DEVOTION
Journal of Research and Community Service

Volume 4, Number 1, January 2023
e-ISSN: 2797-6068 and p-ISSN: 2777-0915

ANTIOXIDANT TEST, TOTAL ALKALOID CONTENT, ANTIMITOSIS TEST AND HPTLC TEST OF CHAMOMILE FLOWER EXTRACT (MATRICARIA CHAMOMILLA L.)

Ferdy Tantowi, David Limanan
Universitas Tarumanagara, Indonesia
E-mail: tantowiferdy@gmail.com, davidlimanan@gmail.com

KEYWORDS
Phytochemicals, DPPH, Alkaloids, Antimitosis, HPTlC

ABSTRACT
Oxygen is one of the supports of multicellular life and is necessary for aerobic metabolism. However, the use of oxygen can produce by-products, one of which is reactive oxygen species (ROS) which can also increase due to external factors such as exposure to ionizing rays, and vehicle smoke. When reactive oxygen species levels are high, the body can experience a condition called oxidative stress, which is a condition that can cause tissue damage. To overcome this, antioxidants are needed, namely compounds that can donate electrons to free radicals to neutralize their effects. Antioxidants are divided into endogenous antioxidants and exogenous antioxidants. Endogenous antioxidants are antioxidants found in the body. Exogenous antioxidants are antioxidants that come from outside the body. Because there is an increase in ROS levels due to external influences, an increase in exogenous antioxidants is needed, one of which is chamomile flowers. This study evaluated qualitative phytochemical levels using the Harnborne method, total antioxidant capacity using the Blois method, total quantitative levels of alkaloids using the Trivedi et al method, BSLT toxicity using the Meyer method, and fingerprint analysis using HPTLC. The obtained chamomile flowers are dried and extracted by maceration using methanol solvent. The test results on chamomile flowers revealed phytochemical content in the form of alkaloids, betasianin, cardioglycosides, coumarins, flavonoids, phenols, quinones, saponins, steroids, tepenoids and tannins. Antioxidant ability 209.27 g/ml; Total alkaloid content of 12.62 g/ml; toxicity 174.39 g/mL; As well as fingerprint analysis chamomile flowers have active ingredients. Therefore, chamomile flowers can serve as a moderate antioxidant with antimitotic activity.

INTRODUCTION

Oxygen is a molecule that greatly affects chemical reactions in the body. Oxygen has four known oxidation states. Namely (O2)n, where n=0 (dioxygen, O2); n=+1 (dioxygen cation, O 2+); n=1 (superoxide ion, O 2•-); n=2 (dianion peroxide, O 2•2−). Superoxide ions are the most common precursors of reactive oxygen species (ROS) metabolites, and ROS is a highly (Hayyan, Hashim, & AlNashef, 2016) reactive oxygen-derived substance such as superoxide and hydrogen peroxide ions. The formation of ROS can be divided into endogenous, that is, the (Murphy et al., 2022) result of by-productsof mitochondrial metabolism, or exogenous, such as exposure to ionizing rays, drugs, pollution of the lingkungan (Cosentino, Plantamura, Cataldo, & Iorio, 2019). High levels of ROS in the body can cause oxidative stress.

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidants. Which can occur due to an increase in ROS levels or a decrease in antioxidant levels. Oxidative stress can cause different types of

macromolecular damage in the body such as (Aguiar et al., 2012) lipids, proteins, carbohydrates, and nucleic acids that (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012) can cause diseases such as Alzheimer's, cancer, autoimmune. Therefore, antioxidants are needed that can prevent damage due to oxidative stress.

Antioxidants can be divided into exogenous and endogenous antioxidants based on their source, enzymatic and non-enzymatic structure, fat-soluble and fat-insoluble, intracellular or extracellular in an organism (Evans & Halliwell, 2001). From Source, endogenous antioxidants are antioxidants produced in the body, while exogenous antioxidants are antioxidants obtained from food sources. Together, exogenous and endogenous antioxidants maintain redox homeostasis such as when regenerating vitamin E by glutathione, or vitamin C which prevents lipid peroxidation processes (Bouayed, Rammal, & Soulimani, 2009).

Chamomile flowers are flowers described by Hippocrates as a medicinal plant having a wide variety of benefits, including: fever, inflammation, muscle spasms, menstrual disorders, ulcers, pain due to rheumatism (Santos-Sánchez, Salas-Coronado, Villanueva-Cañongo, & Hernández-Carlos, 2019). The flowers contain phenolic compounds, in particular flavonoids apigenin, quercetin, patuletin and luteolin. Phenolic compounds are known to have potential as antioxidants and antimitosis, thus prompting research to further find out the antioxidant (Chauhan & Jaya, 2017) and antimitotic capabilities of chamomile flowers, which are expected to be a source of new properties.

RESEARCH METHODS

Manufacture of Chamomile Flower Extract

The sample obtained is dried. Dry samples are crushed using a blender mixer until they become powder or simplicia. Then the extract is made using maceration technique using methanol extract until the top of the simplicia is submerged, the mixture is stirred every morning and evening. After two days, the extract is accommodated, and into the maceration tube is added methanol Back, this process is repeated twice. Then the extract obtained is evaporated using a rotary evaporator.

Research Design

This research is included in in vitro experimental research and bioassay. Research conducted in vitro tests consists of phytochemical tests, antioxidant capacity tests, total alkaloid tests, HPTLC fingerprint analysis and bioassay research in the form of antimitosis tests with BSLT(BLaws, 1958) (Trivedi, Patel, Rathnam, & Pundarikakshudu, 2006) (Meyer et al., 1982)

Antioxidant

In the antioxidant examination, DPPH is used as a radical compound used to measure absorbance in a UV-Vis spectrophotometer. Maximum wavelength examination of DPPH compounds is carried out, then absorbance data of flower extracts with comparison extracts, namely vitamin C, is carried out and calculations are carried out in order to obtain the value of inhibitory concentrate

Determination of the Length of Gelombang Maximum

DPPH with a concentration of 50 µM was taken as much as 3.5 mL and added 0.5 mL methanol then left in a darkroom for 30 minutes. Then the solution is read at a wavelength absorbance of 400-800 nm. The solution is prepared in the form of duplo.

Determination of Standar Vitamin C

Vitamin C with a concentration of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/ mL, and 10 µg/mL is inserted into test tubes of 0.5 mL each and DPPH of 3.5 mL is added. then incubated in a darkroom for 30 minutes and read at maximum wavelength

Penentuan Standar Ekstrak Bunga Chamomile

Chamomile flower extracts with concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL were put in test tubes of 0.5 each and DPPH of 3.5 mL were added. then incubated in a darkroom for 30 minutes and read at maximum wavelength

Penentuan Aktivitas Antioksidan Ekstrak Bunga Chamomile

Antioxidant activity is measured by the absorbance of DPPH radicals inhibited using the formula:

Information:

Abs. Control = radical DPPH uptake of 50 µM at optimal wavelength

Abs. Sample = sample uptake in radical DPPH of 50 µM at optimal wavelength

The antioxidant concentration value of the sample was calculated using the linear equation Y = aX + b then in the variable y entered the number 50 so as to produce IC 50 on variable X.

Alkaloid

Manufacture of Larutan Standar Berberine Chloride

Berberine chloride with a concentration of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL using metaanol solvent. then added 5mL to phosphate with pH 4.7 and 5mL BCG (bromocresol green). The solution is then put into a flask and allowed to stand until two layers are formed. After that, the bottom layer is accommodated and chloroform is added up to 10mL so that the final concentrations of 2 µg / mL, 4 µg / mL, 6 µg / mL, 8 µg / mL, and 10 µg / mL are then measured at one of the concentrations and absorbance checks are carried out at each concentration and the results are made linear line equations.

Uji Kadar Alkaloid Ekstrak Bunga C

hamomile

A total of 50 mg of chamomile flower extract was added with 5mL of phosphate with a pH of 4.7, 3 mL of hydrochloric acid, and 5 mL of BCG. Then the solution is introduced into the separator flask and homogenized and waited until two layers are formed. The bottom is then accommodated and chloroform is added to 10mL. The test was carried out duplo and alkaloid levels were calculated using the linear line equation of berberine chloride solution

Antimitosis

Hatching of artemia salina shrimp larvae for 2 x 24 hours was carried out in a tube given an aerator and illuminated by a lamp. After that, a concentration of chamomile flower extract was made of 50 µg / mL, 100 µg / mL, 150 µg / mL, 200 µg / mL , and 250 µg / mL, respectively. Then in each tube were inserted shrimp larvae that had hatched as many as 10 heads and allowed to stand for 24 hours with a light illuminated. This action is carried out with duplo. Then the mortality percentage of shrimp larvae was carried out at each concentration and a linear line equation curve was made

HPTLC

A total of 10 mg of chamomile flower extract is dissolved with 1 mL of methanol resulting in a concentration of 10mg/mL. then a sample application was carried out on a silica gel plate of 10 µL with a CAMAG Linomat 5 applicator. In addition, solanesol

standards are also used as a terpenoid comparison in samples. After that, the sample was put into the CAMAG Automatic Development 2 development chamber with N-hexan solvent and ethyl acetate in a ratio of 1.5:0.5 until a total volme of 35 mL was obtained and left for 30 minutes. After that, the plate is sprayed with vanillin-phosphoric acid reagent and put in an oven at 100 °C for 10 minutes. Then the plate is removed and checked for documentation with CAMAG TLC Scanner and CAMAG TLC Visualizer.

RESULTS AND DISCUSSION

Phytochemical Test

The results of phytochemical tests on chamomile flower extract showed positive results on alkaloid compounds, betasianin, cardioglycosides, coumarins, flavonoids, phenolics, quinones, saponins, steroids, tepenoids, and tannins (Table 1). Plants that contain phytochemism can function as antioxidants (M. T. Lee, Lin, Yu, & Lee, 2017)

Table 1
Phytochemical Content

Phytochemicals	Methods/Reagents	Chamomile Flower Extract
Alkaloid	Mayer	+
Anthocyanins	NaOH	-
Betasianin	NaOH	+
Kardioglikosida	Keller-Kiliani	+
Koumarin	NaOH	+
Flavonoid	NaOH	+
Glycosides	Modified Borntrager	-
Phenolic	Folin Ciocalteau	+
Kuinon	H2SO4	+
Saponin	Foam Test	+
Steroid	Liebermann-Burchard	+
Tepenoid	Liebermann-Burchard	+
Tannin	Ferric-Chloride	+

Antioxidant Capacity Test

The maximumDPPH wave event is 516 nm with a maximum absorbance of 0.546. Then a review of chamomile flower extract and vitamin C standards was carried out using the wave p. The result is made a curve with the X axis as the concentration and the Y axis is inhibition. The linear line equation of vitamin C is Y = 6.934*X + 12.52 with R^2=0.9988, from the linear equation obtained IC_{50} standard vitamin C is 5.4 µg/mL. and the linear line equation of chamomile flowers is Y = 0.1680*X + 14.61 with R 2 = 0.9801. With the linear line equation, the IC value of $_{50}$ chamomile flower extract was obtained at 209.27 µg / mL.

Table 2
Vitamin C Standards

Concentration	%Inhibition (%)	IC_{50}(µg/mL)
2	26,85	
4	39,11	

6	54,97	5,4
8	67,87	
10	81,81	

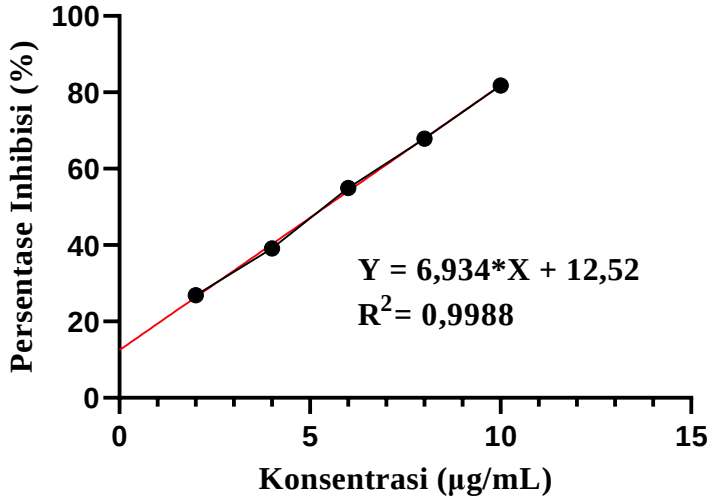

Figure 1
Vitamin C Standard Curve

Table 3
Antioxidant Capacity Test of Chamomile Flower Extract

Concentration	%Inhibition(%)	IC50(µg/mL)
100	29,396	
200	47,161	
300	68,498	209,27
400	86,081	
500	93,956	

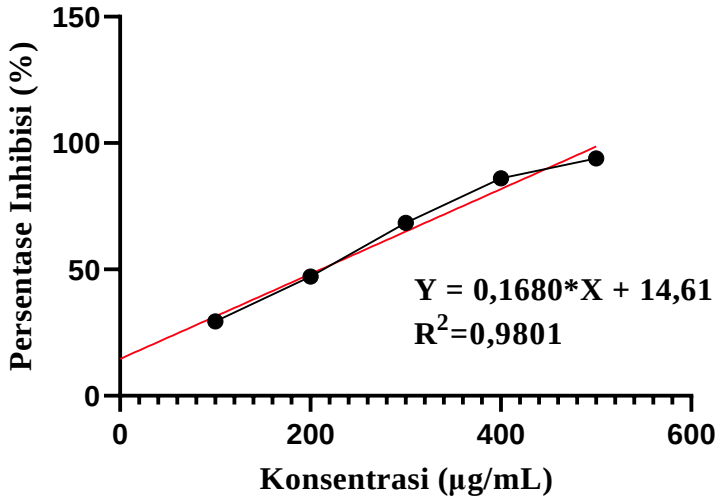

Figure 2
Curve Chamomile Flower Extract

The DPPH method was used in this study to test the total antioxidant capacity. Chamomile flower extract and a comparator standard of vitamin C or ascorbic acid were used to obtain a comparison of the total antioxidant capacity by calculating an IC value of$_{50}$. An IC value of 50 is the concentration required concentration required to inhibit 50% of DPPH activity. The lower the IC value$_{of\ 50}$ the higher the antioxidant capacity in the extract. In this study, the IC value of 50 chamomile flower extract was 209.27 µg / mL and IC $_{50}$ ascorbic acid, namely 5.4 µg / mL which makes chamomile flowers have a moderate antioxidant potential of 101-150 ppm. Based on these results, it can be concluded that ascorbic acid is a more potent source of antioxidants. However, excessive consumption of ascorbic acid can cause indigestion while chamomile flowers can be consumed by people with indigestion (Sukweenadhi, Setiawan, Yunita, Kartini, & Avanti, 2020)(Joon Kyung Lee et al., 2018) (Albrecht, Müller, Schneider, & Stange, 2014)

Alkaloid Test

Berberine chloride testing was carried out as a standard alkaloid and chamomile flower extract with a wavelength of 420 nm to obtain absorbance. Then a linear line equation is made for the *berberine chloride* standard with the X axis as the concentration and the Y axis as the absorbance. Then a curve is made for the standard and the linear equation Y = 0.09105*X – 0.09590 with R^2 = 0.9857 is obtained. From the linear equation can be determined the total alkaloid content of chamomile flowers

Table 4
Standar Berberine Chloride

Standard Levels of Berberine Chloride (µg/mL)	Absorbansi (λ = 420 nm)
2	0,13
4	0,22
6	0,43
8	0,65
10	0,83

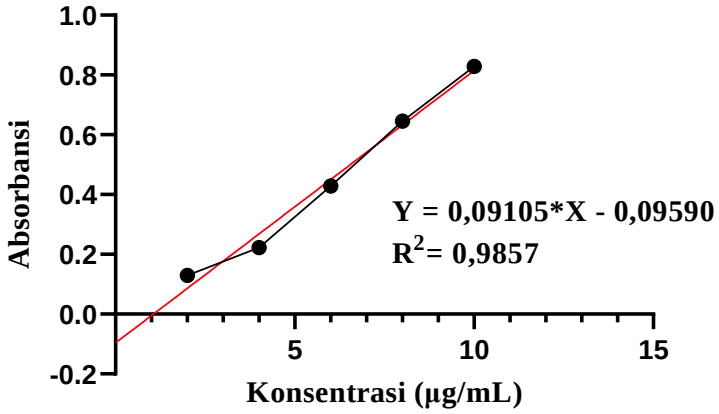

Figure 3
Berberine Chloride Standard Curve

Table 5
Alkaloid Test of Chamomile Flower Extract

Tube	Absorbance	Alkaloid Levels (µg/mL)	Average Rate Alkaloid (µg/mL)
I	0,468	6,1933	
II	0,489	6,4239	6,30

Berberine chloride was used as a standard in this study to obtain alkaloid levels of chamomile flower extract. The standard R^2 value of *berberine chloride* is 0.9857 so it has a high level of confidence. The alkaloid level in the examination was diluted 1: 2, so that the alkaloid content of chamomile flower extract was obtained at 12.6 µg / mL.

Antimitosis

After recording deaths at each concentration, a calculation of the percentage of deaths and a log of larval concentrations were then made. Then a curve is created where the X axis is the concentration log and the Y axis is the percentage of deaths. From the curve, the linear line equation Y = 168.7*X - 323.5 and the value R^2 = 0.9770 are obtained.

Table 6
Antimitosis Test

Concentration (µg/mL)	Concentration Log	%Death	LC_{50} (µg/mL)
100	2	17,30	
150	2,18	39,13	
200	2,3	62,22	174,39
250	2,4	85,41	

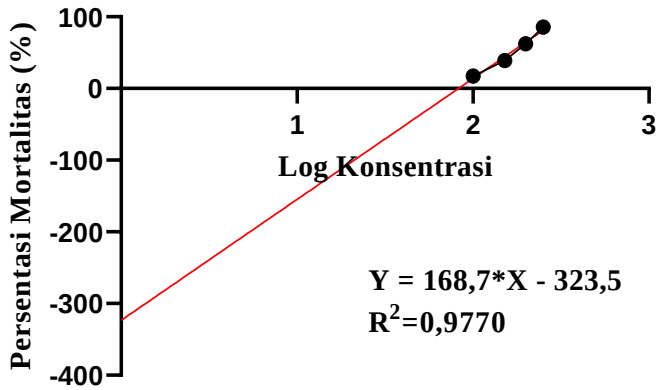

Figure 4
Antimitosis Test Curve

The curve results show that the R^2 result in this study is 0.9770 so it has a high level of accuracy and can be trusted. Based on the linear line equation, the higher the concentration log, the higher the percentage of larval mortality. The LC value of $_{50}$ was obtained, which is the level needed to kill 50% of the flower extract population is 174.39.

The LC value of $_{50}$<1000 has antimitosis activity that makes chamomile flowers have the potential to become antimitosis compounds (Meyer et al., 1982)

HPTLC

Samples are documented before and after derivatization. Prior to derivatization, images were shot at 256 nm, 366 nm, and visible light waves. After derivatization, documentation was carried out using visible light, as well as densitogram analysis of flower extracts and solanesol standards

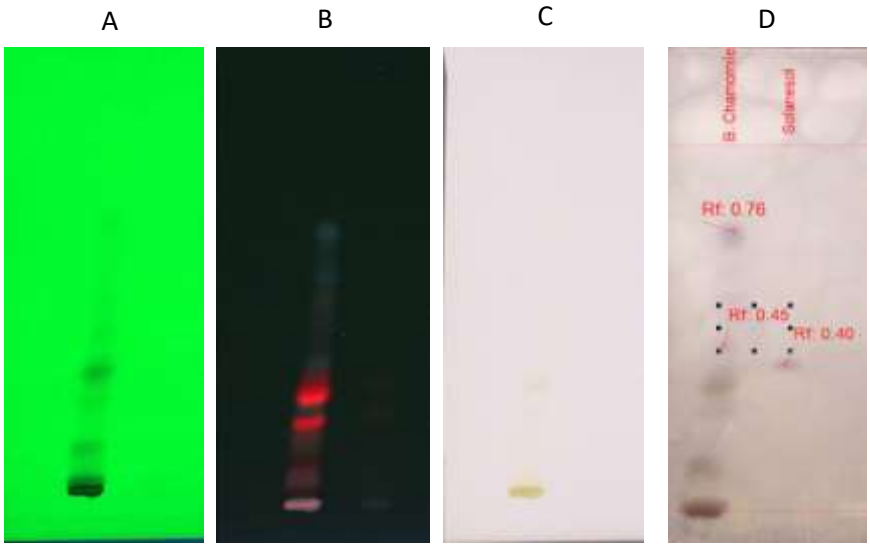

Figure 5
Chromatogram of Chamomile Flowers (left) and Solanesol (right) at 256 nm (A); 366 nm (B); Visible Light (C); and Visible Light After Derivatization

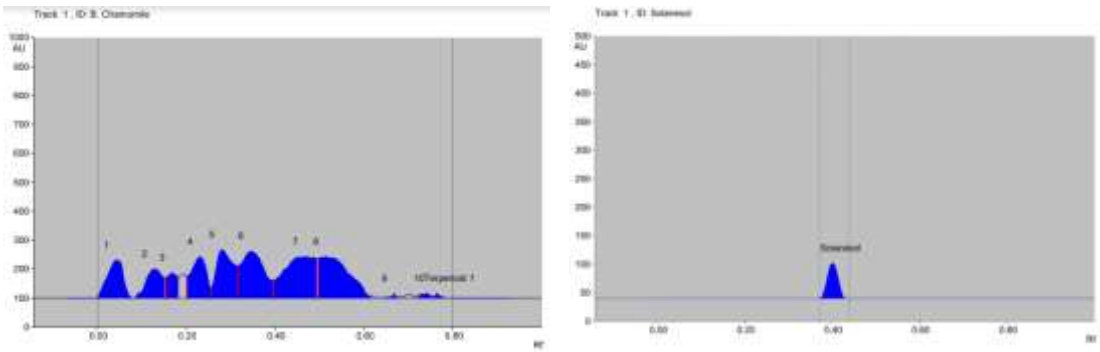

Figure 6
Densitograms of Chamomile Flowers (left) and solanesol (right)

Table 7
Densitogram of Chamomile Flowers

Peak	Rf	High (AU)	Area (AU)	*Assigned Substance*
1	0.04	130.2	3758.8	Unknown
2	0.13	99.6	2650.1	Unknown
3	0.17	86.6	1572.4	Unknown

4	0.23	141.5	3950.3	Unknown
5	0.28	166.8	5174.8	Unknown
6	0.35	160.7	6727.4	Unknown
7	0.47	141.7	8003.8	Unknown
8	0.51	142.1	7798.5	Unknown
9	0.67	13.5	94.1	Unknown
10	0.74	15.0	252.5	Unknown
11	0.76	14.2	125.0	Terpenoid

Table 8
Densitogram Solanesol

Peak	Rf	High (AU)	Area (AU)	*Assigned Substance*
1	0.40	61.6	1237.2	Solanesol

There is a blue band at an Rf value of 0.76 (Figure 5) which indicates that the extract contains terpenoids. However, when compared with solanesol with an Rf value of 0.40 (Table 8, Figure 5D) it can be concluded that chamomile flowers do not contain terpenoid compounds in them. In densitogram testing, 11 peaks were found where 1 peak was terpenoid (at peak 11) and the other 10 peaks were unidentified active compounds (Table 7). In another study, it was found that kandugan bisabolol and chamazulene are derivatives of terpenoids with an Rf range ranging from 0.75-0.9 (Agatonovic-Kustrin & Ortakand, 2015)

CONCLUSION

Chamomile flowers have moderate antioxidant activity with an IC value of 50 209.27 µg / mL, the total alkaloid content with berberine chloride comparison standards is 12.62 µg / mL, the antimitotic activity of flowers gets an LC value of 50 of 174.39 so that it has antimitotic activity, and examination using HPTLC found the presence of 11 active compounds of which one is terpenoid.

REFERENCES

Aguiar, Carlos Clayton Torres, Almeida, Anália Barbosa, Araújo, Paulo Victor Pontes, Abreu, Rita Neuma Dantas Cavalcante De, Chaves, Edna Maria Camelo, Vale, Otoni Cardoso Do, Macêdo, Danielle Silveira, Woods, David John, Fonteles, Marta Maria De França, & Vasconcelos, Silvania Maria Mendes. (2012). Oxidative Stress And Epilepsy: Literature Review. *Oxidative Medicine And Cellular Longevity*, *2012*.

Albrecht, Uwe, Müller, Valentina, Schneider, Berthold, & Stange, Rainer. (2014). Efficacy And Safety Of A Herbal Medicinal Product Containing Myrrh, Chamomile And Coffee Charcoal For The Treatment Of Gastrointestinal Disorders: A Non-Interventional Study. *BMJ Open Gastroenterology*, *1*(1), E000015.

Birben, Esra, Sahiner, Umit Murat, Sackesen, Cansin, Erzurum, Serpil, & Kalayci, Omer. (2012). Oxidative Stress And Antioxidant Defense. *World Allergy Organization Journal*, *5*(1), 9–19.

Bouayed, Jaouad, Rammal, Hassan, & Soulimani, Rachid. (2009). Oxidative Stress And Anxiety: Relationship And Cellular Pathways. *Oxidative Medicine And Cellular Longevity*, *2*(2), 63–67.

Chauhan, Ekta Singh, & Jaya, Aishwarya. (2017). Chamomile An Ancient Aromatic Plant-A Review. *J. Ayurveda Med. Sci*, *2*(4), 251–255.

Cosentino, Giulia, Plantamura, Ilaria, Cataldo, Alessandra, & Iorio, Marilena V. (2019). Microrna And Oxidative Stress Interplay In The Context Of Breast Cancer Pathogenesis. *International Journal Of Molecular Sciences*, *20*(20), 5143.

Evans, Patricia, & Halliwell, Barry. (2001). Micronutrients: Oxidant/Antioxidant Status. *British Journal Of Nutrition*, *85*(S2), S67–S74.

Hayyan, Maan, Hashim, Mohd Ali, & Alnashef, Inas M. (2016). Superoxide Ion: Generation And Chemical Implications. *Chemical Reviews*, *116*(5), 3029–3085.

Lee, Joon Kyung, Jung, Sang Hyuk, Lee, Sang Eun, Han, Joo Hui, Jo, Eunji, Park, Hyun Soo, Heo, Kyung Sun, Kim, Deasun, Park, Jeong Sook, & Myung, Chang Seon. (2018). Alleviation Of Ascorbic Acid-Induced Gastric High Acidity By Calcium Ascorbate In Vitro And In Vivo. *The Korean Journal Of Physiology & Pharmacology*, *22*(1), 35–42.

Lee, M. T., Lin, W. C., Yu, B., & Lee, T. T. (2017). Antioxidant Capacity Of Phytochemicals And Their Potential Effects On Oxidative Status In Animals—A Review. *Asian-Australasian Journal Of Animal Sciences*, *30*(3), 299.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. J., & Mclaughlin, Jerry L. (1982). Brine Shrimp: A Convenient General Bioassay For Active Plant Constituents. *Planta Medica*, *45*(05), 31–34.

Santos-Sánchez, N. Francenia, Salas-Coronado, Raúl, Villanueva-Cañongo, Claudia, & Hernández-Carlos, Beatriz. (2019). Antioxidant Compounds And Their Antioxidant Mechanism. *Antioxidants*, *10*, 1–29.

Sukweenadhi, Johan, Setiawan, Finna, Yunita, Oeke, Kartini, Kartini, & Avanti, Christina. (2020). Antioxidant Activity Screening Of Seven Indonesian Herbal Extract. *Biodiversitas*, *21*(5), 2062–2067.

Trivedi, Priti D., Patel, Brijesh C., Rathnam, Shivprakash, & Pundarikakshudu, Kilambi. (2006). A Validated Liquid Chromatographic Method For Estimation Of Solasodine In Various Solanum Species And Market Formulations. *Journal Of AOAC International*, *89*(6), 1519–1523.