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Research Article

Anti-SARS-CoV-2 and Antioxidant Activities of The Methanolic Extract and Its Fractions of *Marrubium alysson* L. Aerial Parts

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ABSTRACT

The pandemic novel coronavirus disease 2019 (COVID-19) has resulted in enormous economic damage and an exclusive health crisis, so developing a cure for this respiratory infectious disease has proceeded forward at full speed. Natural products and herbal medicines with accepted safety profiles are considered promising sources for discovering new medication leads. Genus *Marrubium* was reported to exhibit potential antiviral and antioxidant activities. Thus, this study aims to evaluate the inhibitory activity of the total methanolic extract and its soluble fractions: (petroleum ether, methylene chloride, and ethyl acetate) of the aerial parts of *Marrubium alysson* L. against SARS-CoV-2 main protease (M^{pro}). Also, they were quantitatively assayed for their phenolics and flavonoids contents, in addition to evaluating their antioxidant activity. The results showed that the petroleum ether fraction has the highest inhibitory activity against SARS-CoV-2 main protease (M^{pro}) enzyme with IC_{50} value of 3.959±0.17 µg/mL compared to the reference tipranavir (IC_{50} 1.89±0.08 µg/mL). The ethyl acetate fraction showed the highest total phenolics and flavonoids contents as well as the highest antioxidant activity (IC_{50} 33.81±0.15 µg/mL) compared to the reference L-ascorbic acid (IC_{50} 30.43±0.14 µg/mL). It could be concluded that soluble fractions of the total methanolic extract of *Marrubium alysson* L. may be used as an alternative source for developing new natural inhibitors against the main protease enzyme and may be suggested for the treatment of COVID-19.

Keywords: *Marrubium alysson* L., Anti-SARS-CoV-2, ABTS antioxidant assay, total phenolics, flavonoids.

1. INTRODUCTION

Marrubium alysson L. belongs to Genus *Marrubium* (family Lamiaceae). The Genus *Marrubium* is a genus of flowering plants, which contains about 40 species. It is located mainly in the temperate regions of the Eurasian Continent and along the Mediterranean Sea.[1] *Marrubium* species as *Marrubium vulgare* has a long history of being used in folk medicine for conditions like fever, skin injuries, inflammatory processes, diseases of the digestive, respiratory, and kidney systems, and as a hepatoprotective.[2-4] Plants belonging to the *genus Marrubium* are famous for their contents of labdane diterpenes, phenylpropanoids, flavonoids, and essential oils.[5-11] *Marrubium alysson* L. plant is a perennial herb with round, cuneate-base, coarsely crenate and fleece-covered leaves, pink or bluish corolla, and fruit-calyx with five spreading star-shaped spines.[12] Previous studies reported that its extracts had anti-inflammatory, antioxidant, analgesic, hypoglycaemic, and antimicrobial activities.[13-15]

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Coronavirus 2 (SARS-CoV-2), a novel strain of the coronavirus family that causes severe acute respiratory syndrome, was the cause of the COVID-19 sickness. It is extremely infectious, pathogenic and has spread rapidly throughout the world, so the World Health Organization (WHO) designated it a pandemic on March 11, 2020. Despite the fact that over hundreds of clinical trials and preclinical studies have been conducted to find treatments against COVID-19, up to date, there is no effective treatment for this disease.[16,17] The SARS-CoV-2 main protease (M^{pro}) enzyme, also known as the chymotrypsin-like protease 3CLpro, is responsible for the release of vital functional peptides during proteolysis, which is crucial to coronavirus replication and the subsequent progression of the virus life cycle.[18] It has been recognized as a significant target for treatment approaches.

Phenolic compounds and flavonoids are considered effective antioxidants owing to their ability to neutralize the free radicals that cause oxidative stress.[19-21] Although synthetic anti-oxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very efficient, they also have several negative side effects, including the development of tumors when used at high dosages and over an extended period of time. As a result, there is growing interest in utilizing naturally occurring antioxidants instead of synthetic antioxidants in food, cosmetic, and pharmaceutical goods.[22]

Plant-derived natural products with therapeutic relevance have recently gained a lot of interest in exploring highly effective natural drugs for several diseases because these products are available and relatively safe. It was reported that several species of *Marrubium*, such as *M. peregrinum* and *M. deserti* showed antiviral activity against different viruses, including coronaviruses.[17,23,24] This encouraged us to evaluate *Marrubium alysson* L. plant growing in Egypt as anti-COVID-19.

This study evaluates the antioxidant and anti-SARS-CoV-2 activities of the total methanolic extract and its soluble fractions of *Marrubium alysson* L. aerial parts to discover potential antioxidant and anti-COVID-19 therapeutics. Moreover, their total phenolics and total flavonoid contents were quantitatively estimated.

It is worth noting that this study is the first one to report the inhibitory activity of the total methanolic extract and its soluble fractions of *Marrubium alysson* L. aerial parts against the SARS-CoV-2 main protease (M^{pro}) enzyme.

2. MATERIAL AND METHODS

2.1. Apparatus and Reagents

Rotary flash evaporator (Büchi, Switzerland), Spekol 11 (Carl Zeiss-Jena) spectrophotometer, ELx800UV (USA), and a fluorescent microtiter plate reader. Folin-Ciocalteu reagent, aluminium chloride reagent, Gallic acid (Sigma, 98%), Quercetin (Sigma, 95%), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) reagent. Inhibition of M^{pro} enzyme activity was measured using the Fluorogenic M^{pro} Assay Kit (Catalog #79955-1); the kit includes purified main protease and is available in a convenient 96-well test plate, main protease assay buffer and fluorogenic substrate.

2.2. Plant material

The aerial parts of *Marrubium alysson* L. were collected from North Coast at the Mediterranean coastal strip, Egypt, in April 2019. The plant was authenticated by Prof. Ibrahim Mashaly, Professor of Ecology, Faculty of Science, Mansoura University, Egypt. Voucher specimens of the plant have been kept in the Pharmacognosy Department of the Faculty of Pharmacy at Mansoura University in Egypt and were coded as MA-2-2019.

2.3. Preparation of the extracts:

The aerial parts of *Marrubium alysson* L. were ground into a fine powder after being air-dried at room temperature in the shade. Extraction was done by macerating the powdered, air-dried aerial parts (2 Kg) in 70% methanol at room temperature (7 x 3.5 L). The dark green viscous residue (220 g) was produced after the collected methanolic extract was evaporated under reduced pressure and allowed to dry in a desiccator over anhydrous $CaCl_2$. The dried methanolic residue (200 g) was dissolved in the least amount of methanol, diluted with a suitable volume of distilled water, and fractionated with petroleum ether 60/80, methylene chloride, and ethyl acetate. Each time, the solvent was dried under reduced pressure, resulting in petroleum ether soluble fraction (35.34 g), methylene chloride soluble fraction (30.23 g), ethyl acetate soluble fraction (13.47 g), and aqueous fraction (111.24 g).

2.4. Biological activities

2.4.1. Fluorogenic substrate assay

Based on the fluorogenic substrate assay,[25,26] the SARS-CoV-2 main protease (M^{pro} or $3CL^{pro}$) enzyme's inhibiting activity has been examined. The principle based on the peptide substrate's C-terminal is connected to a fluorophore (Edans), and a peptide substrate's N-terminal fluorescence quencher (Dabcyl) inhibits the fluorescence signal of Edans, The peptide substrate has low fluorescence as the Dabcyl in the N-

terminal quenching the intensity of Edans' fluorescence in the C-terminal. The substrate was hydrolyzed by the M^{pro}, yielding the non-fluorescent Dabcyl fragment and the intensely florescent Edans fragment. As a result, the fluorescence signal has increased in proportion to the protease activity. The release of fluorescent fragments is inhibited by the main protease (M^{pro}) inhibitor, which reduces the intensity of the fluorescent signal. A fluorescent microtiter plate reader that can read excitation/emission at 360/460 nm is used to measure the intensity of the fluorescence.

2.4.2. Total phenolics determination assay

Using gallic acid as a standard, total phenolics were determined using Folin-Ciocalteu colorimetric method.[27] One milligram of the total methanolic extract and its soluble fractions were dissolved in 1 mL of MeOH, then 4 mL of sodium carbonate (7% *w/v*) were added and mixed completely with 5 mL of Folin-Ciocalteau regent (10% *v/v*), the blue mixture was kept for 30 minutes in a water bath at 40^0C. Then, against a blank (1 mL of methanol was mixed with 4 mL of sodium carbonate (7% *w/v*) and 5 mL of Folin-Ciocalteau regent (10% *v/v*) to create a final volume of 10 mL), the absorbance at 760 nm was measured, every measurement was made in triplicate. The absorbance values at various gallic acid concentrations from (0-1 mg/mL) that were previously prepared in the same manner for the quantitative estimation of phenolics content were used to plot the calibration curve. Phenolics content was expressed as milligrams per gram of gallic acid equivalent (GAE).

2.4.3. Total flavonoids determination assay

Using quercetin as a reference substance, the total flavonoids content of each extract was calculated spectrophotometrically by the aluminum chloride method.[28] This technique is based on the presence of a yellow compound made of flavonoids-aluminum that have absorptivity at around λ 415 nm. 10 mg of the total methanolic extract and its fractions was dissolved in 1 mL of ethanol, then 100 µL of plant extract in ethanol was combined with 100 µL of 20% $AlCl_3$ in ethanol and 50 µL of acetic acid, then diluted with ethanol to 5.0 mL, the intensity of the formed yellow color was estimated at 415 nm and were measured after 40 min. Against control samples (100 µL of plant extract and 50 µL of acetic acid, then diluted with ethanol to 5.0 mL), under the same circumstances, the absorption of a standard quercetin solution of different concentrations from (0-1 mg/mL) in ethanol was evaluated, every measurement was made in triplicate, and a standard curve was produced**.** Flavonoids content was expressed as milligrams per gram of quercetin equivalent (QE).

2.4.4. Free radical scavenging activity (ABTS anti-oxidant assay)

Determination of ABTS Radical Scavenging Activity was carried out as reported.[29,30] Preparing the $ABTS^{\bullet+}$ radical cation (blue-dark green) by adding equal amounts of ABTS (2,2`-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) stock solution (colorless; 7 mM in pure distilled H_2O) and potassium persulfate stock solution ($K_2S_2O_8$; 3.5 mM in pure distilled H_2O), the mixture was kept and left to stand in the dark at room temperature overnight (for 12–16 hours) till the reaction was completed, then the $ABTS^{\bullet+}$ working solution was prepared by diluting the $ABTS^{\bullet+}$ stock solution in pure EtOH until it had an absorbance (A_{blank}) of 0.7±0.02 at a wavelength of 734 nm. The solution was then equilibrated with a temperature control set at 30˚C in an incubator (A_{blank}).

The free radical scavenging activity was evaluated by combining 1.5 mL of the blue-green $ABTS^{\bullet+}$ working solution with varied concentrations ranging from 10 to 100 µg (in distilled H_2O, pure EtOH, or a mixture of both of them according to the solubility) of all test extract and its fractions. After the mixing, the change in absorbance at 734 nm for each test extract (A_{test}) was taken after 15 minutes of their mixing because the steady state was reached after that time in the current experiment. Values are the averages of three separate calculations (as all the measurements were taken three times). The following equation was used to compute the percent decrease in absorbance, which represents the test extract's ability to scavenge $ABTS^{\bullet+}$ radical cations:

$$\% \text{ inhibition} = 100\ (A_{blank}-A_{test})/A_{blank}.$$

After 15 minutes of reaction, the inhibitory concentration (IC_{50}) of the total methanolic extract and its fractions were calculated and compared to that of L-ascorbic acid (taken as the reference and standard antioxidant compound in this assay). The IC_{50} is the concentration of any test extract required to reduce the absorption or amount of $ABTS^{\bullet+}$ radical cations by 50% at a wavelength of 734 nm. Using the GraphPad Prism 6 software (U.S.A., 2015), the antioxidant or anti-$ABTS^{\bullet+}$ IC_{50} value (for each test compound and the reference L-ascorbic acid) was computed. The lower the IC_{50} value, the more potent the test extract as an antioxidant is (i.e., the stronger the antioxidant activity of the test extract is).

3. RESULTS AND DISCUSSION OF BIOLOGICAL ACTIVITIES

3.1. Anti-SARS-COV-2 activity (main protease (M^{pro}) inhibitory activity)

COVID-19 is regarded as a severe public health danger throughout the world as it is an extremely contagious disease, pathogenic, and can spread swiftly. Different strains of this virus have evolved over the past years, leading to increased transmissibility and severity of the disease. Since there are no specific medications to treat COVID-19, it has become vital to develop and produce anti-COVID-19 medications. Natural products and their derivatives have been reliable sources to cure a wide range of illnesses for a very long time in human history.[31]

Therefore, the evaluation of anti-SARS-CoV-2 activity is very important. The anti-SARS-CoV-2 of the total methanolic extract and its fractions of the aerial parts of *Marrubium alysson* L. were examined. The total methanolic extract exhibited weak inhibitory activity against SARS-CoV-2 main protease (M^{pro}) enzyme (IC_{50} 73.89±3.14 µg/mL) in comparison with the reference antiviral compound Tipranavir (IC_{50} 1.89±0.08 µg/mL), which may be attributed to the antagonistic effects of different classes of components as terpenes, flavonoids and phenylpropanoids with each other. However, the petroleum ether fraction demonstrated strong inhibitory activity against SARS-CoV-2 main protease (M^{pro}) enzyme (IC_{50} (3.959±0.17 µg/mL), which may be attributed to the synergistic effect of its components as fatty acids, sterol, and terpenes. Moreover, the methylene chloride and ethyl acetate fractions showed moderate inhibitory activity against SARS-CoV-2 main protease (M^{pro}) enzyme with IC_{50} (11.62±0.49, and 16.1±0.68 µg/mL, respectively) (Table 1). Our findings shed light on the potential use of the *Marrubium alysson* L. plant as a green source of M^{pro} inhibitors.

Table 1: Results of the M^{pro} inhibitory activity of the total methanolic extract of the aerial parts of *Marrubium alysson* L. and its fractions compared with the standard Tipranavir.

Extract/fractions	In vitro COV-M pro IC_{50} (µg/mL)±S.D
Tipranavir (Standard)	1.89±0.08
Methanolic extract	73.89±3.14
Petroleum ether fraction	3.959±0.17
Methylene fraction	11.62±0.49
Ethyl acetate fraction	16.1±0.68

*Tipranavir is used as standard with IC_{50} 1.89±0.08 µg/mL.

3.2. Total phenolics determination assay

Quantitative analysis by UV-visible spectrophotometer after establishing calibration curves with different concentrations of gallic acid. Results shown in (Table 2) revealed that the total methanolic extract of aerial parts of *Marrubium alysson* L. possessed moderate phenolics content. The fractions' results revealed that the ethyl acetate fraction possessed the highest total phenolics content; 226.7 mg /g (GAE), followed by the methylene chloride fraction; 90.5 mg /g (GAE). Finally, petroleum ether fraction possessed the lowest phenolics content, 45.59 mg /g (GAE) (Figure 1, Table 2).

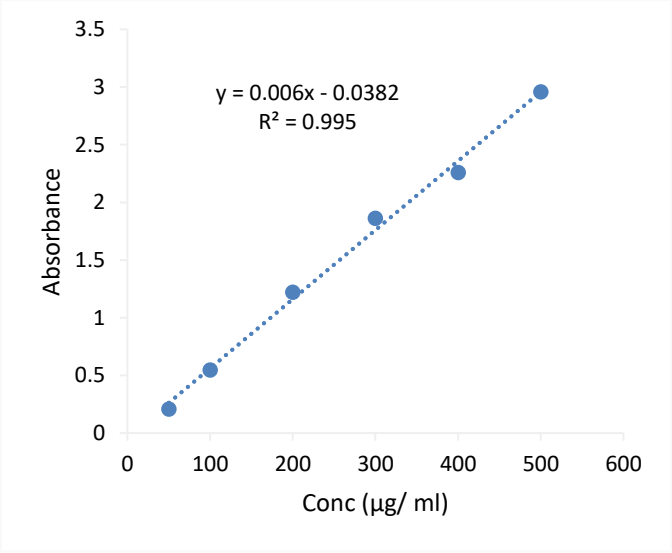

Figure 1: Calibration curve of gallic acid.

Table 2: The total phenolics contents of total methanolic extract of the aerial parts of *M. alysson* L. and its fractions.

Extract/fractions	mg /g (GAE)±S.D
Methanolic extract	73.36±1.73
Petroleum ether	45.59±1.50
Methylene chloride	90.50±1.74
Ethyl acetate	226.70±4.23

3.3. Total flavonoids determination assay

Quantitative analysis by UV-visible spectrophotometer after establishing the calibration curve with different concentrations of quercetin. Results are shown in (Table 3) which revealed that the total methanolic extract of the aerial parts of *Marrubium alysson* L. possessed moderate flavonoid content. The fractions' results revealed that the ethyl acetate fraction possessed the highest total flavonoid content; 316.80 mg /g (QE), followed by the methylene chloride fraction; 200.5 mg /g (QE). The Petroleum ether fraction possessed the lowest flavonoid content, 79.54 mg /g (QE) (Figure 2, Table 3).

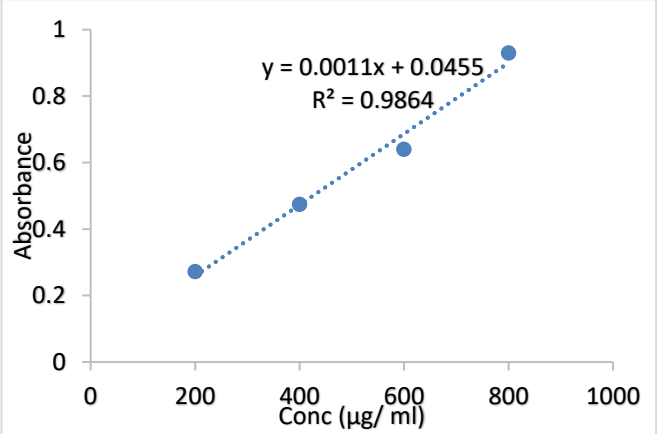

Figure 2: Calibration curve of quercetin

Table 3: The total flavonoid content of the total methanolic extract of the aerial parts of *M. alysson* L. and its fractions.

Extract/fractions	mg /g (QE)±S.D
Methanolic extract	211.40±1.48
Petroleum ether	79.54±1.31
Methylene chloride	200.50±2.52
Ethyl acetate	316.80±4.78

3.4. Free radical scavenging activity (ABTS antioxidant assay)

Numerous antioxidant defense mechanisms in the body provide resistance against free radicals. As a result, oxidative stress results from an imbalance between the body's antioxidant defense system and reactive oxygen species (ROS) production.[32] Polyphenolic compounds and flavonoids in plants are responsible for their antioxidant activity.[19-21] Polyphenols have the ability to scavenge reactive oxygen species that can seriously harm a person's health as these reactive oxygen species play a role in many different diseases, such as cancer, atherosclerosis, and cardiovascular disorders.[33] Therefore, the evaluation of antioxidant activity is very important.

The antioxidant activity of the total methanolic extract of the aerial parts of *Marrubium alysson* L. and its fractions were evaluated using the ABTS method. Results shown in (Table 4) revealed that the ethyl acetate fraction had the greatest radical scavenging activity among the other examined fractions with an IC_{50} value of 33.81±0.15 µg/mL compared to standard ascorbic acid with an IC_{50} value of 30.43±0.14. This could be explained by the ethyl acetate fraction's high phenolics content and high flavonoid content, which are crucial to the observed antioxidant activity.[32] It was followed by total methanolic extract with IC_{50} 79.17±0.35, the methylene chloride fraction and petroleum ether fraction that showed almost the same IC_{50} value 82.48±0.44 µg/mL, 84.44±0.42 µg/mL respectively that considered moderate radical scavenging activity compared to standard ascorbic acid (Table 4).

Table 4: Antioxidant activity (IC_{50}, µg/mL) of total methanolic extract and its fractions of *Marrubium alysson* L. using ABTS antioxidant assay.

Extract/ Fractions	ABTS IC_{50} (µg/mL)±S.D
Ascorbic acid* (Standard)	30.43±0.14
Total methanolic	79.17±0.35
Petroleum ether	84.44±0.42
Methylene chloride	82.48±0.44
Ethyl acetate	33.81±0.15

*Ascorbic acid (ABTS) is used as a standard antioxidant with IC50 30.43±0.14 µg/mL.

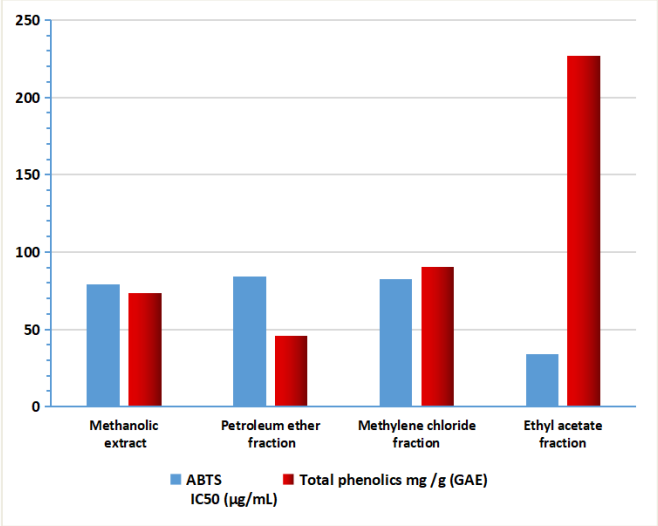

Figure 3: The relation between the ABTS antioxidant activity and total phenolics content of the different extracts of the aerial parts of *Marrubium alysson* L.

From the results listed in (Tables 2,4) and (Figure 3), we can conclude the following:

The antioxidant activity of the total methanolic extract and its fractions is mainly attributed to their phenolics content[20,24], e.g., the ethyl acetate extract had the highest phenolics content and, subsequently, the lowest IC_{50}, which means the highest antioxidant activity. We can conclude that the phenolics content plays a great role in the antioxidant activity, but the antioxidant activity is not restricted to phenolics compounds and may be related to other types of

compounds; the evidence for that is petroleum ether fraction showed moderate antioxidant activity while their phenolics content was very low.

4. CONCLUSION:

Evaluation of the anti-SARS-CoV-2 and antioxidant activities of the total methanolic extract and different soluble fractions of *Marrubium alysson* L. aerial parts were carried out in order to find and explore highly effective natural main protease inhibitors and antioxidant agents. This study indicated that the petroleum ether fraction had the highest inhibitory activity against SARS-CoV-2 main protease (M^{pro}). The ethyl acetate fraction with the highest level of total phenolics and flavonoids showed the highest antioxidant activity. Our result was a preliminary screening for using *Marrubium alysson* L. plant as an anti-COVID-19 and antioxidant drug, and further phytochemical investigation may be required.

CONFLICT OF INTEREST:

The authors confirm that the article content has no conflicts of interest.

5. REFERENCES

1. Mabberley, D. J. (1987). The Plant Book. Cambridge: Cambridge University Press.
2. Schlemper V, Ribas A, Nicolau M, Cechinel Filho V. Antispasmodic effects of hydroalcoholic extract of *Marrubium vulgare* on isolated tissues. *Phytomedicine*. 1996;3(2):211-216. doi:10.1016/S0944-7113(96)80038-9.
3. de Souza MM, de Jesus RAP, Cechinel-Filho V, Schlemper V. Analgesic profile of hydroalcoholic extract obtained from *Marrubium vulgare*. *Phytomedicine*. 1998;5(2):103-107. doi:10.1016/S0944-7113(98)80005-6.
4. Asadi-Samani M, Kafash-Farkhad N, Azimi N, Fasihi A, Alinia-Ahandani E, Rafieian-Kopaei M. Medicinal plants with hepatoprotective activity in Iranian folk medicine. *Asian Pac J Trop Biomed*. 2015;5(2):146-157. doi:10.1016/S2221-1691(15)30159-3.
5. Savona G, Piozzi F, Aránguez LM, Rodríguez B. Diterpenes from *Marrubium sericeum*, *Marrubium supinum* and *Marrubium alysson*. Phytochemistry. 1979;18(5):859-860. doi:10.1016/0031-9422(79)80029-1.
6. Caliş I, Hosny M, Khalifa T, Rüedi P. Phenylpropanoid glycosides from *Marrubium alysson*. *Phytochemistry*. 1992;31(10):3624-3626. doi:10.1016/0031-9422(92)83740-p.
7. Karioti A, Skaltsa H, Heilmann J, Sticher O. Acylated flavonoid and phenylethanoid glycosides from *Marrubium velutinum*. *Phytochemistry*. 2003;64(2):655-660. doi:10.1016/s0031-9422(03)00242-5.
8. Karioti A, Heilmann J, Skaltsa H. Labdane diterpenes from *Marrubium velutinum* and *Marrubium cylleneum*. *Phytochemistry*. 2005;66(9):1060-1066. doi:10.1016/j.phytochem.2005.02.029.
9. Lazari DM, Skaltsa HD, Constantinidis T. Essential oils of *Marrubium velutinum* Sm. and *Marrubium peregrinum* L., growing wild in Greece. *Flavour Fragr J*. 1999;14(5):290-292. doi:10.1002/(SICI)1099-1026(199909/10)14:5.
10. Golmakani H, Rabbani Nasab H, Sharifan M, Kamali H, Yadollahi A. The Essential Oil Composition and Antibacterial Activity of *Marrubium duabense* Murata from North Khorassan Province, Iran. *J Essent Oil Bear Plants*. 2016;19(4):963-971. doi:10.1080/0972060X.2014.998722.
11. Elbali W, Djouahri A, Djerrad Z, et al. Chemical variability and biological activities of *Marrubium vulgare* L. essential oil, depending on geographic variation and environmental factors. *J Essent oil Res*. 2018;30(6):470-487. doi:10.1080/10412905.2018.1493405. doi:10.1080/10412905.2018.1493405.
12. Täckholm, V. (1974) Students' Flora of Egypt. 2nd Edition, Cairo University Publishing, Beirut, 888.
13. Essawy SS, Abo-Elmatty DM, Ghazy NM, Badr JM, Sterner O. Antioxidant and anti-inflammatory effects of *Marrubium alysson* extracts in high cholesterol-fed rabbits. *Saudi Pharm J*. 2014;22(5):472-482. doi:10.1016/j.jsps.2013.12.004.
14. Alaa R, Abd-Alhaseeb MM, Habib ES, Ibrahim AK, Ahmed SA. Screening of *Marrubium alysson* L. extract for pharmacological activity. *J Chem Pharm Res*. 2016;8(4):283-289.
15. Ghazy, M.N. & Bamane, H.F. & Moneib, A.N. & Badr, Jihan. Investigation of biological activities of different extracts of *Marrubium alysson* L. *J Pharm Res*. 2011;1(4):4387-4389.
16. Huang J, Tao G, Liu J, Cai J, Huang Z, Chen JX. Current Prevention of COVID-19: Natural Products and Herbal Medicine. *Front Pharmacol*. 2020;11:588508. doi:10.3389/fphar.2020.588508.
17. Dey D, Dey N, Ghosh S, Chandrasekaran N, Mukherjee A, Thomas J. Potential combination therapy using twenty phytochemicals from twenty plants to prevent SARS-CoV-2 infection: An in silico Approach. *Virusdisease*. 2021;32(1):108-116. doi:10.1007/s13337-021-00658-7.
18. Tahir ul Qamar M, Alqahtani SM, Alamri MA, Chen LL. Structural basis of SARS-CoV-2 $3CL^{pro}$ and anti-COVID-19 drug discovery from medicinal plants. *J Pharm Anal*. 2020;10(4):313-319. doi:10.1016/J.JPHA.2020.03.009.
19. Sharma K, Assefa AD, Kim S, Ko EY, Lee ET, Park SW. Evaluation of total phenolics, flavonoids and antioxidant activity of 18 Korean onion cultivars: a comparative study.

J Sci Food Agric. 2014;94(8):1521-1529. doi:10.1002/jsfa.6450.
20. Yan-Hwa C, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. *J Sci Food Agric*. 2000;80(5):561-566. doi:10.1002/(SICI)1097-0010(200004)80.
21. Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*. 2010;15(6):4324-4333. doi:10.3390/molecules15064324.
22. Kahl R, Kappus H. Toxikologie der synthetischen Antioxidantien BHA und BHT im Vergleich mit dem natürlichen Antioxidans Vitamin E [Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E]. *Z Lebensm Unters Forsch*. 1993;196(4):329-338. doi:10.1007/BF01197931.
23. Haid S, Novodomská A, Gentzsch J, et al. A plant-derived flavonoid inhibits entry of all HCV genotypes into human hepatocytes. *Gastroenterology*. 2012;143(1):213-222.e5. doi:10.1053/j.gastro.2012.03.036.
24. Edziri H, Mastouri M, Aouni M, Verschaeve L. Polyphenols content, antioxidant and antiviral activities of leaf extracts of *Marrubium deserti* growing in Tunisia. *South African J Bot*. 2012;80:104-109. doi:10.1016/J.SAJB.2012.03.001.
25. Morse JS, Lalonde T, Xu S, Liu WR. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. *ChemBioChem*. 2020;21(5):730-738. doi:10.1002/cbic.202000047.
26. Wahab GA, Aboelmaaty WS, Lahloub MF, Sallam A. In vitro and in silico studies of SARS-CoV-2 main protease M^{pro} inhibitors isolated from *Helichrysum bracteatum*. *RSC Adv*. 2022;12(29):18412-18424. doi:10.1039/d2ra01213h.
27. Genwali GR, Acharya PP, Rajbhandari M. Isolation of Gallic Acid and Estimation of Total Phenolic Content in Some Medicinal Plants and Their Antioxidant Activity. *Nepal J Sci Technol*. 2013;14(1):95-102. doi:10.3126/NJST.V14I1.8928.
28. Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. F*ood Sci Tech*. 2007;40(2):344–352. doi:10.1016/j.lwt.2005.09.011.
29. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26(9-10):1231-1237. doi:10.1016/s0891-5849(98)00315-3.
30. Rabie AM, Tantawy AS, Badr SMI. Design, Synthesis, and Biological Evaluation of Novel 5-Substituted-2-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazoles as Potent Antioxidants. *Am J Org Chem*. 2016;6(2):54-80. doi:10.5923/J.AJOC.20160602.02.
31. Huq AKMM, Roney M, Imran S, et al. Virtual screening of bioactive anti-SARS-CoV natural products and identification of 3β,12-diacetoxyabieta-6,8,11,13-tetraene as a potential inhibitor of SARS-CoV-2 virus and its infection related pathways by MD simulation and network pharmacology. *J Biomol Struct Dyn*. 2023;1-14. doi:10.1080/07391102.2023.2176926.
32. Bouterfas K, Mehdadi Z, Elaoufi MM, Latreche A, Benchiha W. Antioxidant activity and total phenolic and flavonoids content variations of leaves extracts of white Horehound (*Marrubium vulgare Linné*) from three geographical origins. *Ann Pharm Fr*. 2016;74(6):453-462. doi:10.1016/j.pharma.2016.07.002.
33. Alimpić A, Oaldje M, Matevski V, Marin PD, Duletić-Laušević S. Antioxidant activity and total phenolic and flavonoid contents of Salvia amplexicaulis Lam. *extracts. Arch Biol Sci*. 2014;66(1):307-316. doi:10.2298/ABS1401307A.