

Ethnomedicinal and Antiviral Study of Plants Used by the Moroccan Population During the COVID-19 Pandemic

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ABSTRACT

Despite the development of chemical drugs, traditional medicine is widely used because of the confidence in the use of plant extracts and the lack of resources. The survey was conducted by online questionnaire, via Google Forms and accessible on the Internet between 28/06/2020 and 14/08/2020. The questionnaire has two parts; one on the plants used as remedies or to prevent COVID-19 and the other part on the socio-demographic determinants associated with the use of these species. The AutoDock Vina bioinformatics tool was used to assess *in silico* the inhibitory potential of the phytochemicals obtained from these species against the main protease (Mpro) of SARS-CoV-2. A total of 1070 informants participated in this investigation. The most represented botanical family was the *Lamiaceae* family, and the three most cited species were lemon, garlic, and clove (with citation numbers 183, 171, and 150; respectively). The *in-silico* study showed that glycyrrhizin (the active ingredient of licorice) was revealed as the most potential inhibitor against SARS-CoV-2 Mpro. The medicinal plants may contain promising antiviral substances, thanks to their richness in phytochemicals. These compounds are diversified and known for their biological activities which could increase the immune response and fight oxidative stress.

Keywords: Medicinal plants, COVID-19, SARS-CoV-2, In silico, Diet, Morocco.

1. INTRODUCTION

Medicinal plants are widely used in different communities and especially among rural populations who are more familiar with the natural medicinal (Létard et al., 2015). According to the World Health Organization, traditional plant medicine, covers the therapeutic needs of about 80% of the population in developing countries (Bousta and Ennabili, 2011). In Morocco, many plant species are classified as medicinal plants and known for their richness in bioactive molecules (Eddouks et al., 2007). A total of 100 medicinal plants used to treat inflammation have been identified (Zouhri et al., 2023), along with 104 medicinal plant species specifically

Momona Ethiopian Journal of Science (MEJS), V17(1):63-81,2025 ©CNCS,Mekelle University,ISSN:2220-184X

Submitted: 27th April 2024 Accepted: 29th October 2024 Published: 6th February 2025



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used for managing hypertension in Morocco (Idm'hand et al., 2022). The therapeutic activity of these plants is directly related to their content of secondary metabolites (Zaluski et al., 2015). The major classes of secondary metabolites include alkaloids, polyphenols, which are very diverse and terpenes (Chomel et al., 2016). All of these natural organic compounds are the basis of the majority of antioxidant properties, antimutagenic activities and protection and defense against microbial infections (Cai et al., 2004; Kumar and Goel, 2019).

With the outbreak of COVID-19 on December 1, 2019, in Wuhan, China, and the lack of efficient treatment against SARS-CoV-2, communities around the world have turned to the use of medicinal plants to try to reduce or prevent COVID-19 infection. This pandemic was caused by SARS-CoV-2 (severe acute respiratory syndrome 2) contains single-stranded (positive-sense) RNA associated with a nucleoprotein within a capsid composed of matrix proteins. A typical CoV contains at least six open reading frames (ORFs) in its genome. All structures and helper proteins are translated from the CoV sgRNA. The four major structural proteins are encoded by ORFs 10 and 11 near the 3' end of the genome (Bahrami and Ferns, 2020).

Currently, studies have been conducted on the naturally occurring components that may have antiviral effects and may prevent coronavirus infection using in silico, in vitro and in vivo studies respectively (Romeo et al., 2021). The in-silico study is based on bioinformatics techniques, including molecular docking, which allows to virtually highlight the inhibitory potential of the studied molecules towards a target protein of SARS CoV-2 (Sharma et al., 2022).

The objective of this study was to identify the plants used by the Moroccan population during the pandemic to prevent covid19 and to carry out an in-silico study by molecular docking techniques on the inhibitory potential of the active components present in the cited plants against the main protease (Mpro) of SARS CoV-2.

2. MATERIALS AND METHODS

2.1. Survey Conduct

The support of this work was a survey by questionnaire put online, via Google forms and accessible on the Internet by the laboratory team within the framework of a research project on the covid (CNRST-UCAM). The questionnaire was made available online from 28.06.2020 to 14.08.2020 and received responses from 1070 respondents. The participants were people capable of using smart devices and accessing the Internet, given the constraints of confinement. We collected data on the main plants used as remedies or to prevent coronavirus disease and

the sociodemographic determinants associated with the use of these remedies. The dataset was assembled in an Excel file. The data were compiled to estimate the number of different species used and their frequencies. A specific bibliographic and web graphical search was used to characterize these plants on a systematic, phytotherapeutic, and phytochemical level.

2.2. In-silico Study

For the in-silico study, the main phytochemical compounds of the cited plants were designated based on the bibliography. The 3D structures of these molecules were extracted from the PubChem database in SDF format and were converted into PDB files using PyMol software (Borrel and Fourches, 2017). All generated structures were prepared by AutoDock Tools version 1.5.6 and the results were saved in PDBQT format (Seeliger and de Groot, 2010).

The crystal structure of the main protease (Mpro) of COVID-19 in complex with the inhibitor N3 (Jin et al., 2020) was obtained from the RCSB PDB database (code PDB: 6LU7). In process, the protein was prepared using AutoDock Tools; water molecules were removed, polar hydrogens and Kollman charges were added (da Silva et al., 2020) and the result obtained was saved in PDBQT format.

AutoDock Vina was used to assess the binding affinity of photochemical compounds to the main protease (Forli et al., 2016). The conformation revealing the lowest binding energy between the phytochemical compound (ligand) and the target protein (Mpro) was characterized as the most stable conformation (good affinity) of the ligands to the receptor (Ahmad et al., 2020). The grid was defined to cover the active site of the main protease (Mpro) of SARS-COV-2. The grid size and spacing value were $40\text{Å} \times 40\text{Å} \times 40\text{Å}$ and 0.375Å , respectively. Discovery-Studio-Visualiser was then used to prepare the anchored poses and 2D interaction models (Ghanimi et al., 2022).

3. RESULTS

3.1. Socio-demographic Characteristics

Among the respondents, 62.1% were women with an average age 32.2 ± 12.8 years and minimum and maximum age being 15 and 71 years respectively. Among respondents, 87.5% have a university degree; 85.1% are living in urban areas; 57.2% are single (57.2%); and majority respondents are civil servants, professionals, and students, while shopkeepers, craftsmen, and workers are only slightly represented. In addition, 22.2% of the respondents received social assistance from the state during the confinement.

3.2. Main Plant Species Used

Figure 1 shows the main plant species used against covid-19 by the Moroccan population and their frequency of use by the respondents. The most used plants were, by decreasing order of citation: lemon (183), garlic (171), clove (150), ginger (88), orange (74), thyme (71), onion (61), eucalyptus (41), mugwort (40), cinnamon (35), turmeric (30), olive (29), Bell pepper, Chilli, Paprika (26), lavender (21), and nigella (16).

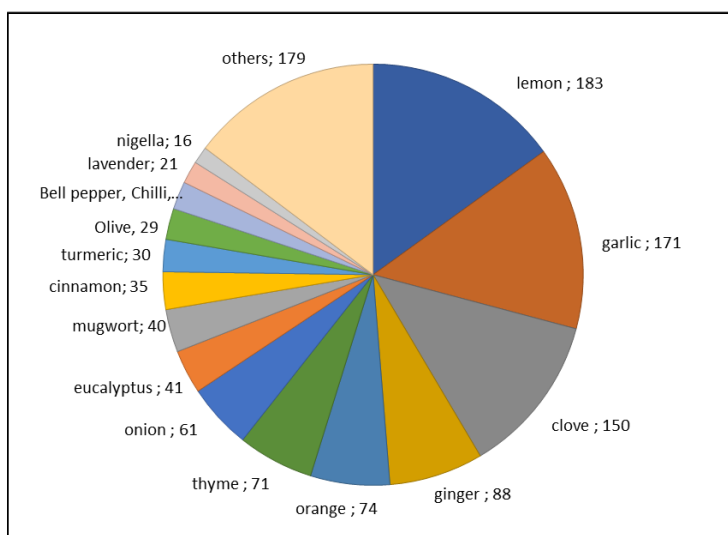


Figure 1. Citation frequencies of the main medicinal plants used to prevent covid-19 by the Moroccan population during the confinement period.

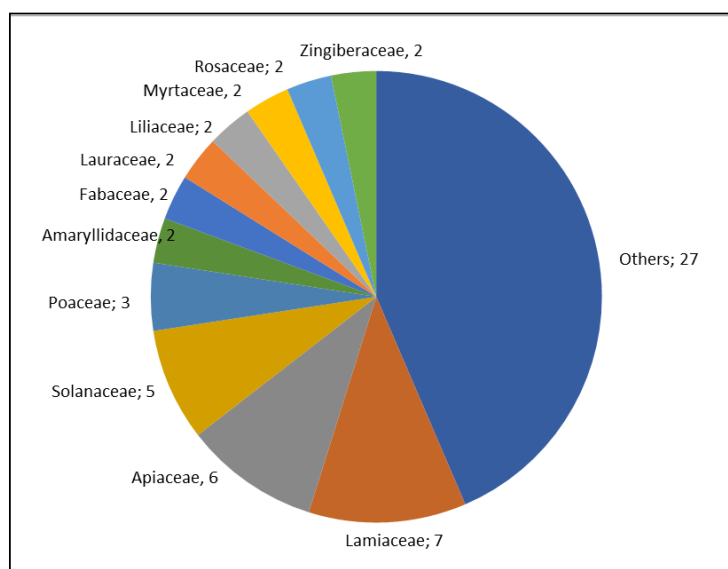


Figure 2. Botanical families of the studied species.

Concerning the botanical families as shown in figure 2, the *Lamiaceae* family is the most represented one with a set of 7 species (spearmint, rosemary, spearmint, marjoram, oregano, basil, and thyme), followed by the *Apiaceae* family with 6 species (parsley, anise,

cumin, fennel, coriander, and carrot). Then the *Solanaceae* family with 5 species and the *Poaceae* family represented 3 species. The rest of the families have between 1 and 2 species (Appendix 1).

The species studied represent one or more therapeutic activities (Fig 3). The immune property is the most dominant with 28 species, suggesting that plants can strengthen the immune system, and stimulate an adequate antiviral response. On the other hand, the anti-inflammatory and antiseptic property comes second (Appendix 1).

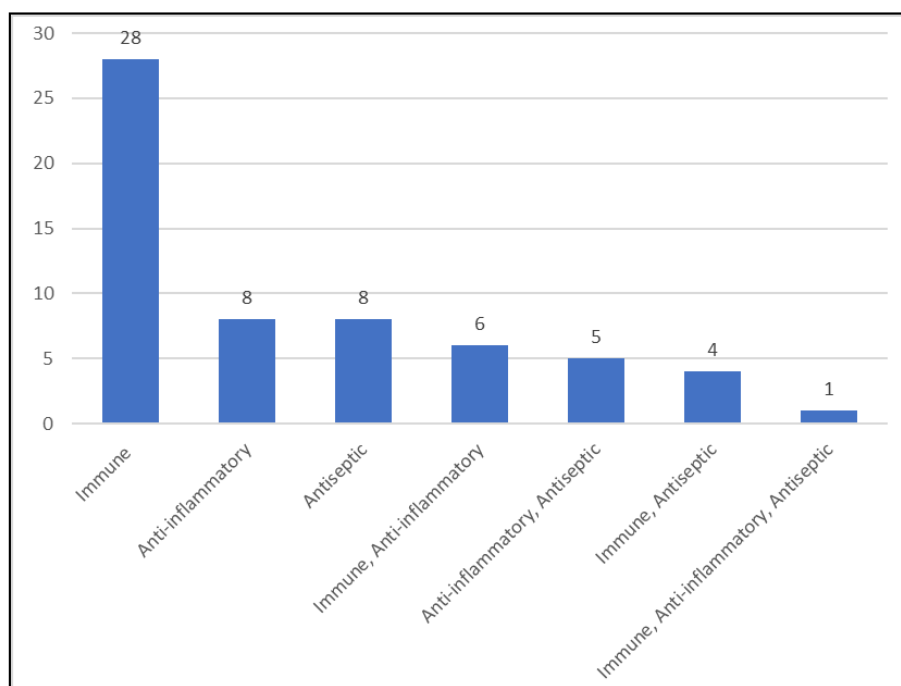


Figure 3. The therapeutic properties of the studied species.

3.3 In-silico Study

Figure 4 represents the molecular interactions of three phytochemicals with the highest inhibitory activity within the active site of Mpro. The active ingredients of the studied plants showed a binding energy that varies between -3.2 kcal/mol and -8.4 kcal/mol. The strongest inhibitory activity against SARS-CoV2 Mpro is the one corresponding to the lowest binding energy (-8.4 kcal/mol). This inhibitory ability was obtained by Naringin which is the active ingredient of grapefruit (*Citrus maxima*) followed by Glycyrrhizin (-8.1 kcal/mol) obtained from *Glycyrrhiza glabra* and then by β -carotene (-7.9kcal/mol) which is the active ingredient of *Cucumis melo* (Fig 4). On the other hand, Sulforaphane which is the active ingredient of *Brassica oleracea* showed the lowest inhibitory potential against Mpro (Appendix 1).

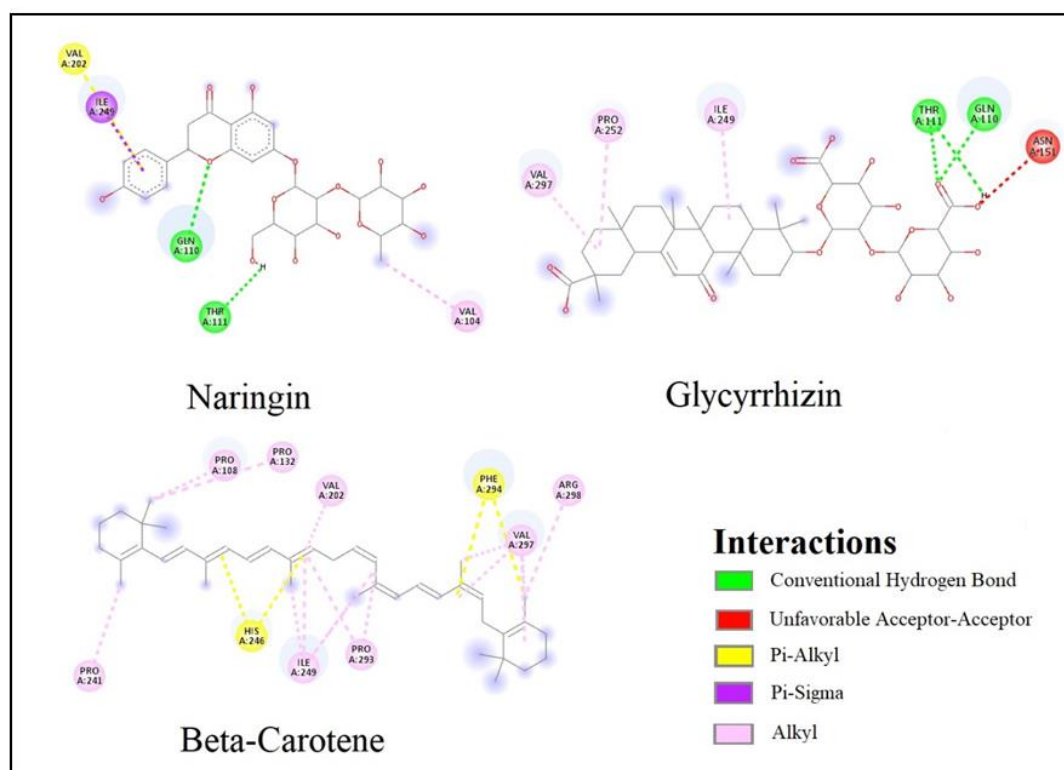


Figure 4. The 2D representation of molecular interactions within the active site of M^{pro} .

4. DISCUSSION

The study conducted in Colombia by Cordoba-Tovar et al. (2022) showed that the first three most used species against covid-19 are respectively; ginger, eucalyptus, and lemon, while garlic was in eighth position (Cordoba-Tovar et al., 2022). A similar study in India showed that the four species; ginger, garlic, clove, and lemon are among the five most used species by the population (Singh et al., 2022). The agreement between these results could reflect the importance of these species in traditional medicine mainly in these regions known for their ethnomedicinal knowledge.

The majority of the species that were cited as being used against COVID-19 belong to the *Lamiaceae* family. This result is consistent with the results of many ethnomedicinal surveys since this family is known for its species-rich in bioactive molecules (Abdelhalim and Hanrahan, 2021). The therapeutic properties of these species are governed by their content of phytochemicals having antioxidant and antimicrobial activity that could act either directly on microbes or indirectly by improving the immune system (EL ALAMI et al., 2020; Singh et al., 2022).

In this work the best active ingredient regarding the inhibitory potential against the main protease (M^{pro}) of SARS-CoV-2 was Naringin, which is in agreement with other studies (Saric et al., 2021; Varughese et al., 2022). On the other hand, the results obtained by Cinatl et al.

(2003), concerning the evaluation of the antiviral activity of some components, revealed that glycyrrhizin (the active ingredient of licorice) is a potential substance to struggle against the SARS CoV virus (Cinatl et al., 2003), which is in agreement with our results. Furthermore, in this paper as well as in other research such as the work of Kar et al. (2020) β -carotene has also shown a high inhibitory potential against this viral protease (Kar et al., 2020).

5. CONCLUSION

The outbreak of coronavirus has led to a strong use of plants to prevent the disease. Despite the massive development of chemical drugs, traditional herbal medicine is widely used by much of the population due to the confidence in the use of plant extracts and the lack of means to cover the costs of modern medicine. The most cited botanical family among the 1070 informants was Lamiaceae, and the three most cited species were lemon, garlic and clove (with 183, 171 and 150 citations respectively). Finally, based on the in silico study, glycyrrhizin (the active ingredient in licorice) showed great promise for further in vitro and in vivo studies to provide solid experimental evidence regarding its activity as a COVID-19 inhibitor.

6. ACKNOWLEDGEMENTS

The authors are very grateful to the local informants who kindly participated in the survey.

7. CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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APPENDIX 1: Main compounds of the studied plants and their inhibitory potential (Affinity) against the Main protease of SARS-CoV-2.

<i>Species</i>	<i>Common Name</i>	<i>Family</i>	<i>Therapeutic properties</i>	<i>Citation Numbers</i>	<i>Active ingredient</i>	<i>Reference</i>	<i>ID</i>	<i>Affinity kcal/mol</i>
<i>Citrus maxima</i> (Burm.) Merr.	Grapefruit	Rutaceae	Immune	1	Naringin	(Hakim et al., 2019)	CID: 442428	-8,4
<i>Glycyrrhiza glabra</i> L.	Licorice	Fabaceae	Anti-inflammatory	2	Glycyrrhizin	(Pastorino et al., 2018)	CID: 14982	-8,1
<i>Cucumis melo</i> L.	Melon	Cucurbitaceae	Immune	1	β -carotene	(Qian et al., 2019)	CID: 5280489	-7,9
<i>Limnospira fusiformis</i> (Voronichin) Nowicka-Krawczyk, Mühlsteinová & Hauer.	Spirulina	Microcoleaceae	Immune	7	β -carotene	(Miranda et al., 1998)	CID: 54603957	-5,7
<i>Citrus × aurantium</i> L.	Orange	Rutaceae	Immune	74	Hesperidin	(Bahmanzadegan et al., 2023)	CID: 10621	-7,6
<i>Rosmarinus officinalis</i> L.	Rosemary	Lamiaceae	Immune, antiseptic	11	Carnosol	(Okamura et al., 1994)	CID: 442009	-7,2
<i>Saussurea costus</i> (Falc.) Lipsch.	Indian Costus	Compositae	Anti-inflammatory, antiseptic	1	Dehydrocostus lactone	(Singh et al., 2017)	CID: 73174	-7,1
<i>Sesamum indicum</i> L.	Sesame	Pedaliaceae	Immune, anti-inflammatory	4	Sesamin	(Kermani et al., 2019)	CID: 72307	-7,1
<i>Marrubium vulgare</i> L.	Marrubine	Lamiaceae	Antiseptic	2	Diterpene lactones	(Béjaoui et al., 2017)	CID: 339816	-7
<i>Phoenix dactylifera</i> L.	Dates	Arecaceae	Immune	3	Epicatechin	(Habib et al., 2014)	CID: 72276	-7
<i>Fragaria × ananassa</i> Duchesne	Strawberry	Rosaceae	Immune	3	Kaempferol-3-glucoside	(Aaby et al., 2007)	CID: 5282102	-7
<i>Salvia officinalis</i> L.	Sage	Lamiaceae	Anti-inflammatory, antiseptic	2	Diterpenes	(Santos-Gomes et al., 2002)	CID: 65390	-6,9
<i>Persea americana</i> Mill.	Avocado	Lauraceae	Immune	1	Catechin	(Dereli et al., 2022)	CID: 9064	-6,8
<i>Triticum aestivum</i> L.	Soft wheat	Poaceae	Immune	3	Rutin	(Rajoria et al., 2015)	CID: 5280805	-6,8
<i>Petroselinum crispum</i> (Mill.) Fuss	Parsley	Apiaceae	Immune	12	Apigenin	(Poureini et al., 2020)	CID: 5280443	-6,7
<i>Artemisia herba-alba</i> Asso	Mugwort	Compositae	Anti-inflammatory, antiseptic	40	Apigenin	(Bourgou et al., 2016)	CID: 5280443	-6,7
<i>Crocus sativus</i> L.	Saffron	Iridaceae	Immune	7	Crocin	(Aung et al., 2007)	CID: 5281233	-6,7
<i>Solanum tuberosum</i> L.	Potato	Solanaceae	Immune	4	Chlorogenic acid	(Griffiths & Bain, 1997)	CID: 1794427	-6,6
<i>Camellia sinensis</i> (L.) Kuntze	Tea	Theaceae	Anti-inflammatory, antiseptic	5	Epigallocatechin	(Abd El-Hack et al., 2020)	CID: 72277	-6,6
<i>Allium cepa</i> L.	Onion	Amaryllidaceae	Immune	61	Quercetin	(Ko et al., 2015)	CID: 5280343	-6,6
<i>Corchorus olitorius</i> L.	Cornet	Malvaceae	Immune	3	Quercetin	(Azuma et al., 1999)	CID: 5280343	-6,6

<i>Malus domestica</i> Borkh.	Apple	Rosaceae	Immune	6	Quercetin	(Liaudanskas et al., 2014)	CID: 5280343	-6,6
<i>Ananas comosus</i> (L.) Merr.	Pineapple	Bromeliaceae	Anti-inflammatory	2	Quercetin	(Mhatre et al., 2009)	CID: 5280343	-6,6
<i>Actinidia deliciosa</i> (A.Chev.) C.F.Liang&A.R.Ferguson	Kiwi	Actinidiaceae	Immune	4	Neochlorogenic acid	(Wojdyło et al., 2017)	CID: 5280633	-6,4
<i>Olea europaea</i> L.	Olive	Oleaceae	Antiseptic	29	Oleuropein	(Nediani et al., 2019)	CID: 56842347	-6,3
<i>Piper nigrum</i> L.	Pepper	Piperaceae	Immune	6	Piperine	(Meghwal & Goswami, 2013)	CID: 638024	-6,3
<i>Vitis vinifera</i> L.	Grape	Vitaceae	Immune	2	Caftaric acid	(Tourtoglou et al., 2014)	CID: 6440397	-6,2
<i>Juglans nigra</i> L.	Walnut	Juglandaceae	Immune	2	Hydrojuglone-glucoside	(Burtin et al., 1998)	CID: 78012803	-6,2
<i>Hordeum vulgare</i> L.	Barley	Poaceae	Immune	2	Ferulic acid	(Dvořáková et al., 2008)	CID: 445858	-5,9
<i>Triticum durum</i> Desf.	Durum wheat	Poaceae	Anti-inflammatory	4	Ferulic acid	(Yilmaz & Koca, 2017)	CID: 445858	-5,9
<i>Beta vulgaris</i> L.	beet	Amaranthaceae	Immune	3	Ferulic acid	(Abd El-Ghffar et al., 2019)	CID: 17100	-5,9
<i>Salix alba</i> L.	Willow	Salicaceae	Anti-inflammatory	2	Salicin	(Maistro et al., 2019)	CID: 439503	-5,7
<i>Capsicum annuum</i> L.	Bell pepper	Solanaceae	Immune	26	Capsaicin	(Díaz et al., 2004)	CID: 1548943	-5,7
<i>Ophiocordyceps sinensis</i> (Berk.) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora	Cordyceps	Ophiocordycipitaceae	Immune	1	Cordycepin	(Chen et al., 2017)	CID: 6303	-5,7
<i>Daucus carota</i> L.	Carrots	Apiaceae	Immune	1	Carotol	(Özcan & Chalchat, 2007)	CID: 442347	-5,6
<i>Curcuma longa</i> L.	Turmeric	Zingiberaceae	Immune, Anti-inflammatory	30	Curcumin	(Araujo & Leon, 2001)	CID: 969516	-5,6
<i>Peganum harmala</i> L.	Peganum	Nitrariaceae	Anti-inflammatory	4	Harmaline	(Berrougui et al., 2006)	CID: 3564	-5,6
<i>Nasturtium officinale</i> R.Br.	Watercress	Brassicaceae	Immune, Anti-inflammatory	8	Sinapic acid	(Zeb, 2015)	CID: 637775	-5,5
<i>Cuminum cyminum</i> L.	Cumin	Apiaceae	Anti-inflammatory	6	Cuminaldehyde	(Morshedi et al., 2015)	CID: 326	-5,4
<i>Zingiber officinale</i> Roscoe	Ginger	Zingiberaceae	Immune, anti-inflammatory and antiseptic	88	Shogaol	(Connell & Sutherland, 1969)	CID: 5281794	-5,4
<i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry	Cloves	Myrtaceae	Immune, anti-inflammatory	150	Eugenol	(Rana et al., 2011)	CID: 3314	-5,3
<i>Origanum compactum</i> Benth.	Oregano	Lamiaceae	Antiseptic	3	Carvacrol	(Aboukhalid et al., 2016)	CID: 10364	-5,3
<i>Foeniculum vulgare</i> Mill.	Fennel	Apiaceae	Immune, antiseptic	3	Trans-anethole	(Saharkhiz & Tarakeme, 2011)	CID: 129704194	-5,3
<i>Tagetes erecta</i> L.	Carnation	Compositae	Immune, anti-inflammatory	2	Alpha-Terthienyl	(Kagan et al., 1980)	CID: 65067	-5,2
<i>Solanum lycopersicum</i> L.	Tomato	Solanaceae	Immune	7	Lycopene	(Figueira et al., 2017)	CID: 446925	-5,2
<i>Nigella sativa</i> L.	Nigella	Ranunculaceae	Immune, antiseptic	16	Thymoquinone	(Abdel-Fattah et al., 2000)	CID: 10281	-5,2

<i>Eucalyptus globulus</i> Labill.	Eucalyptus	Myrtaceae	Antiseptic	41	Eucalyptol	(Almas et al., 2021)	CID: 2758	-5,1
<i>Cinnamomum verum</i> J.Presl	Cinnamon	Lauraceae	Antiseptic, immune	35	Cinnamaldehyde	(Ramazani et al., 2020)	CID: 637511	-5
<i>Mentha pulegium</i> L.	Poppy Mint	Lamiaceae	Anti-inflammatory	1	Pulegone	(Stoyanova et al., 2005)	CID: 442495	-5
<i>Trigonella foenum-graecum</i> L.	Fenugreek	Fabaceae	Anti-inflammatory	9	Sotolon	(Das & Panda, 2015)	CID: 62835	-5
<i>Thymus saturejoides</i> Coss.	Thyme	Lamiaceae	Antiseptic	71	Thymol	(Nordine et al., 2016)	CID: 6989	-5
<i>Citrus limon</i> (L.) Osbeck	Lemon	Rutaceae	Anti-inflammatory	183	Limonene	(Paw et al., 2020)	CID: 22311	-4,7
<i>Mentha spicata</i> L.	Spearmint	Lamiaceae	Anti-inflammatory	7	Menthol	(Mahboubi, 2017)	CID: 1254	-4,6
<i>Illicium verum</i> Hook.f.	Badian	Schisandraceae	Anti-inflammatory, antiseptic	6	Anethole	(Bhadra et al., 2011)	CID: 637563	-4,5
<i>Pimpinella anisum</i> L.	Anise	Apiaceae	Antiseptic	11	Anethole	(Obaid et al., 2017)	CID: 637563	-4,5
<i>Ocimum basilicum</i> L.	Basil	Lamiaceae	Immune	1	Estragole	(Rodrigues et al., 2016)	CID: 8815	-4,5
<i>Spinacia oleracea</i> L.	Spinach	Amaranthaceae	Immune	2	Linalool	(Issazadeh et al., 2021)	CID: 1128495	-4,1
<i>Coriandrum sativum</i> L.	Coriander	Apiaceae	Antiseptic	2	Linalool	(Gastón et al., 2016)	CID: 6549	-4,1
<i>Lavandula stoechas</i> L.	Lavender	Lamiaceae	Antiseptic	21	Linalool	(Özcan et al., 2018)	CID: 6549	-4,1
<i>Allium sativum</i> L.	Garlic	Amaryllidaceae	Immune, anti-inflammatory	171	Allicin	(Shang et al., 2019)	CID: 65036	-3,3
<i>Brassica oleracea</i> L.	Broccoli	Brassicaceae	Immune	1	Sulforaphane	(Mahn & Reyes, 2012)	CID: 5350	-3,2