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Antioxidant Activity of the Herbal Combination of Black Cumin (*Nigella sativa* L.) with Soursop Leaves (*Annona muricata* L.) or Celery (*Apium graveolens* L.)

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Abstract

Excessive free radicals in the body can cause various degenerative diseases; antioxidants can reduce the impact of free radical injury. Several plants have been tested for antioxidant activity, including black cumin (Nigella sativa L.), soursop leaves (Annona muricata L.), and celery (Apium graveolens L.). Black cumin is easy to find in several finished products on the market in Indonesia alone or in combination with other ingredients, including soursop leaves and celery. This study aimed to explore the interaction of laboratory-prepared and commercial combinations of black cumin extract with soursop or celery extract. The antioxidant activity of each extract was determined, and the combined antioxidant activity was calculated. The interactions of the combinations were identified based on the interaction factor values. The antioxidant activities were measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent. The results revealed nearly all exhibited strong antioxidant activity, except for black cumin–based commercial combination preparations, which demonstrated weak had antioxidant activity. Moreover, all combination extracts—laboratory made or commercial—had an IF value of > 1. Conclusion: The combination of black cumin extract with soursop or celery leaf extract resulted in an antagonistic effect (IF > 1) on the antioxidant ability.

***Keywords**: Nigella_sativa L., Annona_muricata L., Apium_graveolens L, antioxidant, combination*

INTRODUCTION

Unhealthy eating patterns accompanied by frequent exposure of the body to dangerous substances can increase the amount of free radicals, which cause oxidative stress and the formation of harmful compounds, eventually leading to degenerative diseases (Munteanu & Apetrei, 2021). Endogenous antioxidant enzymes, including glutathione peroxidase, catalase, superoxidase, and dismutase, can neutralize the effects of oxidative stress. Exogenous antioxidant compounds can strengthen the defense against free radical injury, especially when endogenous antioxidants are unable to provide adequate protection (Ahmad et al., 2014). Numerous plants have been demonstrated to possess antioxidant activity, including black cumin (*Nigella sativa* L.), soursop leaves (*Annona muricata* L.), and celery (*Apium graveolens* L.) (Tiji et al., 2021; Gueffai et al., 2022; Ilango et al., 2021; Liu et al., 2020; Kooti & Daraei, 2017; Gyesi et al., 2019). Their antioxidant activity (IC_{50} value) was determined to be 94.1 ± 5.6, 141.127, and 34.l56 ± 0.014 μg/mL, respectively (Sasikumar et al., 2020; Hasmila et al., 2019; Al Aboody, 2020).

Herbal or natural medicines or supplements are generally used in combination to increase their potency through a synergistic effect (Zhou et al., 2016), as well as reduce the dosage, decrease the side effects, and/or increase the types of active ingredients to increase the body's resistance (Prakash et al., 2009). For example, the combination of black cumin extract with *Psidium guajava* and *Foeniculum vulgare* extract exhibited increased inhibitory activity (Divya et al., 2020). The combination of celery with *Thymus vulgaris* L. and *Coriandrum sativum* L. extract exhibited a synergistic increase in antioxidant activity (Crespo et al., 2019), whereas that of celery

leaves with *Moringa* leaves decreases antioxidant activity (Natsir et al., 2019). Black cumin is easily found in several finished products on the market alone or in combination with other ingredients in Indonesia (Rulianti & Astuti, 2017). However, no study has investigated the antioxidant activity of the combination of black cumin with soursop leaves or celery.

In the current study, we explored the effect of the commercially available and laboratory-made combinations of black cumin extract with soursop or celery leaf extract on antioxidant parameters. This effect was determined using the interaction factor value and comparing the measured activity of the sample with the theoretical activity of the sample based on the dose of each combination (Durak et al., 2014).

RESEARCH METHODOLOGY

Chemicals

All chemicals and solvents used were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH) obtained from Sigma Aldrich (Singapore) and sodium chloride, ferric chloride, ethanol, and methanol from Merck. Distilled water was used to prepare all reagents and solutions.

Extract Preparation

The plant samples (seeds and leaves), obtained from Bogor area. Black cumin and soursop leaves samples determined at Unit Konservasi Budidaya Biofarmaka (UKBB) Pusat Studi Biofarmaka Tropika LPPM IPB University and celery leaves at Direktorat Pengelolaan Koleksi Ilmiah National Research and Innovation Agency (BRIN) Indonesia. The samples were first cleaned separately with tap water, rinsed with distilled water, and then dried to a constant weight in an oven at 40 °C for 76 h. The dried sample was crushed and sifted with a 40-mesh siever. Each 100 g of powdered plant sample was macerated (in triplicate) by dissolving it in 1000 mL of 96% ethanol in three remacerations. Following a 24-h soak, the extract was filtered using a Whatman filter paper. The filtered solvent was then removed by evaporating under reduced pressure at 55°C in a rotary evaporator, and the dried extract was weighed and then refrigerated (at 4°C) until analysis. Extraction yield (%) was calculated as $W_{\text{extract}}/W_{\text{powdered sample}}$, where W_{extract} indicates the weight of the dry extracts, and $W_{\text{powdered sample}}$ indicates the weight of powdered seeds or leaves.

Combination Products in the Market

Antioxidant activities were analyzed from commercial products containing a combination of *Nigella sativa* L. extract with *Apium graveolens* L. extract in a 9:10 ratio (Product 1) or *Annona muricata* L. extract in a 3:2 ratio (Product 2). Both products were purchased in the market in Bogor City, Indonesia.

Qualitative Phytochemical Tests

Qualitative tests were conducted to identify phytochemicals such as alkaloids, flavonoids, saponins, and tannins, as described elsewhere (Shaikh & Patil, 2020).

Ash Content Determination

A crucible containing 5 g of the sample was placed in a muffle furnace and heated to 550 °C for 6 h or until the sample turned gray. The dish was removed from the muffle furnace using crucible tongs and placed in a desiccator to cool. The ash was weighed again after cooling, and its weight was calculated from the difference (Offor et al., 2014).

Moisture Content Determination

After thorough cleaning, the Petri dish was placed in the oven to dry. Next, 5 g of the sample was placed in a Petri dish that had been previously weighed, and the sample was dried for 2 h at 105 °C in the oven. Before being weighed once more, the dish and dry sample were moved to a desiccator to cool to

Table 1. Moisture Content, Ash Content, and Extraction Yield

Sample	Moisture content (%)	Ash value (%)	Extraction yield (%)
Nigella sativa L. extract	7.435 ± 0.148	1.185 ± 0.290	29.56 ± 2.53
Annona muricata L. extract	5.235 ± 0.078	1.845 ± 0.092	20.38 ± 1.89
Apium graveolens L. extract	6.950 ± 1.117	2.880 ± 0.368	25.98 ± 2.46
Product 1	5.853 ± 0.237	3.215 ± 0.189	-
Product 2	6.324 ± 0.163	2.834 ± 0.253	-

Note: Product 1 contain a mixture of *Nigella sativa* L extract and *Apium graveolens* L. extract (9:10). Product 2 contains a mixture of *Nigella sativa* L extract and *Annona muricata* L. extract (3:2).

Table 2. Qualitative Analysis Extract

Sample	Alkaloids	Flavonoids	Saponins	Tannins
Nigella sativa L. extract	+	+	+	+
Annona muricata L. extract	+	+	+	+
Apium graveolens L. extract	+	+	+	+
Product 1	+	+	+	+
Product 2	+	+	+	+

Note: Product 1 contain a mixture of *Nigella sativa* L extract and *Apium graveolens* L. extract (9:10). Product 2 contains a mixture of *Nigella sativa* L extract and *Annona muricata* L. extract (3:2).

room temperature. Until a consistent weight was achieved, the experiments were repeated (Offor et al., 2014).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the extract was evaluated as described by Sasikumar et al. (2020). Using the appropriate solvents, extracts (1 mL) of varying concentrations (10–240 μg/mL) were added to a DPPH solution (1 mL, 0.1 mM) in methanol, diluted to 10 mL with methanol, and vortexed. The absorbance was measured at 517 nm in a UV-Vis double beam spectrophotometer (Jasco V-730) against a blank (methanol) following 20 min of reaction at 25 °C. Moreover, 1 mL of methanolic DPPH solution diluted to 10 mL methanol was used as a control. The extract's ability to scavenge DPPH was measured using its IC_{50} value, which represents the concentration of the extract required to quench DPPH radicals by 50%.

$$\% \text{ of inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} x\ 100\% \quad (1)$$

Where $A_{control}$ is the absorbance of DPPH without extract and Abs_{sample} is the absorbance of the sample extract.

Interaction Factor Determination

Interaction factor (IF) was used to compare the measured activity of a combination of samples (A_M) with the theoretically calculated mixture activity (A_T) (which is based on the dosage response of individual components at different concentrations).

$$IF = A_M/A_T \quad (2)$$

IF values of <1, 1, and >1 indicate synergistic, additive, and antagonistic interactions, respectively (Durak et al., 2014).

Table 3. **Comparison IF Sample**

Sample	Actual IC_{50}	Theoretical IC_{50}	Interaction Factor
Combination 1	95.15	75.132	1.272 ± 0.012
	96.02	74.705	
	93.94	74.365	
Combination 2	71.68	67.095	1.065 ± 0.021
	72.26	66.611	
	69.71	66.858	
Product 1	164.76	67.095	2.498 ± 0.041
	169.06	66.611	
	167.08	66.858	
Product 2	204.15	75.362	2.722 ± 0.020
	205.71	74.928	
	202.28	74.586	

Note: Combination 1 contains *Nigella sativa* L extract and *Annona muricata* L. extract (1:0.7), Combination 2 contains *Nigella sativa* L extract and *Apium graveolens* L. extract (0.9:1), Product 1 contains *Nigella sativa* L extract and *Apium graveolens* L. extract (9:10) (commercial), and Product 2 contains *Nigella sativa* L extract and *Annona muricata* L. extract (3:2) (commercial)

RESULT AND DISCUSSION

The sample was extracted with 96% ethanol solvent to extract the active compounds. Black cumin leaves extracted with 98% ethanol produce an antioxidant capacity of 74.9% (Dorra et al., 2019). Soursop leaves extracted with 96% ethanol displayed antioxidant activity, with IC_{50} being 20.75 ± 0.28 µg/mL (Nguyen et al., 2020). Celery leaves extracted with 96% ethanol resulted in 90% DPPH inhibition (Liu et al., 2020). The solvent 96% ethanol can produce extracts that have strong antioxidant activity (Hasmila et al., 2019). The extraction yield, water content, and ash content of the resulting extracts are presented in **Table 1**.

The water and ash content of *Nigella sativa* L., *Annona muricata* L., and *Apium graveolens* L. were consistent with those reported in previous studies (Albakry et al., 2022, Haron et al., 2014, Victor et al., 2021; Onuoha et al., 2021). The results of the qualitative analysis (**Table 2**) revealed that black cumin extract, soursop leaves, and celery leaves as well as combination extracts purchased on the market contained alkaloids, flavonoids, saponins, and tannins, also in accordance with previous research. Black cumin extract contains alkaloids, tannins, phenols, steroids, flavonoids, and cardiac glycosides (Dalli et al., 2021). Soursop leaf extract contains steroids, terpenoids, alkaloids, flavonoids, polyphenols, steroids, coumarins, carbohydrates, carotenoids, and ascorbic acid (Hasmila et al., 2019; Nguyen et al., 2020; Muthu & Duraij, 2015). Celery leaf extract contains terpenes, phenols, steroids, glycosides, flavonoids, and alkaloids (Khalil et al., 2015).

Antioxidant activity was defined as the ability of the extract to reduce DPPH free radicals, resulting in a color change of the sample from purple to yellow. The color formed can be measured at a maximum wavelength of 517 nm (Munteanu & Apetrei, 2021). **Figure 1** illustrated the IC_{50} values of all the extracts and products used. The IC_{50} of single extracts is higher than that of combination extracts and commercial combinations of extracts. Among the single extracts, celery leaves had the highest antioxidant activity (IC_{50} value).

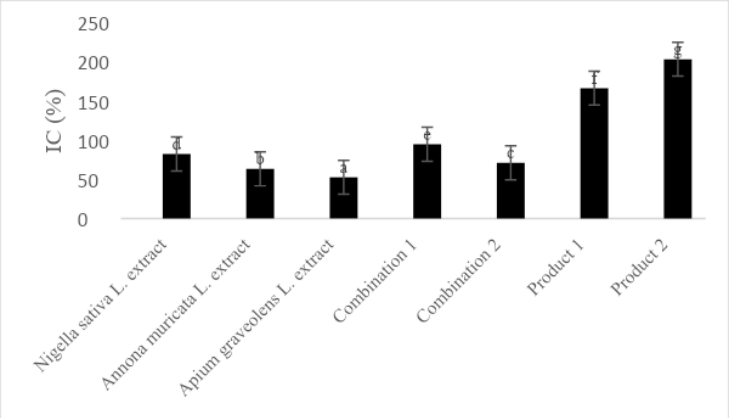

Figure 1. IC_{50} Value Extract Single and Combination
Note: Combination 1 contains *Nigella sativa* L extract and *Annona muricata* L. extract (1:0.7), Combination 2 contains *Nigella sativa* L extract and *Apium graveolens* L. extract (0.9:1), Product 1 contains *Nigella sativa* L extract and *Apium graveolens* L. extract (9:10), and Product 2 contains *Nigella sativa* L extract and *Annona muricata* L. extract (3:2). Grup with different superscript have significantly different ($p<0.05$)

The antioxidant activity of polyphenolic compounds, including flavonoids and tannins, is related to their ability to inactivate free radicals by donating electrons or hydrogen to free radicals and is associated with the presence of phenol groups in the polyphenol structure (Olszowy, 2019). The types of phenolic and flavonoid compounds contained in *Nigella sativa* include quercetin, kaempferol 3-glucosyl galactosyl glucoside, quercetin 3-galactosyl glucoside, trigillin quercetin-3-glucospide, acidic phenolics, phenolics, vanillic acid, hydroxybenzoic acid, syringic acid, and p-coumaric acids (Ahmad et al., 2021). The phenolic compounds in soursop leaf extract include gallic acid, rutin, naringenin, vanillin, and eugenol (Carmona et al., 2020). In celery leaves, compounds that exert antioxidant activity include phenols, caffeic acid, ferulic acid, tannin, saponin, p-coumaric acid, coumarin, steroids, alkaloids, kaempferol, luteolin, apigenin, essential oils, and sesquiterpene alcohols (Ozmatara, 2020). The interactions that occur in the combination are traced by determining the interaction factor, which can be calculated by comparing the measured and theoretical antioxidant activity values (**Table 3**).

The results revealed that both combination extracts had an IF value > 1. Several researchers have stated that IF > 1 indicates that the combination is antagonistic (Durak et al., 2014), but some have categorized an IF value of 1–4 as having no interaction (Caesar & Cech, 2019). Extracts generally comprise many compounds, making it often challenging to determine the exact compound responsible for a particular activity of the extract. For example, the interaction of the antioxidant activity of the polyphenolic compounds quercetin and resveratrol with caffeic acid shows antagonistic activity, whereas the interaction of quercetin with caffeic acid shows an antagonistic interaction and quercetin and resveratrol show antagonist activity (Kurin et al., 2012). The composition of the extract also varies depending on the plant source, extract preparation method, and extract storage. To enhance the benefits of natural product mixtures and ensure that they are safe, they must be characterized comprehensively for identifying all the compounds and their contributions to the extract's biological activity (Caesar & Cech, 2019).

CONCLUSION

The combination of black cumin extract with soursop or celery leaves resulted in an antagonistic effect (IF>1) in terms of antioxidant ability.

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References

Ahmad, M. F., Ahmad, F. A., Ashraf, S. A., Saad, H. H., Wahab, S., Khan, M. I., Ali, M., Mohan, S., Hakeem, K.R., & Athar, M. T., 2021. An Updated Knowledge of Black Seed (*Nigella sativa* Linn.): Review of Phytochemical Constituents and Pharmacological Properties. Journal of Herbal Medicine, 25, 100404.

Ahmad, S., Arshad, M. A., Ijaz, S., Khurshid, U., Rashid, F., & Azam, R., 2014. Review on Methods Used to Determine Antioxidant Activity. International Journal of Multidisciplinary Research and Development, 1(1), 35-40.

Al Aboody, M. S., 2021. Cytotoxic, Antioxidant, and Antimicrobial Activities of Celery (*Apium graveolens* L.). Bioinformation, 17(1), 147.

Albakry, Z., Karrar, E., Ahmed, I. A. M., Oz, E., Proestos, C., El Sheikha, A. F., Oz, F., Wu, G., & Wang, X., 2022. Nutritional Composition and Volatile Compounds of Black Cumin (*Nigella sativa* L.) Seed, Fatty Acid Composition and Tocopherols, Polyphenols, and Antioxidant Activity of Its Essential Oil. Horticulturae, 8(7), 575.

Balderrama-Carmona, A. P., Silva-Beltrán, N. P., Gálvez-Ruiz, J. C., Ruíz-Cruz, S., Chaidez-Quiroz, C., & Morán-Palacio, E. F., 2020. Antiviral, Antioxidant, and Antihemolytic Effect of *Annona muricata* L. Leaves Extracts. Plants, 9(12), 1650.

Bat-Özmatara, M., 2020. The Antioxidant Activity of *Apium graveolens*. International Journal of Food Engineering Research, 6(1), 17-33.

Caesar, L. K., & Cech, N. B., 2019. Synergy and Antagonism in Natural Product Extracts: When 1+ 1 Does Not Equal 2. Natural Product Reports, 36(6), 869-888.

Crespo, Y. A., Sánchez, L. R. B., Quintana, Y. G., Cabrera, A. S. T., Del Sol, A. B., & Mayancha, D. M. G., 2019. Evaluation of the Synergistic Effects of Antioxidant Activity on Mixtures of the Essential Oil from *Apium graveolens* L., *Thymus vulgaris* L. and *Coriandrum sativum* L. using Simplex-Lattice Design. Heliyon, 5, e01942

Dalli, M., Azizi, S. E., Kandsi, F., & Gseyra, N., 2021. Evaluation of the in vitro Antioxidant Activity of Different Extracts of *Nigella sativa* L. Seeds, and the Quantification of Their Bioactive Compounds. Materials Today: Proceedings, 45, 7259-7263.

Divya, R. K., & Revathi, K., 2020. Antioxidant Activity in Aqueous and Methanol Extract of Combinations of *Psidium guajava, Foeniculum vulgare* and *Nigella sativa*. Annals of the Romanian Society for Cell Biology, 349-363.

Dorra, N., El-Berrawy, M., Sallam, S., & Mahmoud, R., 2019. Evaluation of Antiviral and Antioxidant Activity of Selected Herbal Extracts. Journal of High Institute of Public Health, 49(1), 36-40.

Durak, A., Gawlik-Dziki, U., & Pecio, Ł., 2014. Coffee with Cinnamon–Impact of Phytochemicals Interactions on Antioxidant and Anti-Inflammatory in vitro Activity. Food Chemistry, 162, 81-88.

Durodola, O., 2021. Proximate and Some Phytochemical Constituents of Three West African Vegetable Spices. Eurasian Journal of Food Science and Technology, 5(1), 59-66.0

Gueffai, A., Gonzalez-Serrano, D. J., Christodoulou, M. C., Orellana-Palacios, J. C., Ortega, M. L. S., Ouldmoumna, A., Kiari, F.Z., Loannou, G.D., Kapnissi-Christodoulou, C.P., Moreno, A., & Hadidi, M., 2022. Phenolics from Defatted Black Cumin Seeds (*Nigella sativa* L.): Ultrasound-Assisted Extraction Optimization, Comparison, and Antioxidant Activity. Biomolecules, 12(9), 1311.

Haron, H., Grace-Lynn, C., & Shahar, S., 2014. Comparison of Physicochemical Analysis and Antioxidant Activities of *Nigella sativa* Seeds and Oils from Yemen, Iran and Malaysia. Sains Malaysiana, 43(4), 535-542.1

Hasmila, I., Natsir, H., & Soekamto, N. H., 2019. Phytochemical Analysis and Antioxidant Activity of Soursop Leaf Extract (*Annona muricata* Linn.). In Journal of Physics: Conference Series (Vol. 1341, No. 3, p. 032027). IOP Publishing.

Ilango, S., Nirmaladevi, R., & Jayachandran, P., 2021. In vitro Antioxidant Activity of *Annona Muricata* Leaves. Journal of Advanced Scientific Research, 12(01 Suppl 1), 32-41

Khalil, A., Nawaz, H., Ghania, J. B., Rehman, R., & Nadeem, F., 2015. Value Added Products, Chemical Constituents and Medicinal Uses of Celery (*Apium graveolens* L.)–A Review. International Journal of Chemical and Biochemical Sciences, 8(2015), 40-48.

Kurin, E., Mučaji, P., & Nagy, M., 2012. In vitro Antioxidant Activities of Three Red Wine Polyphenols and Their Mixtures: An Interaction Study. Molecules, 17(12), 14336-14348.

Liu, D. K., Xu, C. C., Zhang, L., Ma, H., Chen, X. J., Sui, Y. C., & Zhang, H. Z., 2020. Evaluation of Bioactive Components and Antioxidant Capacity of Four Celery (*Apium graveolens* L.) Leaves and Petioles. International Journal of Food Properties, 23(1), 1097-1109.

Liu, D. K., Xu, C. C., Zhang, L., Ma, H., Chen, X. J., Sui, Y. C., & Zhang, H. Z., 2020. Evaluation of Bioactive Components and Antioxidant Capacity of Four Celery (*Apium graveolens* L.) Leaves and Petioles. International Journal of Food Properties, 23(1), 1097-1109.

Munteanu, I. G., & Apetrei, C., 2021. Analytical Methods Used in Determining Antioxidant Activity: A Review. International Journal of Molecular Sciences, 22(7), 3380.

Muthu, S., & Durairaj, B., 2015. Evaluation of Antioxidant and Free Radical Scavenging Activity of Annona muricata. European Journal of Experimental Biology, 5(3), 39-45.

Natsir, H., Wahab, A. W., Budi, P., Arif, A. R., Arfah, R. A., Djakad, S. R., & Fajriani, N., 2019. Phytochemical and Antioxidant Analysis of Methanol Extract of Moringa and Celery Leaves. In Journal of Physics: conference series (Vol. 1341, No. 3, p. 032023). IOP Publishing.

Nguyen, M. T., Nguyen, V. T., Minh, L. V., Trieu, L. H., Cang, M. H., Bui, L. B., Le, X.T., & Danh, V. T., 2020. Determination of the Phytochemical Ccreening, Total Polyphenols, Flavonoids Content, and Antioxidant Activity of Soursop Leaves (*Annona muricata*

Linn.). In IOP Conference Series: Materials Science and Engineering (Vol. 736, No. 6, p. 062011). IOP Publishing.

Offor, I. F., Ehiri, R. C., & Njoku, C. N., 2014. Proximate Nutritional Analysis and Heavy Metal Composition of Dried *Moringa oleifera* Leaves from Oshiri Onicha LGA, Ebonyi State, Nigeria. Journal of Environmental Science, Toxicology and Food Technology, 8(1), 57-62.

Olszowy, M., 2019. What is Responsible for Antioxidant Properties of Polyphenolic Compounds from Plants?. Plant Physiology and Biochemistry, 144, 135-143.

Onuoha, C. H., Nwachukwu, C. C., Nwachukwu, R. T., Nwogu, C. G., Chukwudoruo, C. S., & Ujowundu, F. N., 2021. Comparative Evaluation of Proximate Composition and Anti-Sickling Potential of *Annona muricata* Linn Seeds and Leaves. AROC in Natural Product Research, 1(2), 29-35.

Prakash, O., Singh, G. N., Singh, R. M., Madan, S., & Mathur, S. C., 2009. Interactions of Herbal Extract Combinations Against Free Radical Scavenging Activity. Pharmaceutical Biology, 47(8), 729-733.

Rulianti, M. R., & Astuti, V., 2017. Uji Perbandingan Kandungan Antioksidan Produk Jinten Hitam yang Beredar di Kota Palembang dengan Metode DPPH. JPP (Jurnal Kesehatan Poltekkes Palembang), 12(1), 27-35.

Sasikumar, J. M., Erba, O., & Egigu, M. C., 2020. In vitro Antioxidant Activity and Polyphenolic Content of Commonly Used Spices from Ethiopia. Heliyon, 6(9). E05027

Shahidi, F., & Zhong, Y., 2015. Measurement of Antioxidant Activity. Journal of functional foods, 18, 757-781.2

Shaikh, J. R., & Patil, M., 2020. Qualitative Tests for Preliminary Phytochemical Screening: An Overview. International Journal of Chemical Studies, 8(2), 603-608.

Tiji, S., Benayad, O., Berrabah, M., El Mounsi, I., & Mimouni, M., 2021. Phytochemical Profile and Antioxidant Activity of *Nigella sativa* L Growing in Morocco. The Scientific World Journal, 2021, 1-12.

Zhou, X., Seto, S. W., Chang, D., Kiat, H., Razmovski-Naumovski, V., Chan, K., & Bensoussan, A., 2016. Synergistic effects of Chinese Herbal Medicine: a Comprehensive Review of Methodology and Current Research. Frontiers in Pharmacology, 7, 201.