	1.22	-	2.8	$C_{15}H_{24}$	Bicyclic sesquiterpene
β-Elemene	14.14	11.5	11.39	$C_{15}H_{24}$	Monocyclic sesquiterpene
β-Caryophyllene	10.15	11.3	15.56	$C_{15}H_{24}$	Bicyclic sesquiterpene
γ- Elemene *o*-Menth-8-ene	1.39	1.65	-	$C_{15}H_{24}$	Monocyclic sesquiterpene
α-Humulene	0.31	0.6	2.57	$C_{15}H_{24}$	Monocyclic sesquiterpene
Germacrene-D	0.54	1.9	0.70	$C_{15}H_{24}$	Monocyclic sesquiterpene
Isoledene	-	-	1.23	$C_{15}H_{24}$	Sesquiterpenoids
α-Amorphene	-	-	3.23	$C_{15}H_{24}$	Sesquiterpenoids
α-Selinene	3.66	-	-	$C_{15}H_{24}$	Sesquiterpenoid
Caryophylleneoxide	8.15	2.13	-	$C_{15}H_{24}O$	Bicyclic sesquiterpene
Sphathulenol	0.14	0.19	1.37	$C_{15}H_{24}O$	Bicyclic sesquiterpene
Total	61.24	51.07	43.33		

This profile variation of the samples can be attributed to the fact that EO components are known to readily transform into one another by oxidation, isomerization, cyclization, or dehydrogenation reactions induced either enzymatically or chemically, due to their structural link within the same chemical group[30]. There have been many reports about the composition of EOs from the different parts of *A. Squamosa*).

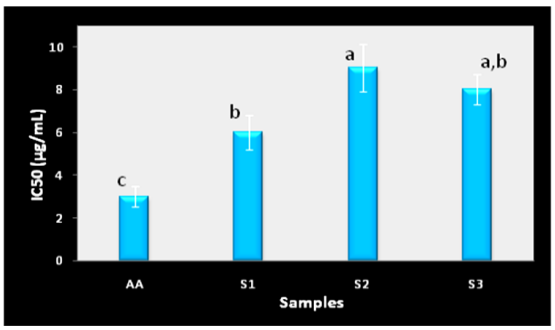

Figure 2: IC_{50} values of samples A and B compared with ascorbic acid (AA).

Values with different superscripts in a column differ significantly (p<0.05)

For instance, the chemical profile of EO from the leaves of *A. Squamosa* growing in Badagary (Nigeria) was mainly composed of (E)-caryophyllene (38.9%) and eugenol (30.2%)[31]. This research work aims to study the effect of the extraction factor on the volatile compounds occurring in AS. The GC-MS method highlighted the difference in the content of the EO whether it was extracted and conserved in the refrigerator or preserved in the leaves.

The chemical composition of EOs and plant secondary metabolites, in general, is affected by different abiotic factors, namely climate, growing conditions, or harvest time are the most studied[32]. Sesquiterpenes isolated from EOs are among compounds with promising antimicrobial activity[35,36]. These encompass β-caryophyllene, a sesquiterpene extensively present in EOs, which possesses anti-inflammatory and anticarcinogenic activities[37]. Its oxygenated form caryophyllene oxide, present at 8.15% in the obtained EOAS, owns high antimicrobial properties[38]. Furthermore, β-elemene presents good antitumor and antiinflammatory activities without obvious cytotoxicity or clinical side effects[39].

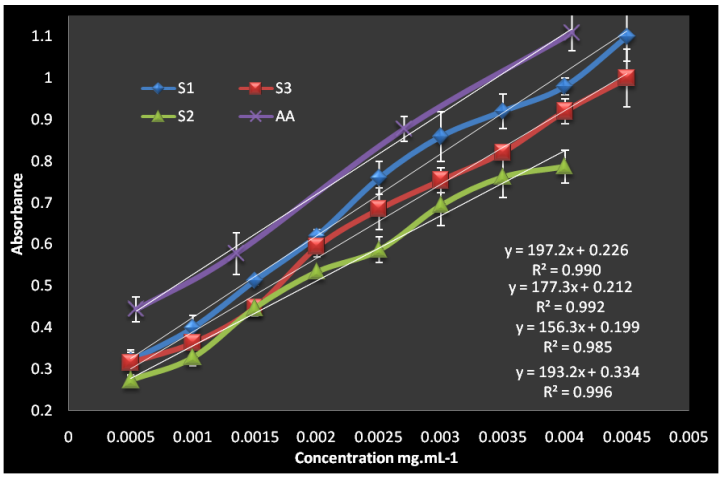

Figure 3: Reducing power of the different samples in comparison with ascorbic acid as a reference.

DPPH assay

In the antioxidant test of essential oils, much positive control can be used, such as quercetin, trolox, α-tocopherol, ascorbic acid, and many others herein ascorbic acid was used in the two tests. The three assessed EOs were able to reduce the stable, purple-colored radical DPPH˙ to yellow colored DPPH-H, thus samples S1, S2 and S3 had IC_{50} values of 6, 9, and 8 µg.mL^{-1} respectively (Figure 2) and varied significantly (p<0.05). The positive control (ascorbic acid) had an IC_{50} value of 3 µg.mL^{-1}. The highest antioxidant activity was obtained with the sample with the lowest IC_{50}.

Table 2: Major components of leaves from *Annona* species essential oils reported from some regions of the world.

Major compounds (%)	Geographic regions		References
(E)-Caryophyllene (38.9), eugenol (30.2), δ-cadinene (6.0), caryophyllene oxide (5.0), α-humulene (4.3)	Nigeria	EOAM	31
β-Caryophyllene (24.5), β-Cubebene (13.0), β-Elemene (5.9), α-Cadinol (5.2), α-Terpinene (4.6)	Vietnam	EOAS	33
β-Caryophyllene (40), β-elemene (14.4), α-santalene (9.5), (Z)-hex-3-enol (5.2), δ-cardinene (4.8)	Cameroon	EOAM	34
Germacrene-D (22.01), trans-caryophyllene (12.12), bicyclogermacrene (2.80), α-copaene (2.12), and humulene (1.15), as well as phytol (2.22) and squalene (1.3).	Saudi Arabia	ASAS	24
Spathulenol (43.7), limonene (20.5), caryophyllene oxide (8.1) and a pinene (5.5)	Brazil	EO *A. vepretorum*	25

Among the samples, S1 revealed the lowest IC_{50}, this means that sample S1 has more ability to inactivate free radicals leading to more antioxidant activity. Based on the previous GC-Ms results, this could be explained by the fact that the number of secondary metabolites (such as phenolic compounds, terpenoids) present in the ASEO was influenced by several factors including duration of conservation, and chemical variability. The obtained IC_{50} values are in good agreement with those obtained for a different part of *A. squamosa*[40]. The IC_{50} is lower than the 1.33 mg.mL^{-1} reported for seed oil[41]. In addition, the results showed that it outperforms EO from the leaf of its peers *A. muricata* (244.8 µg.mL^{-1})[42].

FRAP method

The antioxidant potential of plant extracts or EOs may be determined by their reducing power[43]. The reducing power of ASEO was determined for samples S1, S2, and S3 at different concentrations (Figure 3). It was observed that the absorbance of all samples gradually increases with the increasing concentration of oil. Also, the capacity of the extracts to reduce Fe^{3+} to $Fe^{2+}$is lower than that of ascorbic acid, and sample S1 had the greatest reducing power. The reducing power of the three ASEOs is found lesser than the positive control compound, and there was a significant difference at p<0.05.

Table 3: Pearson's correlation coefficients.

	Sample	DPPH	FRAP
Sample	1		
DPPH	0.87831	1	
FRAP	0.812564	0.992349	1

Current findings were, qualitatively, in agreement with the observation obtained with AS leaves extracts[37,38,44,45], where they found that the reducing power of ascorbic acid exceeds that of AS extracts. In other words, the reducing power of *A. squamosa* extracts referred to its electron transfer capacity in a redox reaction, leading to the neutralization of free radicals and forming stable products. It has been reported that the reducing power of extracts probably depends on the

hydrogen-donating ability present in terpenoids and phenolic compounds. As a result, antioxidants can be thought of as reductants, and the inactivation of oxidants.

Significant correlations were obtained between the ASEO and the antioxidant activities via two different assays. Table 3 shows the correlation between DPPH and FRAP assays of ASEOs obtained along different storage conditions. A positive correlation exists between the way of conservation and DPPH(r=0.88) and FRAP (r=0.81) assays. So, there is a high possibility of the same reasons, same mechanisms or same bioactive compounds influence the antioxidant activity ASEO with DPPH and FRAP assays. On the other side, it merits featuring that, a significant correlation was obtained between the two antioxidant assays, and various examinations have likewise revealed this relationship[46,47].

Limitations

Besides the limited number of samples, the limitation of the study was the biological activities tests, restricted to antioxidant tests, which might cover other potential biological effects, such as anticancer and antimicrobial.

CONCLUSIONS

The phytochemical content and antioxidant activity of *A. squamosa* essential oils were influenced by the time of conservation. In this work, the phytochemical screening using GC-MS revealed that different oil of ASEO contain sesquiterpenoids. In addition, many compounds present in EO obtained from fresh samples disappeared with time even with conservation at -18°C. Same results were obtained with conserved plant materials. The results of antioxidant activity showed that freshly prepared EO samples from freshly dried leaves had exerted the best antioxidant activity. Thus, EO of fresh samples can have an interesting application in versatile areas such as the pharmaceutical and food industries. Further studies can be performed to reveal the reactions responsible for the biological activities present in the ASEOs. Evaluation of the anticancer activity of sample S1 against cell lines can be done in the future since antioxidant is correlated to anticancer activity.

ACKNOWLEDGEMENTS

The authors are grateful to the Lebanese University (Faculty of Pharmacy and Faculty of Sciences) Lebanon for providing gall chemicals and products necessary to carry out this work. The GC-MS spectra were performed at the Lebanese Agricultural Research Institute Laboratory. The assistance of the staff is gratefully appreciated.

AUTHOR CONTRIBUTIONS

Sara H: writing original draft, literature survey. **Ali J:** investigation, data collection, data interpretation. **Ghassan I:** methodology, conceptualization. **Edmomd C:** formal analysis, literature review. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

REFERENCES

1. Karunamoorthi K, Jegajeevanram K, Vijayalakshmi J, Mengistie E. Traditional medicinal plants: A source of phytotherapeutic modality in resource-constrained health care settings. J Evidence-Based Comp Alt 2013; 18:67–74. *https://doi.org/10.1177/2156587212460241*
2. Kumar M, Changan S, Tomar M, *et al*. Custard apple (*Annona squamosa* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting biological activities. Biomolecules 2021; 11:614. *https://doi.org/10.3390/biom11050614*
3. Kumar R, Roopan SM, Prabhakarn A, Khanna VG, Chakroborty S. Agricultural waste *Annona squamosa* peel extract: Biosynthesis of silver nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2012; 90:173–176. *https://doi.org/10.1016/j.saa.2012.01.029*
4. Chen YY, Ma CY, Wang ML, *et al*. Five new ent-kaurane diterpenes from *Annona squamosa* L. pericarps. Natural Product Research 2020; 34:2243-2247. *https://doi.org/10.1080/14786419.2019.1582048*
5. Ibrahim F, Jaber A, Ibrahim G, Cheble E. Antioxidant activity and total phenol content of different plant parts of lebanese *Annona squamosa* linn. Int J Pharmacy Pharmaceut Sci 2020; 100–105. *https://doi.org/10.22159/ijpps.2020v12i8.36992*
6. Kar S, Gupta P, Gupta J. Essential oils: Biological activity beyond aromatherapy. Natural Prod Sci 2018; 24:139. *https://doi.org/10.20307/nps.2018.24.3.139*
7. Mancianti F, Ebani VV. Biological activity of essential oils. Molecules 2020; 25:678. *https://doi.org/10.3390/molecules25030678*
8. Baser KHC, Buchbauer G. Handbook of Essential Oils : Science, Technology, and Applications. CRC Press; Boca Raton/London/New York. 2009. *https://doi.org/10.1201/9781420063165*
9. Baritaux O, Richard H, Touche J, Derbesy M. Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flavour Frag J 1992; 7:267–271. *https://doi.org/10.1002/ffj.2730070507*
10. Mockutë D, Bernotienë G, Judþentienë A. Storage-induced changes in essential oil composition of *Leonurus cardiaca* L. plants growing wild in Vilnius and of commercial herbs. Chemija 2005; 2:29-32.
11. Yuan Y, Huang M, Pang Y-X, *et al*. Variations in essential oil yield, composition, and antioxidant activity of different plant organs from *Blumea balsamifera* (L.) DC. at different growth times. Molecules 2016; 21:1024. *https://doi.org/10.3390/molecules21081024*
12. Chavan MJ, Shinde DB, Nirmal SA. Major volatile constituents of Annona squamosa L. bark. Natural Prod Res 2006; 20:754-757. *https://doi.org/10.1080/14786410500138823*
13. Meira CS, Guimarães ET, Macedo TS, *et al*. Chemical composition of essential oils from *Annona vepretorum* Mart. and *Annona squamosa* L. (Annonaceae) leaves and their antimalarial and trypanocidal activities. J Essent Oil Res 2015; 27:160–168.

https://doi.org/10.1080/10412905.2014.982876
14. Chen Y-Y, Peng C-X, Hu Y, *et al*. Studies on chemical constituents and anti-hepatoma effects of essential oil from *Annona squamosa* L. pericarps. Nat Prod Res 2017; 31:1305–1308. *https://doi.org/10.1080/14786419.2016.1233411*
15. Ma C, Chen Y, Chen J, Li X, Chen Y. A review on Annona squamosa L.: phytochemicals and biological activities. The American J Chinese Med 2017; 45:933–964. *https://doi.org/10.1142/S0192415X17500501*
16. Padhi LP, Panda SK, Satapathy SN, Dutta SK. *In vitro* evaluation of antibacterial potential of *Annona squamosa* L. and *Annona reticulata* L. from Similipal Biosphere Reserve, Orissa, India. J Agri Tech 2011; 7:133–142
17. Mohamad N, Majid EM, Falah A sadi, *et al*. Antibacterial, antioxidant and antiproliferative activities of the hydroalcoholic extract of the Lebanese *Annona squamosa* L. seeds. Int Res J Pharm 2017; 8:1-7. *https://doi.org/10.7897/2230-8407.08011*
18. Gamble JS. Flora of the Presidency of Madras. Nature 1921; 108:464-464. *https://doi.org/10.1038/108464b0*
19. British pharmacopoeia 1980. Her Majesty's Stationery Office, Atlantic House, Holborn Viaduct. London. *https://doi.org/10.1002/jps.2600691142*
20. Sirivibulkovit K, Nouanthavong S, Sameenoi Y. Paper-based DPPH Assay for Antioxidant Activity Analysis. Analyt Sci 2018; 34:795–800. *https://doi.org/10.2116/analsci.18P014*
21. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. *In vitro* antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. Ayu 2013; 34:209–214. *https://doi.org/10.4103/0974-8520.119684*
22. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese J Nutrition Diet 1986; 44:307–315
23. Shen VK, Siderius DW, Krekelberg WP, Hatch HW. NIST Standard Reference Simulation Website, NIST Standard Reference Database Number 173, National Institute of Standards and Technology, Gaithersburg MD, 20899. *http://doi.org/10.18434/T4M88Q*
24. Al-Nemari R, Al-Senaidy A, Semlali A, *et al*. GC-MS profiling and assessment of antioxidant, antibacterial, and anticancer properties of extracts of *Annona squamosa* L. leaves. BMC Complementary Medicine and Therapies 2020; 20:296. *https://doi.org/10.1186/s12906-020-03029-9*
25. Araújo C de S, Oliveira AP de, Lima RN, *et al*. Chemical constituents and antioxidant activity of the essential oil from leaves of *Annona vepretorum* Mart. (Annonaceae). Pharmacog Magaz 2015; 11:615. *https://doi.org/10.4103/0973-1296.160462*
26. Dutra LM, Costa EV, Moraes VR de S, *et al*. Chemical constituents from the leaves of *Annona pickelii* (Annonaceae). Biochem Syst Ecol 2012; 41:115–118. *https://doi.org/10.1016/j.bse.2011.12.011*
27. Bartley JP, Jacobs AL. Effects of drying on flavour compounds in Australian-grown ginger (*Zingiber officinale*). J Sci Food Agri 2000; 80:209–215. *https://doi.org/10.1002/(SICI)1097-0010(20000115)80:2<209::AID-JSFA516>3.0.CO;2-8*
28. Raghavan B, Abraham KO, Shankaranarayana ML, Koller WD. Studies on flavor changes during drying of dill (*Anethum sowa* Roxb.) leaves. J Food Qual 1994; 17:457–466. *https://doi.org/10.1111/j.1745-4557.1994.tb00166.x*
29. Fournier G, Leboeuf M, Cavé A. Annonaceae essential oils: A review. J Essent Oil Res 1999;11:131-142. *https://doi.org/10.1080/10412905.1999.9701092*
30. Turek C, Stintzing FC. Stability of essential oils: a review. Comp Rev Food Sci Food Safety 2013; 12:40–53. *https://doi.org/10.1111/1541-4337.12006*
31. Owolabi MS, Ogundajo AL, Dosoky NS, Setzer WN. The cytotoxic activity of *Annona muricata* leaf oil from Badagary, Nigeria. American J Essent Oil Natural Prod 2013; 1: 1-3.
32. Benckiser G, Schnell S. Biodiversity in agricultural production systems. Boca Raton, US: CRC Press, 2006. *https://doi.org/10.1201/b13577*
33. Thang TD, Dai DN, Hoi TM, Ogunwande IA. Study on the volatile oil contents of Annona glabra L., *Annona squamosa* L., *Annona muricata* L. and *Annona reticulata* L., from Vietnam. Nat Prod Res 2013; 27:1232–1236. *https://doi.org/10.1080/14786419.2012.724413*
34. Jirovetz L, Buchbauer G, Ngassoum MB. Essential oil compounds of the *Annona muricata* fresh fruit pulp from Cameroon. J Agri Food Chem 1998; 46:3719–3720. *https://doi.org/10.1021/jf980204n*
35. Drage S, Mitter B, Muchugi A, Jamnadass RH, Sessitsch A, Hadacek F. Antimicrobial drimane sesquiterpenes and their effect on endophyte communities in the medical tree Warburgia ugandensis. Front Microbiol 2014; 5:13. *https://doi.org/10.3389/fmicb.2014.00013*
36. Sieniawska E, Sawicki R, Golus J, *et al*. *Nigella damascena* L. Essential oil- A valuable source of β-elemene for antimicrobial testing. Molecules 2018; 23:256. *https://doi.org/10.3390/molecules23020256*
37. Tellez MR, Canel C, Rimando AM, Duke SO. Differential accumulation of isoprenoids in glanded and glandless *Artemisia annua* L. Phytochem1999; 52:1035-1040. *https://doi.org/10.1016/S0031-9422(99)00308-8*
38. Guillén MD, Cabo N, Burillo J. Characterisation of the essential oils of some cultivated aromatic plants of industrial interest. J Sci Food Agri 1996; 70:359–363. *https://doi.org/10.1002/(SICI)1097-0010(199603)70:3<359::AID-JSFA512>3.0.CO;2-0*
39. Xie Q, Li F, Fang L, Liu W, Gu C. The antitumor efficacy of β-elemene by changing tumor inflammatory environment and tumor microenvironment. BioMed Res Int 2020; 2020:e6892961. *https://doi.org/10.1155/2020/6892961*
40. Mariod AA, Abdelwahab SI, Elkheir S, *et al*. Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. Acta Sci Polon, Tech Alim 2012; 11:249-58.
41. Adesanwo JK, Akinloye AA, Otemuyiwa IO, Akinpelu DA. Chemical characteristics and biological activities of *Annona squamosa* fruit pod and seed extracts. J Explor Res Pharmacol 2021; 6(1):5-15. *https://doi.org/10.14218/JERP.2020.00019*
42. Gyesi JN, Opoku R, Borquaye LS. Chemical composition, total phenolic content, and antioxidant activities of the essential oils of the leaves and fruit pulp of *Annona muricata* L. (Soursop) from Ghana. Biochem Res Int 2019; 2019:1-9. *https://doi.org/10.1155/2019/4164576*
43. Vasyliev GS, Vorobyova VI, Linyucheva OV. Evaluation of reducing ability and antioxidant activity of fruit pomace extracts by spectrophotometric and electrochemical methods. J Analyt Methods Chem 2020; 2020:8869436. *https://doi.org/10.1155/2020/8869436*
44. El-Chaghaby GA, Ahmad AF, Ramis ES. Evaluation of the antioxidant and antibacterial properties of various solvents extracts of *Annona squamosa* L. leaves. Arabian J Chem 2014; 7:227–233. *https://doi.org/10.1016/j.arabjc.2011.06.019*
45. Tomar RS, Sisodia S. Estimation of phenolic content, total flavonoids and *in-vitro* antioxidant activity of Annona squamosa Linn. and *Bougainvillea glabra* Choisy. J Global Pharma Tech 2013; 5:11–14.
46. Chaves N, Santiago A, Alías JC. Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants 2020; 9:76. *https://doi.org/10.3390/antiox9010076*
47. Dudonné S, Vitrac X, Coutière P, Woillez M, Mérillon JM. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J Agri Food Chem 2009; 57:1768-1774. *https://doi.org/10.1021/jf803011r*