Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1326 - 1334
Received 15 April 2021; Accepted 05 May 2021.

Revealing the Active Compounds Caper Roots Using GC-MS and Evaluation of the Effectiveness of Antioxidants

Abeer H. Abed[1], Ali F Almehemdi[2*]
1- M.SC. postgraduate, college of pedagogy for pure science university of Anbar, Ramadi-IRAQ
2- Prof. Department of conservation agriculture, center of desert studies, university of Anbar, Ramadi-IRAQ
*E-MAIL: ds.dr.ali.fadaam@uoanbar.edu.iq. ORCID: 0000-0003-1840-1681.

Abstract:

In this study, the active ingredients and anti-oxidation activity were revealed in the roots of the caper plant (*Capparis spinose L.).* The active ingredients of ethanol and methanol extract of the roots of the Caper plant were characterized by GC-MS, which also revealed another eleven chemical active compounds for the methanol extract. The highest part of those compounds was N-Nonadecane with 32.96%, whereas the active compound 2-Methyltetracosane gave the lowest ratio at % 1.28. The ethanol extract gave the highest spreading two compounds, Heneicosanoic % 84.12 then Octacosane with %15.88. The anti-oxidation activity for roots of Caper was measured via ABTS and DPPH methods with various concentrations: 12, 25, 50, 100 and 200 µg ml^{-1}. Results show that methanol extract possesses a very high activity against the radicals ABTS and DPPH at 200 µg ml^{-1} concentration. The activity was 74.92 and it increases with the increase of the extract concentration.

Introduction:

The majority of natural plants are considered anti-oxidants by nature and it is produced from medical plants whether they are wild plants or domestically grown. They are less dangerous to humans' health than being artificial (Howes et al, 2020) the increase of anti-oxidant numbers against an array of microscopic creatures and their resistance to those anti-oxidants aroused a deep concern in the medical field. Therefore, demands increased swiftly to demand natural safe compound that can be used as nutrients in the first place and as a mean to guarantee protective measures and a solution for such a global issue, in addition to the worrying evolution of those microscopic creatures resisting the manufactured drugs (Garcia et al, 2020). Thus, the anti-oxidants are considered antibiotics that can protect the body from damages by the free oxidizing roots. They can multiply the oxidizing effort for the tissues indirectly and reinforce the natural cell defenses, or halting the functionality of free roots (Sharma et al, 2020). Among those plants is the Caper *(Capparis Spinosa L.).* It is a common shrubbery perennial plant known in some areas as the Wild Spinosa. It is known to be a part of the Capparaceae family. Their shrubs are wild or domestically grown. It is spread in various places around the world, especially in the Mediterranean Sea (Chedraoui et al., 2017). It has spreading branches some of which might

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1326 - 1334
Received 15 April 2021; Accepted 05 May 2021.

reach a four-meter length (Chedraoui et al., 2017). The Caper plant can be found in the countries of the Mediterranean Sea spanning the areas of the desert in North Africa as well as the dry areas in the mid and western of Asia (Tlili et al., 2010). The roots of Caper are used as a cure for digestive system disorders, earache, skin diseases, kidney and liver diseases (Mollica et al., 2017). The roots system was used in Iran in folk medicine as a diuretic and recreational, and a cure for inflammations, Malaria, joints disorders, and Cancer (Rana et al., 2012). Throughout the previous studies about the chemical compounds extracted from Caper and different plants, the backbone components are Alkaloids, fatty acids, Phenol, Flavonoids, Vitamins, and glycosides. It is also known that those components have a beneficial biological activity because they are antioxidants, anticancer, antimicrobials, and antiseptics (Tlili et al, 2015) polyphenol is one of the vital chemical components present in the fruits, leaves, roots, and flowers of the plants. Therefore, they were known in folk medicine to be a cure for many illnesses such as toothache and kidney failure. Thus, the purpose of this study is to reveal some of the active components in the methanol and ethanolic extract of roots and testing of the ant oxidization activity.

Materials and Methods:

Plant Material:

Caper plants were collected from the Kilo-35 area west of Ramadi, Al-Anbar in the western part of Iraq. Samples were treated in a lab, washed, and cleaned from dust; roots were separated and taken for extraction. The plants were examined by Dr. Ali F Almehemdi in the Desert Test Center. A sample of the plant was taken and documented by writing some information such as date of collection, the scientific name of the plant, family, and name of the collector. Then, it was saved for records.

Plant Extract:

The roots of Caper were dried and ground by a power grinder. Then, 50 gm of the powder was taken and mixed with 200 ml of alcoholic solvent (ethanol and methanol) separately at room temperature. After that, samples were placed in an Ultrasonic Bath for 20 minutes. The mixture was mixed later and filtered with a filtration paper with a porosity of 0.45 ul under vacuum. A condenser was used to increase the concentration of the mix. Finally, the mix was transferred into a dried and pre-weighted evaporating dish (Quintao et al, 2013).

Gas Chromatography / Mass Spectrometry Analysis:

The active compounds in the roots were identified with GC-2011 Plus equipped with a DB-5MS column. The length is 30m and the internal diameter is 0.25mm) the temperatures of the injector and the detector were set at 250 and 330 C respectively. The temperature of the oven started to climb at 100 C by 5 degrees per minute, whereas the temperature of the heat source of the system started to increase up to 260 C. Helium gas (99.99%) was used as a carrier. The mass spectrum was measured within the range of 50-550 amu and the ratios of the components were illustrated in a chart. The chart was drawn by a computer connected to the system, known as the absorbance chart. The Y-axis represents the strength of the signal, whereas the X-axis represents the retention time. The active components were identified by comparison with the stored library in

the computer linked to the (GC-MS) device.

Anti-oxidization Tests for Alcohol Extracts:

DPPH Assay:

DPPH is a light or dark purple solution that can stabilize the free radicals. It changes color to yellow when exposed to antioxidants. The characteristic is known in stabilizing DPPH radical through taking the hydrogens from the hydroxides and it is highlighted through removing the purple color for DPPH and changing it to the yellow color of (2.2 DPPH-H Diphenyl-1-Picrlhydrazyne) as a result of stabilizing it via DPPH assay protocol (Patel et al, 2011).

The activity of the extracts was estimated using the Visible light spectroscopy on a wavelength of 518 nm. The ratio was calculated via the following equation:

Ratio of sample activity = 100 X (Ab – EV / Ab sample – (Ab—ev))

Where:

Ab-ev: ratio of absorption of the control sample

Ab sample: ratio of absorption of the sample

ABTS Test:

ABTS is a mixed blue-green colored solution. It is produced as a result of losing an electron from a Nitrogen atom via Potassium Persulphate. That forms the radical (ABTS.+), which is absorbed in 743 nm wavelength. With antioxidants, this radical gives a hydrogen atom hence the color disappears from the solution (Ilyasov et al, 2020) ABTS test depends on measuring the ability of the antioxidant to eliminate the Radical ABTS.+ after reacting ATBS1 Potassium Persulphate K_2SO_5 out of (2.45nm in methanol as a 50% concentration. Then, the absorption is measured at a 734 wavelength. Also, Ascorbic Acid can be used as a comparison to our sample and the ratio is going to be calculated in s similar method as the DPPH.

Methodology:

A series of dilutions for alcohol extracts was made using methanol and ethanol 12,25,50,100,200 mg/ml. ABTS was diluted to give an absorption of about (0.02$\pm$ 0.7) at 734nm. 3 ml of the solution was added to 0.3 ml of each of the extract concentrations. The negatively controlled vials were prepared and contained the ABTS and Methanol: DMSO solutions. The prepared concentrations from plant extracts were then added as three replicas for each concentration. The positively controlled vials were prepared and contained the ABTS and Ascorbic Acid. The absorption was measured using UV-vis spectroscopy with a wavelength of 734 nm. The ratio was measured similarly to the aforementioned DPPH test (Ilyasov et al, 2020).

Statistical Analysis:

The analysis was carried out, charts were made by finding the Mean Standard Error and driving the analysis to one direction to evaluate the antioxidant, and relying on Sidak's multiple

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1326 - 1334
Received 15 April 2021; Accepted 05 May 2021.

comparison test.

Results:

GC-MS Analysis:

The results of the analysis for Methanol extract of Caper plant using GC-MS that the extract contains many active components. Depending on the position of the active component, peak area, retention time, molecular weight, the components were as follows: N-Nonadecane (%32.96), Heneicosane (%15.13), Oxalic isobutyl nonyl ester (%10.57), trichlorooctadecyl acid, Silane (%8.52), 9-Nonadecene (%8.26), deoxy-Celidoniol (%7.73), 2-octyl-1-Decanol (%4.47), Pentacosane (%3.07), dodecyl 2-propyl ester, Sulfurous acid (%1.51), and the component 2-Methyltetracosane gave the lowest ratio (%1.289). Results were demonstrated in table 1 and figure 1. On the other hand, the results of the ethanol for roots of Caper using GC-MS were demonstrated in table 2 and figure 2. The prominent compounds were Heneicosanoic (%84.12), then Octacosane (%15.88).

Table 1 retention times, molecular formula, molecular weights, qualities and areas of active compounds in methanolic extract of caper root using GC/MS

Peak	Compound	Rt. (min)	MF	MW g mol^{-1}	Qual.	Area%
1	Pentacosane	18.64	C_25H_{52}	352.68	78	3.07
2	1-Decanol, 2-octyl-	19.84	C18H38O	270.5	64	4.47
3	Oxalic acid, isobuty1 nony1 ester	20.81	$C_{15}H_{28}O_4$	272.38	72	10.57
4	Celidoniol, deoxy-	23.08	$C_{29}H_{60}$	424.79	86	7.73
5	2-Methyltetracosane	24.07	$C_{25}H_{52}$	352.68	72	1.28
6	Silane, trichlorooctadecyl-	25.24	$C_{18}H_{37}Cl_3Si$	387.9	90	8.52
7	9-Nonadecene	28.76	$C_{19}H_{38}$	266.5	96	8.26
8	N-Nonadecane	29.19	$C_{19}H_{40}$	268.5	97	32.96
9	Heptacosane	31.18	$C_{27}H_{56}$	380.7	78	2.81
10	Heneicosane	32.92	$C_{21}H_{44}$	296.6	97	15.13
11	Sulfurous acid, dodecyl 2-propy ester	34.68	$C_{21}H_{44}O_3S$	376.3	72	1.51

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1326 - 1334
Received 15 April 2021; Accepted 05 May 2021.

Table 2 retention times, molecular formula, molecular weights, qualities and areas of active compounds in ethanolic extract of caper root using GC/MS

Peak	Compound	Rt. (min)	MF	MW g mol^{-1}	Qual.	Area%
1	Octacosane	29.35	$C_{28}H_{58}$	394.8	72	15.88
2	Heneicosanoic	32.23	$C_{21}H_{42}O_2$	326.6	64	84.12

Anti-oxidization activity using DPPH:

Results illustrated in fig 1 and table 3 demonstrate that all extracts showed anti-oxidization activity with the highest level, 74.92 at 200 µg ml^{-1}, and the lowest value 14.93 at 12.5 µg $ml^{-1}$of concentration for methanol extract. The highest anti-oxidization activity of ethanol extract was 42.05 at 200 µg $ml^{-1}$and the lowest value was 17.63 at 12.5 µg $ml^{-1}$of concentration. The results also showed that methanol extract has a higher anti-oxidization activity against the radical DPPH at 200 µg $ml^{-1}$and other concentrations compared to ethanol extract. The highest DPPH IC50 activity for ethanol extract was 402.8 and the lowest was 74.3.

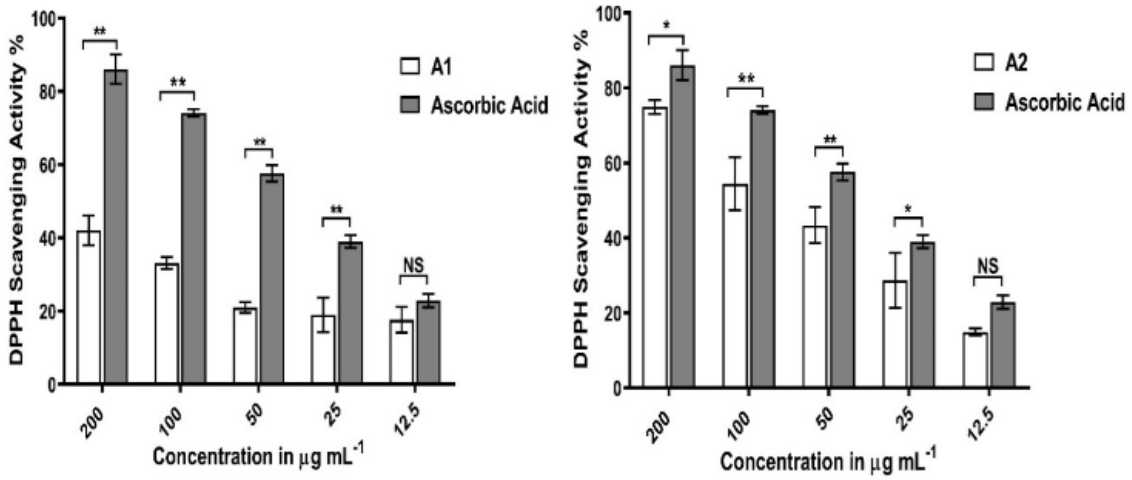

Fig 1 antioxidant potentiality of caper root ethanolic (A1) and methanolic (A2) extracts via DPPH

Table3 effect of caper root ethanolic and methanolic extracts on antioxidant potentiality via DPPH

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1326 - 1334
Received 15 April 2021; Accepted 05 May 2021.

Compound	Concentrations (µg ml^{-1}) (Mean±SE)					
	12.5	25	50	100	200	IC_{50}
Ascorbic Acid	22.90±1.06	39.00±1.00	57.60±1.27	74.07±0.58	86.03±2.33	36.09
Methanol	14.93±0.52	28.10±4.24	43.44±2.78	54.44±4.07	74.92±1.10	74.3
Ethanol	17.63±2.02	18.98±2.72	20.95±0.83	33.14±0.92	42.05±2.34	402.8

Anti-Oxidization Activity ABTS:

Results listed in table 4 and figure 2 show that the two alcohol extracts (Methanol and Ethanol) showed the highest anti-oxidization value (66.20) at 200 µg ml^{-1}, and the lowest value (14.93) at 12.5 µg ml^{-1}. On the other hand, ethanol extract values were at its best (31.25 at 200 µg ml^{-1} and the lowest value was (14.93) at 12.5 µg ml^{-1}. In addition, results for methanol extract showed higher activity compared to ethanol extract against the free radical ABTS at 200 µg ml^{-1} and other concentrations. Inhibition activity (IC_{ABTS}) achieved the highest value (145.4) with ethanol extract and the lowest (50.74) with methanol extract of caper plant.

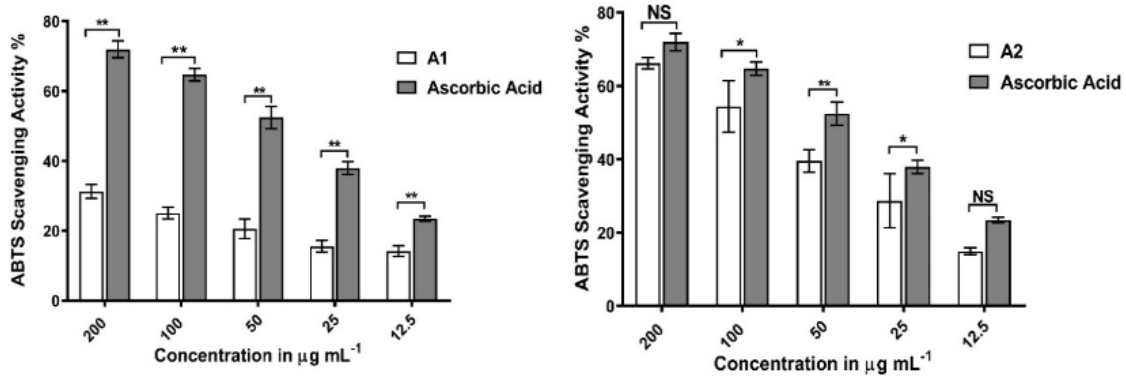

Fig 2 antioxidant potentiality of caper root ethanolic (A1) and methanolic (A2) extracts via ABTS

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1326 - 1334
Received 15 April 2021; Accepted 05 May 2021.

Table4 effect of caper root ethanolic and methanolic extracts on antioxidant potentiality via ABTS

Compound	Concentrations (µg ml^{-1}) (Mean±SE)					
	12.5	25	50	100	200	IC_{50}
Ascorbic Acid	23.43±0.433	37.93±1.04	52.43±1.81	64.70±1.04	71.97±1.37	25.22
Methanol	14.93±0.52	28.70±4.24	39.58±1.78	54.44±4.07	66.20±0.87	50.74
Ethanol	14.16±0.89	15.51±0.97	20.56±1.59	25.04±0.97	31.25±1.14	145.4

Discussion:

GC-MS Analysis:

Results of GC-MS analysis for methanol extract of the roots of caper showed that the extract contains 11 active compounds: N-Nonadecane, Heneicosane, Isobutyl nonyl ester Silane, Oxalic Acid, Trichlorooctadecyl, Nonadecene-9, Celidoniol, deoxy, Decanol, Octyl-1, Pentacosane, dodecyl 2-propyl ester, Sulfurous acid, and 2-Methyltetracosane. On the other hand, the results of the analysis for ethanol extract showed two components, Heneicosanoic and Octacosane. Both components show a large concentration and spreading area. Those components are considered natural pesticides and antibiotics (Caboni, 2012). The components Oxalic acid, Isobutyl nonyl ester, Heneicosane, and Octacosane are also considered natural pesticides, anti-oxidants, and antibiotics (Al-Snafi, 2020). Methanol extract components have high activity in eliminating cancerous cells (Alazzam et al, 2020).

Anti- oxidization activity DPPH:

Methanol extract has a higher anti-oxidization activity against the radical DPPH compared to ethanol activity at 200 µg ml^{-1} and other concentrations. The study shows that the anti-oxidization activity for the roots extracts using DPPH increases with the increase of concentration. This is called the Dose-Dependent Pattern, i.e the activity increases with the increase of the concentration of the extract. This agrees with other conclusions, (Allaith, 2016) when he studied the methanol, water, hexane extracts for Caper, and (Okur et al, 2020) when he studied anti-oxidization activity for methanol extract and increasing the activity when the concentration increases up to %10. Also, (El-Hady et al, 2021) showed that anti-oxidization activity for methanol extract increases with the increase of concentration at 0.5 and 1 then stabilizes with the increase, which is contrary to our demonstrated results.

Anti-Oxidization Activity ABTS:

Table 3 and figure 3 showed that methanol extract has higher anti-oxidization activity compared to ethanol extract against the radical ABTS at 200 µg ml^{-1} and other concentrations, 100, 50,25, 12.5 µg ml^{-1}. The study shows that anti-oxidization activity increases with the increase of extract. This comes in agreement with (Journal et al, 2021) when they studied the leaves of *Launae glomerata.* (Hematian et al, 2020) studied the ethanol extract for the leaves and fruit of the caper plant where the increase of the concentration of the extract at 100 µg ml^{-1} increased the anti-oxidization activity as well.

References

1. Al-Snafi, Ali Esmail. 2020. Phenolics and Flavonoids Contents of Medicinal Plants, as Natural Ingredients for Many Therapeutic Purposes-A Review. *IOSR Journal Of Pharmacy* 10(July): 42–81. www.iosrphr.org.
2. Alazzam, S. A., Sharqi, M. M., & Almehemdi, A. F. (2020). Phytochemical analysis, antioxidant and cytotoxic potential of *Rumex vesicarius* extracts. *Medico-Legal Update*, *20*(2), 449–454. doi: 10.37506/mlu.v20i2.1146
3. Allaith, Abdul Ameer A. 2016. Assessment of the Antioxidant Properties of the Caper Fruit (*Capparis Spinosa* L.) from Bahrain. *Journal of the Association of Arab Universities for Basic and Applied Sciences* 19: 1–7. http://dx.doi.org/10.1016/j.jaubas.2014.07.001.
4. Caboni, Pierluigi, Sarais, G., Aissani, N., Tocco, N., Liori, B., Carta, A. & Angioni, A. 2012. Nematicidal Activity of 2-Thiophenecarboxaldehyde and Methylisothiocyanate from Caper (*Capparis Spinosa*) against Meloidogyne Incognita. *Journal of Agricultural and Food Chemistry* 60(30): 7345–51.
5. Chedraoui, S., Abi-Rizk, A., El-Beyrouthy, M., Chalak, L., Ouaini, N., & Rajjou, L.2017. *Capparis Spinosa* L. in A Systematic Review: A Xerophilous Species of Multi Values and Promising Potentialities for Agrosystems under the Threat of Global Warming. *Frontiers in Plant Science* 8(October): 1–18.
6. El-Hady, N. A. El-Sayed, A.I., El-Saadany, S.S., Deligios, P.A., & Ledda, L. 2021. "Exogenous Application of Foliar Salicylic Acid and Propolis Enhances Antioxidant Defenses and Growth Parameters in Tomato Plants." *Plants* 10(1): 1–13.
7. Fici, Silvio. 2015. A Taxonomic Revision of the *Capparis Spinosa* Group (Capparaceae) from Eastern Africa to Oceania. *Phytotaxa* 203(1): 24–36.
8. Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., & Terekhov, R. P. (2020). ABTS/PP decolorization assay of antioxidant capacity reaction pathways. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21031131
9. Garcia, J., Afonso, A., Fernandes, C., Nunes, F.M., Marques, G., & Saavedra, M.J. 2020. Comparative Antioxidant and Antimicrobial Properties of *Lentinula* Edodes Donko and Koshin Varieties against Priority Multidrug-Resistant Pathogens. *South African Journal of Chemical Engineering* (October).
10. Harb, Amal M., and Jamil N. Lahham. 2020. Cellulose Content in Selected Plant Species along the Dead Sea Coast and the Southern Desert of Jordan. *Jordan Journal of Biological Sciences* 13(3): 343–47.
11. Howes, M. R., Quave, C.L., Collemare, J., Tatsis, E.C., Twilley, D., Lulekal, E., Farlow, A., Li, L., Cazar, M., Leaman, D.J., Prescott, T.A.K., Milliken, W., Martin, C., De

Canha, M.N., Lall, N., Qin, H., Walker, B.E., Vasquez-Londono, C., Alkin, B.[….] and Nic Lughadha, E. 2020. Molecules from Nature: Reconciling Biodiversity Conservation and Global Healthcare Imperatives for Sustainable Use of Medicinal Plants and Fungi. *Plants, People, Planet* 2(5): 463–81.

12. Ilyasov, Igor R., Vladimir L. Beloborodov, Irina A. Selivanova, and Roman P. Terekhov. 2020. ABTS/PP Decolorization Assay of Antioxidant Capacity Reaction Pathways. *International Journal of Molecular Sciences* 21(3).
13. Mollica, A., Zengin, G., Locatelli, M., Stefanucci, A., Mocan, A., Macedonio, G., Carradori, S., Onaaolapo, O., Onaolapo, A., Adegoke, J., Olaniyan, M., Aktumsek, A. & Novellino, E. 2017. Anti-Diabetic and Anti-Hyperlipidemic Properties of *Capparis spinosa* L.: In Vivo and in Vitro Evaluation of Its Nutraceutical Potential. *Journal of Functional Foods* 35: 32–42. http://dx.doi.org/10.1016/j.jff.2017.05.001.
14. Okur, M. E., Karadag, A.E., Ozhan, Y., Sipahi, H., Ayla, S., Daylan, B., Kultur, S.,& Demirci, F. 2021. Anti-Inflammatory, Analgesic and in Vivo-in Vitro Wound Healing Potential of the *Phlomis rigida* Labill. Extract. *Journal of Ethnopharmacology* 266.
15. Patel, M. and Patel, J.V.. 2011. In Vitro Antioxidant Activity of Coumarin Compounds by DPPH, Super Oxide and Nitric Oxide Free Radical Scavenging Methods. *Journal of advanced pharmacy education & research* 1: 52–68.
16. Quintão, F. J.O., Tavares, R.S.N., Vieira-Filho, S.A., Souza, G.H.B., & Santos, O.D.H. 2013. Hydroalcoholic Extracts of *Vellozia squamata*: Study of Its Nanoemulsions for Pharmaceutical or Cosmetic Applications. *Brazilian Journal of Pharmacognosy* 23(1): 101–7. http://dx.doi.org/10.1590/S0102-695X2013005000001.
17. Rahnavard, R. and Nastaran R. 2016. A Review on the Medical Effects of *Capparis spinosa*. *Advanced herbal medicine* 2(1): 44–53. http://herbmed.skums.ac.ir/article_20857_0.html.
18. Rana, J. C., Pradheep, K., Chaurasia, O.P., Sood, S., Sharma, R.M., Singh, A.,& Negi R. 2012. Genetic Resources of Wild Edible Plants and Their Uses among Tribal Communities of Cold Arid Region of India. *Genetic Resources and Crop Evolution* 59(1): 135–49.
19. Sharma, Kanika, Sanjay Guleria, Vijay K. Razdan, and Vikash Babu. 2020. Synergistic Antioxidant and Antimicrobial Activities of Essential Oils of Some Selected Medicinal Plants in Combination and with Synthetic Compounds. *Industrial Crops and Products* 154(May): 112569. https://doi.org/10.1016/j.indcrop.2020.112569.
20. Tlili, N., Mejri, H., Aouer, F., Saadaoui, E., Khaldi, A. & Nasri, N. 2015. Phenolic Profile and Antioxidant Activity of *Capparis Spinosa* Seeds Harvested from Different Wild Habitats. *Industrial Crops and Products* 76: 930–35. http://dx.doi.org/10.1016/j.indcrop.2015.07.040.
21. Tlili, Nizar, Abdelhamid Khaldi, Saida Triki, and Sergi Munné-Bosch. 2010. Phenolic Compounds and Vitamin Antioxidants of Caper (*Capparis Spinosa*). *Plant Foods for Human Nutrition* 65(3): 260–65.