Progress in Engineering Application and Technology Vol. 4 No. 2 (2023) 984-995

PEAT

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/peat
e-ISSN : 2773-5303

Formatted Table

Formatted Table

Determination of Antioxidant Activity of Senna Alexandrina Leaves and Phoenix Dactylifera Fruits for Food Preservation

Muhammad Nourhakeem Muhamad Mahir[1], 'Aisyah Mohamed Rehan[1]*, Noor Akhmazillah Mohd Fauzi[1]

[1]Department of Chemical Engineering Technology, Faculty of Chemical Engineering Technology,
University Tun Hussein Onn Malaysia, 86400 Pagoh, Johor,
MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/peat.2023.04.02.103
Received 14 January 2023; Accepted 12 February 2023; Available online 12 February 2023

Abstract: A sustainable approach in food preservation is by using natural resources, contributing to the overall sustainability of food supply chain. Synthetic preservatives such as nitrates, sorbate, and phenolic antioxidants can cause harmful effects to consumers such as allergy reaction. Preserving food using natural products containing antioxidant or antimicrobial properties can be an alternative to other preservation methods i.e., thermal, or chemical methods. These methods can increase food shelf life from spoilage microorganism that alter the food texture, edibility, and nutritional values (antimicrobial) and delaying or preventing rancidity of food due to oxidation (antioxidant). *Senna alexandrina*, an ornamental shrub that grows natively in Egypt and commonly used as an herbal tea, and *Phoenix dactylifera* commonly known as date palms are being the focus of this study, aimed to determine its' antioxidant activity for food preservation application. The highest antioxidant activity for TPC is observed in *S. alexandrina* leaves extract dissolved in methanol for each concentration tested. This study showed that methanol is the most effective solvent for phenolic extraction. Accordingly, the highest antioxidant activity in DPPH scavenging assay is observed from 1 mL *S. alexandrina* leaves extract with methanol solvent (65.59 %). Meanwhile, the highest antioxidant activity for *P. dactylifera* fruit extract is observed from 5 mL extract in ethanol solvent (83.53 %). The presence of antioxidant compound was determined by the presence of peaks of hydroxyl (-OH) group using infrared FTIR spectroscopy. In addition, observation of both *S. alexandrina* and *P. dactylifera* extracts' application on homemade bread showed promising results, with less mould growth compared to homemade bread without any additive, as well as in comparison to homemade bread with BHA added. Further studies on these findings will be beneficial to explore the potential of these natural food preservatives.

*Corresponding author: aisyahr@organization.edu.co

Keywords: Natural Antioxidant Food Preservation, Antioxidant, Date Palms, Microorganism

1. Introduction

Under the Tenth Schedule (Regulation 24) from Malaysia Food Regulations 1985, permitted antioxidants that may be added to specified food includes synthetic antioxidants i.e. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) with a maximum of 200mg/kg (Schedules Under Food Regulations 1985). Studies showed that large doses of BHA may have interactions with steroid hormones, changing testosterone levels, and resulting in underdeveloped sex organs and reproductive dysfunction (Jeong et al, 2005). Besides, large amounts of BHT have been reported to cause skin, eye and respiratory irritation in humans (NCBI, PubChem, n.d).

Large doses of BHT can also cause kidney, liver and lung tissue damage when applied topically in rats (Lanigan and Yamarik, 2002). Even though lower approved levels of these synthetic antioxidants were generally considered safe (in Malaysia and in the US), vulnerable populations such as infants, children, pregnant or breastfeeding mothers may need to avoid them as a precaution. In many countries, there are certain limits on additives or synthetic preservatives for food which needs to be disclosed to consumers. Japan prohibited the use of BHA and BHT, while in Australia they are prohibited for minors. In Europe, TBHQ is banned. Hence, there are demands for a more organic and natural options for food antioxidants. Thus, this study may be able to provide a sustainable option of natural food antioxidants.

This method is preferred is because it is less consuming time and easy to handle. This study has be done by using two type of solvents which are ethanol and methanol on several different concentrations of extracts. The only problem by using this solvent compared to the water is the less purity of solution obtained after the extraction process.

According to a study from the Department of Floriculture, Ornamental Horticulture and Garden Design, Faculty of Agriculture (El-Shatby), University of Alexandria, Alexandria, Egypt, the phenolic profile of the medicinal plant *Senna alexandrina* can be determined by high -performance liquid chromatography with diode array detection method. Method used in term of antioxidant activity in this study is by DPPH and β-carotene-linoleic acid assays. [1]

With the significant concentration of phenolic compounds based on the rate of the bioactivities of this plant extract, it has the potential to be utilized in food preservation. [1] Another research from the Department of Biology, College of Sciences, Princess Nourah bint Abdulrahman University has demonstrated the positive effects of arbuscular mycorrhizal fungi (AMF) on the increased accumulation of sennoside A and B in *Senna alexandrina* and *Senna italic* determined by high performance liquid chromatography method. [2]

The seeds of Deglet Nour and Khoaut Allig variety had similar values of 27698 and 26905 µmol TE (Trolox equivalents) per 100 g of date seed powder on fresh weight basis. This finding corresponds to the significant level of total phenolic content and total flavonoid content observed in this study. [4] Otherwise for *Phoenix dactylifera*, date cultivation methods were introduced to Malaysia in late 2010. [5]

At high temperatures, antioxidants may even be lost via volatilization, and the ensuing volatiles might damage the oil's stability during thermal processing. Compounds having a greater polarity are less volatile. As a result of its low polarity, BHT has the most volatility. [14] As a result, the materials utilised are nearly pure and easily accessible in huge amounts. Ascorbic acid, citric acid, oleic acid, glutathione-derived, and superoxide dismutase are a few examples. [15]

The objectives of this study are to perform extraction of *Senna alexandrina* leaves and *Phoenix dactylifera* fruits by using Soxhlet method, perform antioxidant assays on extracts of *Senna alexandrina* leaves and *Phoenix dactylifera* fruits and to compare antioxidant activities of *Senna alexandrina* leaves extract and *Phoenix dactylifera* fruits extract, in individually with synthetic antioxidant.

1.1 Food preservative involved in food industry

Food preservatives used to ensure the safety of the food consumed as well as to ensure that the nutritional content and food quality are maintained without spoilage from microorganisms, either through physical, chemical or enzymatic processes. Chemical preservatives are also classified under food additives. This refers to materials other than basic food items that cover aspects such as processing, storage packaging and production. Whereas, the natural food preservatives that are widely used today is salt and sugar. [9]

1.2 Extraction techniques and identification of phenolic contents

Liquid-liquid extraction (Soxhlet method) involves a solvent to extract the phenolic compounds present in parts of the plant such as leaves and fruits. Among its advantages are its simplicity and the low cost. Thus, the results of such phenolic polarity will be found in various variations. [3] UV-visible spectroscopy, near-infrared reflectance spectroscopy, nuclear magnetic resonance and DPPH scavenging activity can be applied to purify and identify them.

Antioxidants are also a preventative agent of diseases associated with oxidative stress. It also serves as one of the main materials in producing metal nanoparticles which are also known as a green synthesis material. [10] Among the antioxidants are flavonoids that have examples such as apigenin, quercetin, luteolin and catechins that are able to address issues related to disease infections such as swine epidemic diarrhea virus (PEDV) and gastroenteritis virus (TGEV). [11][12]

2. Materials and Methods

In this chapter, the chemical, reagent, material and method used for determination of antioxidant activity of *Senna alexandrina* leaves and *Phoenix dactylifera* fruits will be discussed. To ensure this can be achieved, an appropriate extraction process such as Soxhlet was used.

2.1 Methods

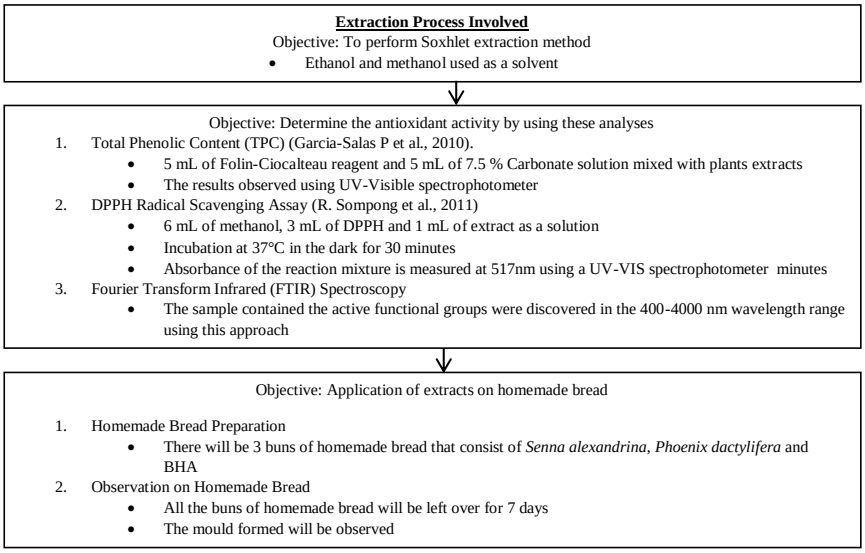

Figure 2.1: Flowchart of the experimental procedure

5 g of *Senna alexandrina* leaves and *Phoenix dactylifera* fruits were weighted by using an analytical balance and placed into 5 different teabags (25 g of samples for one run of extraction). In addition, methanol (250 mL) is used as a solvent. Next, the teabags will be entered into the Soxhlet chamber. Adjusted the heating mantle until achieve 60°C and allowed it to reflux for two hours at a steady rate so that the Soxhlet fills up. After about 30 minutes, the Soxhlet will be full and the solution will flushed through into the round bottom flask. The solution of extract gradually becomes more concentrated until the reflux is finished.

The total phenolic content presented in extracted *Phoenix dactylifera* and *Senna alexandrina* were determined by using Folin-Ciocalteu reagent. For the standard curve, the Gallic acid of 1 mL, 2 mL, 3 mL, 4 mL and 5 mL are mixed with ethanol of 4 mL, 3 mL, 2 mL, 1 mL and 0 mL in order to provide to concentration of 100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL and 500 mg/mL. The samples were produced by added 0.2 mL of extract of Gallic acid, 2.8 mL distilled water, 0.5 mL Folin-Ciocalteu and 2 mL of Na2CO4 while the blank is distilled water that added with Folin-Ciocalteu and Na2SO4.

After 5 minutes, 4 ml of Na2CO3 were added to the mixture. After incubation in water bath for 30 minutes at 40°C, the absorbance's were determined at 650 nm by using UV-VIS spectrophotometer. The test was performed in duplicates and the result is averaged. The antioxidant activity of the extracted

Phoenix dactylifera and *Senna alexandrina* as control are evaluated by using DPPH radical scavenging assay. For standard curve, 2 g ascorbic acid added with 20 mL of distilled water as a solution.

6 mL of methanol, 3 mL of DPPH and 1 mL of extract as a solution. Then, 2 mL and 20 mL distilled water. Next, repeat the step as for the standard curve. The DPPH solution should be protected from light by covering the flask with aluminium foil. The next step is followed by incubation at 37°C in the dark for 30 minutes.

2.2 Equations

The absorbance of the reaction mixture is measured at 517nm using a UV-VIS spectrophotometer. The test is performed in duplicates and the result is averaged. The scavenging activity is calculated as:

$$DPPH\ scavenging\ activity\ (\%\ inhibition) = \frac{OD\ control - OD\ sample}{OD\ control} \times 100$$

Equation 1.1

3. Results and Discussion

In this study, antioxidant activity of *Senna alexandrina* leaves and *Phoenix dactylifera* fruits by two analyses methods which are Total Phenolic Content (TPC) and the DPPH radical scavenging activity while the extraction method are Soxhlet extraction method and the solvents used for this experiment are ethanol and methanol. Next, the extracted antioxidants from *Senna alexandrina* leaves and *Phoenix dactylifera* fruits were compared with prepared butylated hydroxyanisole (BHA) by analyzing the homemade bread using FTIR.

3.1 Results and discussion

3.1.1 Total Phenolic Content (TPC)

Table 3.1: Comparison of total phenolic content in mg GAE/g of each antioxidant at different concentration and solvent

Concentration of extracts (mL), Solvent	Sample	Average Absorbance (%)	Standard Deviation	Error	Total Phenolic Content (mg GAE/g)
1 mL, Methanol	*P. dactylifera*	0.101	1.69967E-17	9.81308E-18	10.1
2 mL, Methanol	*P. dactylifera*	0.133	0.00057735	0.000333333	13.3
3 mL, Methanol	*P. dactylifera*	0.197	0.00057735	0.000333333	19.8
4 mL, Methanol	*P. dactylifera*	0.209	0	0	20.9
5 mL, Methanol	*P. dactylifera*	0.247	0	0	24.7
1 mL, Ethanol	*P. dactylifera*	0.129	0.002081666	0.00120185	12.9
2 mL, Ethanol	*P. dactylifera*	0.201	0.001732051	0.001	20.1
3 mL, Ethanol	*P. dactylifera*	0.288	0.001732051	0.001	28.8
4 mL, Ethanol	*P. dactylifera*	0.391	0	0	39.2
5 mL, Ethanol	*P. dactylifera*	0.533	0.001	0.00057735	53.3
1 mL, Methanol	*S. alexandrina*	0.675	1.35974E-16	7.85046E-17	67.5
2 mL, Methanol	*S. alexandrina*	1.042	0.00057735	0.000333333	104.2
3 mL, Methanol	*S. alexandrina*	1.237	0	0	123.7
4 mL, Methanol	*S. alexandrina*	1.395	2.71948E-16	1.57009E-16	139.5
5 mL, Methanol	*S. alexandrina*	1.489	0.00057735	0.000333333	148.9
1 mL, Ethanol	*S. alexandrina*	0.208	0.00057735	0.000333333	20.8
2 mL, Ethanol	*S. alexandrina*	0.331	0.00057735	0.000333333	33.1
3 mL, Ethanol	*S. alexandrina*	0.467	0	0	46.7
4 mL, Ethanol	*S. alexandrina*	0.638	0	0	63.8
5 mL, Ethanol	*S. alexandrina*	0.686	0	0	68.6

Total phenolic content (TPC) for different antioxidants were calculated after obtained their concentration from standard curve of gallic acid,y=0.001x. From Table 3.1, this test distinguished by using different concentration of extracts and type of solvents. For the concentration of 1 mL, the order of value of TPC in mg GAE/g from the highest to lowest are 67.5, 20.8, 12.9 and 10.1 for *S. alexandrina* in methanol (SM), *S. alexandrina* in ethanol (SE), *P. dactylifera* in ethanol (PE) and *P. dactylifera* in

methanol (PM) respectively. Thus, its shows that *S. alexandrina* have higher value of TPC than *P. dactylifera* in extracts concentration of 1 mL.

Next, for the 2 mL concentration, the orders of value of TPC in mg GAE/g from the highest to lowest are 104.2, 33.1, 20.1 and 13.3 for SM, SE, PE and PM respectively. In addition for 3 mL concentration, the orders of value of TPC in mg GAE/g from the highest to lowest are 123.7, 46.7, 28.8 and 19.8 for SM, SE, PE and PM. For the 4 mL concentration, the orders of value of TPC in mg GAE/g from the highest to lowest are 139.5, 63.8, 39.2 and 20.9 for SM, SE, PE and PM.

Last but not least, for the 5 mL concentration; the orders of value of TPC in mg GAE/g from the highest to lowest are 14.9, 68.6, 53.3 and 24.7 for SM, SE, PE and PM. In the case of wheat and barley grain results reported by Zhou and Yu showed that the contents of TPC were affected by the extraction solvents with the following order from high to low: acetone > ethanol > methanol, which can be used to explain lower amount of phenols in this experimental using ethanol rather than using acetone. [6]

3.1.2 DPPH Free Radical Scavenging Assay

Table 3.2: Comparison of DPPH Scavenging Activity (% inhibition) of each antioxidant at different concentration and solvent

Concentration (mL), Solvent	Sample	Average Absorbance (%)	Standard Deviation	Error	DPPH Scavenging Activity (% inhibition)
1 mL, Methanol	*P. dactylifera*	0.265	0	0	22.059
2 mL, Methanol	*P. dactylifera*	0.319	0.00057735	0.000333333	6.176
3 mL, Methanol	*P. dactylifera*	0.175	0.00057735	0.000333333	48.529
4 mL, Methanol	*P. dactylifera*	0.012	0.00057735	0.000333333	96.471
5 mL, Methanol	*P. dactylifera*	0.14	0.00057735	0.000333333	58.824
1 mL, Ethanol	*P. dactylifera*	0.165	0.001154701	0.000666667	51.471
2 mL, Ethanol	*P. dactylifera*	0.231	0.00057735	0.000333333	32.059
3 mL, Ethanol	*P. dactylifera*	0.084	0.00057735	0.000333333	75.294
4 mL, Ethanol	*P. dactylifera*	0.063	0.00057735	0.000333333	81.471
5 mL, Ethanol	*P. dactylifera*	0.056	0	0	83.529
1 mL, Methanol	*S. alexandrina*	0.117	0	0	65.588
2 mL, Methanol	*S. alexandrina*	0.092	0.00057735	0.067549981	72.941
3 mL, Methanol	*S. alexandrina*	0.088	1.69967E-17	0.053116225	74.118
4 mL, Methanol	*S. alexandrina*	0.097	1.69967E-17	0.050806824	71.471

5 mL, Methanol	*S. alexandrina*	0.163	0.00057735	0.056002976	55.882
1 mL, Ethanol	*S. alexandrina*	0.137	0.00057735	0.000333333	52.059
2 mL, Ethanol	*S. alexandrina*	0.087	0.00057735	0.000333333	59.706
3 mL, Ethanol	*S. alexandrina*	0.074	0.00057735	0.000333333	74.412
4 mL, Ethanol	*S. alexandrina*	0.101	0.00057735	0.000333333	78.235
5 mL, Ethanol	*S. alexandrina*	0 .163	1.69967E-17	9.81308E-18	70.294

DPPH Scavenging Activity (% inhibition) for different antioxidants was calculated by using: $DPPH\ scavenging\ activity\ (\%\ inhibition) = \frac{OD\ control - OD\ sample}{OD\ control} \times 100$ From Table 3.2, this test differentiated by using different concentration of extracts and type of solvents. The orders of value of DPPH scavenging activity (% inhibition) for 1 mL extract concentration from the highest to lowest are 65.588, 52.059, 51.471 and 22.059 for SM, SE, PE and PM. Next, the orders of value of DPPH scavenging activity (% inhibition) for 2 mL extract concentration from the highest to lowest are 72.941, 59.706, 32.059 and 6.176 for SM, SE, PE and PM.

In addition, the orders of value of DPPH scavenging activity (% inhibition) for 3 mL extract concentration from the highest to lowest are 74.118, 75.294, 74.412, 48.529 and 6.176 for SM, PE, SE and PM. Next, the orders of value of DPPH scavenging activity (% inhibition) for 4 mL extract concentration from the highest to lowest are 96.471, 78.235, 81.471, 71.471, for PM, SE, SM and PE. Last but not least, the orders of value of DPPH scavenging activity (% inhibition) for 5 mL extract concentration from the highest to lowest are 83.529, 70.294, 58.824, 55.882 for PE, SE, PM and SM. The DPPH radical is a preferred substrate for measuring antioxidant activity fast due to its stability in the radical state and simplicity of assay. According to recent research, phenolic chemicals extracted from plants may be responsible for the antioxidant action, particularly flavonoids, due to -OH groups, which possess redox properties. [7]

3.1.3 Fourier-transform Infrared Spectroscopy Analysis

Table 3.3: FTIR results of Senna alexandrina

S. No	Wave number cm^{-1} (in methanol solvent)	Wave number cm^{-1} (in ethanol solvent)	Wave number cm^{-1} (reference article)	Functional group assignment	Expected phytocompounds identified
1	3295.0	3332.2	>3000	sp^2 C-H stretch	Alkenes, Aromatic compounds
2	2944.6	2974.4	3600-3200 cm (strong and broad) 3500-3350 cm^{-1}, 3600-2500 cm^{-1}	O-H stretch, N-H stretch, OH of –CO2H	Alcohols, Amines, Carboxylic acids, Alkynes
3	2832.8	2888.7	3600-3200 cm-1 (strong and broad) 3500-3350 cm-1, 3600-2500 cm-2	O-H stretch, N-H stretch, OH of –CO2H	Alcohols, Amines, Carboxylic acids, Alkynes
4	1651.2	1654.9	3600-3200 cm-1 (strong and broad) 3500-3350 cm-1, 3600-2500 cm-1 3333-3267 cm-1	O-H stretch, N-H stretch, OH of –CO2H	Alcohols, Amines, Carboxylic acids, Alkynes
5	1449.9	1379.1	1680-1620 cm-1 (often weak)	C=C stretch	Alkenes
6	1110.7	1088.4	1680-1620 cm-1 (often weak)	C=C stretch	Alkenes
7	1073.5	1043.7	1680-1620 cm-1 (often weak)	C=C stretch	Alkenes
8	1013.8	879.7	1680-1620 cm-1 (often weak)	C=C stretch	Alkenes

Based on research article of Khadim, S., 2022, the wave number of methanol and ethanol solution with *Senna alexandrina* extract are 3295.0 cm^{-1} and 3332.2 cm^{-1} respectively shows the functional group of sp^2 C-H stretch and alkenes, aromatic compounds were expected phytocompounds at first series number [9][13]. For second, third and fourth series number, the wave numbers of methanol and ethanol solution with *Senna alexandrina* extract are 2944.0 cm^{-1} and 2974.4 cm^{-1}, 2832.8 cm^{-1} and 2888.7 cm^{-1}, 1651.2 cm^{-1} and 1654.9 cm^{-1} respectively indicates that the functional group of O-H

stretch, N-H stretch, OH of –CO2H and alcohols, amines, carboxylic acids and alkynes were expected phytocompounds.

For fifth, sixth, seventh, eighth series number, the wave numbers of methanol and ethanol solution with *Senna alexandrina* extract are 1449.9 cm^{-1} and 1379.1 cm^{-1}, 1110.7 cm^{-1} and 1088.4 cm^{-1}, 1073.5 cm^{-1} and 1043.7 cm^{-1}, 1013.8 cm^{-1} and 879.7 cm^{-1} respectively indicates that the functional group of C=C stretch and alkene were expected phytocompounds.

3.1.4 Observation of extracts and BHA on homemade bread

At the seventh day, all the bread already rotten and became smelly. The worst in term of rotten is the blank bread due to the absent of any addition of extract of antioxidant. Second, the SM bread already indicated several type of mould (black and white mould) and followed by SE. Next, the BHA bread also showed some white mould on top of the bread. Last but not least, both breads of PM and PE does not show any significant mould on top of the breads even though already have an unpleasant smell. All breads cannot undergoes any analysis such as DPPH assay, TPC or GC MS analysis due to the concern of the antioxidant content of the bread already mixed up other ingredients.

From the observation, we can see that the order of antioxidant activity from the highest to the lowest are *Phoenix dactylifera* in methanol solution, *Phoenix dactylifera* in ethanol solution, BHA, *Senna alexandrina* in ethanol solution, *Senna alexandrina* in methanol and finally the bread of the blank.

4. Conclusion

This study proposed to perform the determination of antioxidant activity of *Senna alexandrina* leaves and *Phoenix dactylifera* fruits extracts. *Senna alexandrina* has the maximum antioxidant activity for TPC, and methanol is used as the solvent in each concentration. This study proves that the content of TPC were affected by the extraction solvents with the following order from high to low due to the lower amount of phenols. The most highest antioxidant activity in DPPH scavenging assay by using UV-VIS spectroscopy is *Senna alexandrina* where methanol as a solvent and in 1mL concentration of extract in value of 65.59% whereas *Phoenix dactylifera* where ethanol as a solvent and in 5 mL concentration of extract in value of 83.53%. Next, the presence of antioxidant compound which were determined by using FTIR. The presence of antioxidant compound was determined by the presence of peaks of hydroxyl (-OH) group that is one of the functional group of phenolic compound by using infrared FTIR spectroscopy. Last but not least, both application of *S. alexandrina* and *P. dactylifera* on homemade bread may increase the lifespan of bread compared to the blank bread and even healthier compared to the BHA.

Acknowledgement

The author would also like to thank the Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia for its support.

References

[1] Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., Ali, H. M., Elshikh, M. S., Abdel-Salam, E. M., El-Esawi, M., & El-Ansary, D. O. (2018). Bioactivities of Traditional Medicinal Plants in Alexandria. Evidence-Based Complementary and Alternative Medicine, 2018. https://doi.org/10.1155/2018/1463579

[2] Alzain, M. N., Alatar, A. A., Alqarawi, A. A., Mothana, R. A., Noman, O. M., Herqash, R. N., Alsheddi, E. S., Farshori, N. N., & Alam, P. (2020). The influence of mycorrhizal fungion the accumulation of sennosides A and B in *Senna Alexandrina* and *Senna Italica*. Separations, 7(4), 1–13. https://doi.org/10.3390/separations7040065

[3] Jiao T, Li C, Zhuang X, Cao S, Chen H, Zhang S. The new liquid-liquid extraction method for separation of phenolic compounds from coal tar. Chemical Engineering Journal. 2015;266:148-155

[4] Mistrello, J., Sirisena, S. D., Ghavami, A., Marshall, R. J., & Krishnamoorthy, S. (2014). Determination of the antioxidant capacity, total phenolic and flavonoid contents of seeds from three commercial varieties of culinary dates. International Journal of Food Studies, 3(1), 34-44

[5] Kamarubahrin, A. F., & Haris, A. (2020). Nutritional and Potential Planting of Date Palm: Review of Recent Trends and Future Prospects in Malaysia. International Journal of Fruit Science, 20(S3), S1097–S1109. https://doi.org/10.1080/15538362.2020.1775160

[6] K. Zhou and L. Yu, "Effects of extraction solvent on wheat bran antioxidant activity estimation," LWT - Food Sci. Technol., vol. 37, no. 7, pp. 717–721, 2004. [4] J. U. Duncombe, "Infrared navigation - Part I: An assessment of feasibility," IEEE Trans. Electron. Devices, vol. ED-11, pp. 34-39, Jan. 1959 (Example for a journal article)

[7] Borkataky M, Kakoti B, Saikia L. Influence of total phenolic content and total flavonoid content on the DPPH radical scavenging activity of Costus speciosus (Koen Ex. Retz.) Sm. South Asian J Exp Biol. 2015; 4(5):261-266.

[8] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013.

[9] Cassens, R. G. (2008). Meat preservation: preventing losses and assuring safety. John Wiley & Sons.

[10] Flieger, J., Flieger, W., & Baj, J. (2021). Antioxidants: Classification , Natural Sources , Activity / Capacity

[11] Liang, W.; He, L.; Ning, P.; Lin, J.; Li, H.; Lin, Z.; Kang, K.; Zhang, Y. (+)-Catechin inhibition of transmissible gastroenteritis coronavirus in swine testicular cells is involved its antioxidation. Res. Vet. Sci. 2015, 103, 28 33.

[12] Diniz, L.R.L.; Bezerra Filho, C.D.S.M.; Fielding, B.C.; de Sousa, D.P. Natural Antioxidants: A Review of Studies on Human and Animal Coronavirus. Oxid. Med. Cell. Longev. 2020, 1–14.

[13] Khadim, S. (2022). Journal of Plant Biochemistry and Physiology Total Phenolic Profile and Antiviral Activity of *Senna Alexandrina*. 10(1000289), 1–9. https://doi.org/10.35248/2329-9029.22.10.289

[14] Freeman I.P., Padley F.H., W.L. Sheppard. J. Am Oil Chem Soc. 1973; 50: 101p.

[15] Pokorny J., Korczak J.. Preparation of Natural Antioxidant. In: Antioxidants in Food: Practical Applications, ed. Pokorny, J, Yanishlieva, N, and Gordon, M. England: Woodhead Publishing Ltd. Abington. 2001.