Science Midwifery, Vol 10, No. 5, December 2022 ISSN 2086-7689 (Print) | 2721-9453 (Online)

Contents lists available at IOCS

Science Midwifery

journal homepage: www.midwifery.iocspublisher.org

Antioxidant Activity of Ethanol and Methanol Extracts of Black Turmeric (Curcuma Caesia Roxb.) Using the DPPH (2,2-Diphenyl-1-Picrylhidrazyl) Method

Ni Nyoman Wahyu Udayani[1*], Ketut Agus Adrianta[2], IGAA Kusuma Wardani[3]

[1,2,3] Departemen Farmakologi dan Farmasi Klinis, Fakultas Farmasi, Universitas Mahasaraswati, Denpasar

ARTICLE INFO

Article history:

Received Nov 21, 2022
Revised Nov 30, 2022
Accepted Dec 22, 2022

Keywords:

Antioxidant
Curcuma caesia (Roxb.)
DPPH *(2,2-Difenil-1-Pikrilhidrazil)*
UV-Vis Spectrophotometry

ABSTRACT

Free radicals are highly reactive compounds in the body that can cause degenerative diseases. One mechanism that can counteract free radicals is antioxidants. Natural antioxidants can be obtained from traditional drinks made from plants that contain active antioxidant compounds such as black turmeric (*Curcuma caesia* Roxb.) from the Zingiberaceae family. This study aims to determine the antioxidant activity of black turmeric extract. Before testing the antioxidant activity, qualitative and quantitative analysis were carried out. Qualitative analysis was carried out by phytochemical screening. The antioxidant activity of black turmeric extract was tested using the DPPH method. This test begins with the manufacture of extracts and preparation of test samples, then measurement of free radical scavenging activity using a UV-Vis spectrophotometer is carried out. Based on the results of the percentage measurement, the linear regression equation for the ethanol extract was obtained, namely y = 0.1526x + 0.9105 and the value of R2 = 0.9965, and for the methanol extract, namely y = 0.2376x + 1.6809 and the value of R2 = 0.996. According to the linear regression equation, black turmeric extract has antioxidant activity, which is categorized as a very weak antioxidant with an IC50 of 321,687 ppm for ethanol extract and 203, 363 ppm for methanol extract.

Corresponding Author:

Ni Nyoman Wahyu Udayani,
Departemen Farmakologi dan farmasi Klinis,
Universitas Mahasaraswati Denpasar,
Jalan Kamboja No 11A, Denpasar, 80231, Bali,
Email: udayani.wahyu@unmas.ac.id

INTRODUCTION

Natural products have been used for a long time to treat and relieve the symptoms of different diseases once they are sources of bioactive compounds (Juliana Isis Araújo Pereira, 2022). Today most people are not aware of the existence of free radicals that are harmful to the human body. Environmental factors such as pollution, excessive Ultraviolet (UV) light intensity, temperature, chemicals and nutritional deficiencies can cause the human body to be exposed to free radicals. Free radicals are compounds that have unpaired electrons. Free radicals can cause various diseases such as cancer, diabetes, cardiovascular disease, and other generative diseases (Das S, Mondal P,

2013), (Akar Z, Küçük M, 2017). One mechanism that can counteract or reduce free radicals is antioxidants (Atasoy, N., & Yücel, 2021). Antioxidants are substances that can prevent or slow damage to living cells caused by free radicals, which are unstable molecules the body produces as a reaction to environmental and other pressures (Shalaby, 2019). Antioxidants based on the source are classified into two, namely natural antioxidants and synthetic antioxidants (Flieger J, Flieger W, Baj J, 2021). Natural antioxidants are the result of the extraction of natural ingredients, while synthetic antioxidants are antioxidants obtained from chemical reactions (Lourenço SC, Moldão-Martins M, 2019), (Setiawan, Vendra, 2021), (Jelita Rahma Hidayati, Ervia Yudiati., 2019). Currently, the use of synthetic antioxidants is starting to be limited due to their negative impact on health, so it is necessary to use safer alternatives of antioxidants derived from natural or herbal ingredients (Das S, Mondal P, 2013). Indonesia is a tropical country that has abundant biodiversity (Kristina von Rintelen, Evy Arida, 2017). This abundant biological potential needs to be explored and utilized for the health and welfare of the Indonesian people. The World Health Organization (WHO) states that about 80% of the world's population uses traditional medicines derived from plants for both prevention and treatment (Verma, R.K., G. Mishra, P. Singh, K.K. Jha, 2011a). Based on empirical data, it has been proven that traditional medicine using natural ingredients is very safe and effective to improve the health of many people and is preventive (preventive) and healing (curative) (Tilaar, Martha dan Widjaja, 2014).

One plant that has the potential as a natural antioxidant is black turmeric (Curcuma caesia Roxb.). Black turmeric (Curcuma caesia Roxb.) has potential as a medicinal plant because it contains bioactive compounds such as flavonoids, phenols and alkaloids (Fong, 2012). *Curcuma caesia* Roxb. (black turmeric) is a perennial, underutilized, medicinal herb, which belongs to the family Zingiberacea and endangered to South east Asia (Angana Borah, Manabi Paw, 2019). *Curcuma caesia* Roxb (*C. caesia,* Zingiberacea) is a perennial herb with bluish black rhizomes commonly known as black turmeric and are traditionally used in treatment of various ailments and metabolic disorders like leukoderma, asthma, tumours, piles, bronchitis, etc (Mamta Yadav, 2019).In various studies, it is known that some secondary metabolites of plants are able to act as antioxidants. According to research by Zuraidah (Zuraidah, Sulistiyani, 2015), flavonoids are a group of secondary metabolites produced by plants which are included in the large group of polyphenols. Flavonoids have the ability to scavenge free radicals and inhibit lipid oxidation. Flavonoids and phenols have a linear contribution to antioxidant activity, so the higher the levels, the better the antioxidants. There are many methods for determining free radical activity, one of the methods commonly used is in vitro using the DPPH (2,2-diphenyl-1-picrylhydrazil) method. The parameter used is IC50, which is the sample concentration that can cause the absorbance of DPPH to decrease by half which is calculated based on the linear regression equation (Melannisa, Rosita. Muhammad Da'I, 2011). Based on the above explanation, related to the phytochemical content of black turmeric (Curcuma caesia Roxb.) it is necessary to conduct research on the antioxidant activity of black turmeric extract (Curcuma caesia. Roxb.) with ethanol and methanol as solvents.

RESEARCH METHOD

Making black turmeric extract, black turmeric extract was prepared using the maceration method. Each black turmeric powder was weighed as much as 50 g, dissolved in 250 ml of 80% ethanol solvent, and put in an Erlenmeyer 500 ml of a mixture of black turmeric powder with solvent then shaken 2 times for 5 minutes and macerated for 2 x 24 hours. The solution was filtered using whatman paper no. 42. The filtrate obtained was then evaporated using a rotary vacuum evaporator with the aim of evaporating the solvent mixed with the material during the extraction process.

Preparation of 0.1 mM. DPPH test solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (BM 394.32 g/mol) powder was weighed as much as 0.0039 g, then dissolved slightly with methanol p.a then put into a 100 ml volumetric flask, then the volume was made up to the mark with methanol p.a then homogenized. Determination of the maximum wavelength of 0.1 mM . DPPH, pipette 2 mL of 0.1 mM DPPH solution, put in a 10 mL volumetric flask, added with methanol p.a to the mark, then homogenized and allowed to stand for 30 minutes at room temperature. The absorption was measured at a wavelength of 400-800 nm by using a UV-Vis spectrophotometer. Preparation of gallic acid standard solution, weighed as much as 0.01 grams of gallic acid p.a then put into a 100 ml volumetric flask. Add methanol p.a into the volumetric flask up to the mark and then homogenized to obtain a 100 ppm ascorbic acid solution. Furthermore, from the stock solution of 100 ppm, a concentration variation of 0 was made; 0.4; 0.8; 1,2; 1.6 and 2.0 ppm as much as 25 ml. then 0.5 ml of each concentration was pipetted and then put into a measuring flask and added with 3.5 ml of 0.1 mM DPPH solution. The mixture was then incubated for 30 minutes in a place protected from light. Each concentration was then measured its absorption at the maximum wavelength using a UV-vis spectrophotometer

Preparation of black turmeric extract solution, weighed 0.01 grams of the sample and then extracted using 10 ml of methanol p.a, the solution was then homogenized and centrifuged for 15 minutes with a rotation of 3000 rpm. The solution obtained was then filtered and the filtrate was added to a methanol solution to a volume of 10 ml to obtain a sample solution of 1 mg/ml. 0.5 ml of the solution was pipetted and then put into a reaction tube. 3.5 ml of 0.1 Mm DPPH solution was added and then incubated for 30 minutes in a place protected from light. Measure the absorption at the maximum wavelength using a spectrophotometer. The inhibitory percentage values were calculated by comparing the absorbance values of the control and test samples (Sudarshana Borah & Sharma, 2020).

Calculation of antioxidant capacity, from the measurement results of the standard gallic acid absorption and the sample, the antioxidant capacity was calculated. Before calculating the antioxidant capacity, the concentration of the sample was determined based on the equation of the line obtained from the vitamin-c standard. Furthermore, after obtaining the concentration, the antioxidant capacity of each sample was measured. Calculation of antioxidant capacity is carried out with the following formula :

$$\%\text{immersion} = \frac{\text{control absorbance - test sample absorbance}}{\text{control absorbance}} \text{ x } 100\%$$

Information:
Control absorbance = absorbance of control solution at maximum wavelength
Sample absorbance = absorbance of the test solution at maximum wavelength
From the percentage value of attenuation at each concentration, then a regression curve is made, so that the equation y = bx + a will be obtained and the IC50 value will be obtained by linear regression calculations where the extract concentration (ppm) is the abscissa (x axis) and the percentage damping value is as ordinate (y axis). The IC50 value is obtained from the calculation of the 50% damping percent.

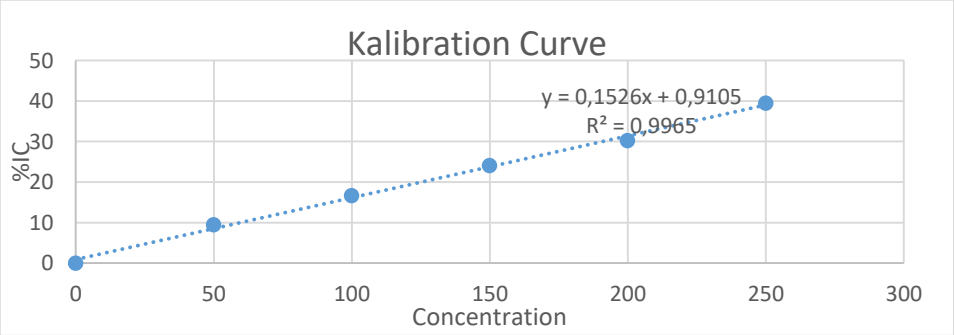
RESULTS AND DISCUSSIONS

Indonesia is a tropical country that has abundant biodiversity. This abundant biological potential needs to be explored and utilized for the health and welfare of the Indonesian people. Based on empirical data, it has been proven that traditional medicine using natural ingredients is very safe and effective in improving the health standards of many people and is both preventive and curative (Tilaar 2014). One of the plants that has the potential as an antioxidant is the rhizome of

black turmeric (Curcuma caesia Roxb.) which comes from the Zingiberaceae family. Black turmeric (Curcuma caesia Roxb.) has potential as a medicinal plant because it contains bioactive compounds such as flavonoids, phenols and alkaloids which can fight free radicals such as DPPH (Fong 2012), (Pakkirisamy M, Kalakandan SK, 2017). Curcuma caesia have got wide range of attention on various antipyretic, analgesic, antimutagenic, neuropharmacological, antibacterial and antifungal pharmacological activities. It is well known for its peculiar characteristics other than yellow turmeric. With the increasing trend on natural products in modern phytomedicines, the antioxidant and cytotoxic activities of Curcuma caesia Roxb. rhizome fractions were evaluated (Munawaroh & Handayani, 2010)(Mina K.C, 2021). Testing the antioxidant activity of black turmeric ethanol extract was carried out using the UV-Vis spectrophotometer method (Munteanu, I.G.; Apetrei, 2021). The absorbance value of DPPH at each concentration of the test solution can be seen in table 4.1.

Table 1 Absorbance of DPPH on the concentration of black turmeric ethanol extract

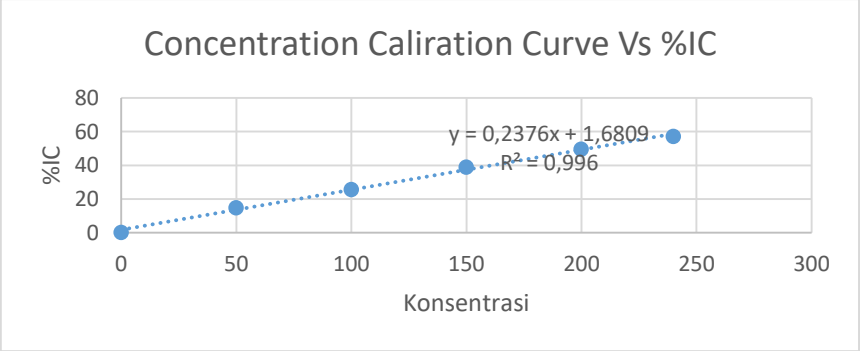
Concentration mg/L	Abs	IC (%)	Linier Regression Equation	IC_{50} (ppm)
0	0,727	0		
50	0,658	9,491		
100	0,606	16,644	0.1526x + 0.9105	321,687
150	0,552	24,072	R^2 = 0.9965	
200	0,507	30,261		



Picture. 1 Linear regression equation curve of black turmeric ethanol extract

Table 2 Absorbance of DPPH on the concentration of black turmeric methanol extract

Concentration mg/L	Abs	IC (%)	Linier Regression Equation	IC_{50} (ppm)
0	0,689	0		
50	0,587	14,804		
100	0,513	25,544	y = 0.2376x +1.6809	203,363
150	0,421	38,897	R^2 = 0.996	
200	0,348	49,492		
240	0,295	57,184		



Picture. 2 Linear regression equation curve of black turmeric methanol extract

The antioxidant activity of black turmeric extract was tested using the DPPH method as measured by a UV-Vis spectrophotometer. The DPPH method was used in this study because it is one of the methods for determining the antioxidant activity of free radical scavengers that is simple, fast, easy and sensitive and only requires a small sample to evaluate the antioxidant activity of certain compounds or plant extracts, so it is widely used to test the ability of compounds that contain compounds. acts as an electron donor (Melannisa, Rosita. Muhammad Da'I, 2011). Furthermore, for testing the antioxidant activity, the concentration of the extract was made, namely 0.0; 237.6 ; 475.2 ; 712.8 ; and 950.4 ppm. The purpose of making variations in the concentration of this test solution is to determine a calibration curve with a graded concentration by connecting the concentration of the test solution with its absorption, so that a linear regression equation will be obtained to calculate the concentration value of the extract which is able to inhibit the oxidation process by 50% (IC50) (Tutik, 2015)(Tutik, 2015).

Testing of antioxidant activity begins with measuring the wavelength of the standard solution of DPPH whose absorption spectrum is observed at a wavelength of 450-550 nm using a UV-vis spectrophotometer. The maximum wavelength of the standard solution obtained is 517 nm, where at that wavelength DPPH provides a strong absorption and changes in absorbance occur for each unit of the largest concentration.

Measurement of the antioxidant activity of the black turmeric extract test solution against DPPH free radicals was carried out by looking at the absorbance value of each concentration at a wavelength of 517 nm. Based on the measurement results, the results showed that there was a change in absorbance at each concentration, where the higher the concentration, the lower the absorbance value and vice versa, the smaller concentration had a larger absorbance value. Furthermore, the calculation of the percentage of attenuation is carried out which shows the results that the larger concentration of the test sample solution has a greater ability to reduce free radicals than the smaller concentration of the test sample. Then from the percentage of attenuation, a curve of the relationship between the concentration of the test solution and the percentage of attenuation was made. So that the linear regression equation is obtained, namely y = 0.0628x + 2.3596, with R2 = 0.9911. Where y in this equation is the absorbance, while x is the concentration of the sample solution being tested, the x value obtained is the amount of concentration needed to be able to reduce 50% of DPPH radical activity.

From the results of standard measurements of gallic acid obtained a linear regression equation y = -0.1598x + 0.5055 with a correlation coefficient value (r2) = 0.9941. The standard standard curve for gallic acid can be seen in Figure 3.

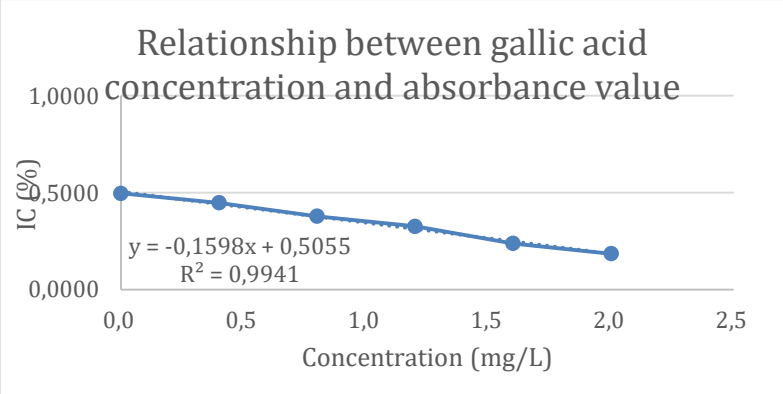


Figure 3. Standard Gallic Acid Standard Linear Regression Curve

The results of the regression curve obtained indicate a close relationship between the concentration of attenuation. Based on the literature, the accepted work area can be seen visually through the absorbance versus concentration curve of several standard concentrations with R values still showing values above 0.9. Based on the linear regression curve between the concentration of the test sample solution and the percentage of attenuation, the R2 value is 0.9911, so it can be seen that the value of R = 0.957 which is close to +1 (positive value) illustrates that with increasing concentration of the test sample, the greater the antioxidant activity (Tutik, 2015)

Furthermore, to get the IC50 value, namely the concentration of the test solution capable of inhibiting the oxidation process by 50%, calculations were carried out from the linear regression equation. The IC50 value was obtained after replacing y = 50. The IC50 value obtained showed activity as an antioxidant. Based on the literature, the smaller the IC50 value, the higher the antioxidant activity. Specifically, a compound is said to be a very strong antioxidant if the IC50 value is less than 50 ppm, strong for IC50 is 50-100 ppm, moderate if IC50 is 100-150 ppm, and weak if IC50 is 151-200 ppm (Verma, R.K., G. Mishra, P. Singh, K.K. Jha, 2011b), . From the calculation results of the IC50 value of black turmeric ethanol extract obtained by 321.687 and IC50 of black turmeric methanol extract obtained by 203.363 ppm, it can be said that black turmeric extract has antioxidant activity in the very weak category. Based on the IC value of50, a compound is said to be a very strong antioxidant of the value IC50 less than 50, strong (50-100), moderate (100-150), and weak (151-200). The smaller the value IC50 the higher the antioxidant activity (Utami, 2020). The identified compounds of the plant extracts possessing several health benefits may be helpful in promoting the species for pharmaceuticals and multiple industrial uses (Om Prakash Arya., 2022).

CONCLUSION

Based on the results obtained, it can be concluded that: Black Turmeric Extract (Curcuma caesia Roxb.) has very weak antioxidant activity. The IC50 value of the black turmeric ethanol extract was 321,687 and the IC50 of the black turmeric methanol extract was 203.363 ppm. The rhizomes of Curcuma caesia have got wide range of attention on various antipyretic, analgesic, antimutagenic, neuropharmacological, antibacterial and antifungal pharmacological activities. It is well known for its peculiar characteristics other than yellow turmeric. With the increasing trend on natural products in modern phytomedicines, the antioxidant and cytotoxic activities of Curcuma caesia Roxb. rhizome fractions were evaluated. In the next research, it is necessary to conduct similar research with different methods.

ACKNOWLEDGEMENTS

We thank you for the support of facilities from the Laboratory of Natural Materials and Pharmacology, Faculty of Pharmacy, Mahasaraswati University Denpasar, which has provided research facilities and the parties involved in this research.

References

Akar Z, Küçük M, D. H. (2017). A new colorimetric DPPH scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 640–647.

Angana Borah, Manabi Paw, et. a. (2019). Chemical composition, antioxidant, anti-inflammatory, anti-microbial and in-vitro cytotoxic efficacy of essential oil of Curcuma caesia Roxb. leaves: An endangered medicinal plant of North East India, Industrial Crops and Products. *Www.Sciencedirect.Com/Science/Article/Pii/S0926669018310872)*, *129*, 448–454. https://doi.org/10.1016/j.indcrop.2018.12.035.

Atasoy, N., & Yücel, U. M. (2021). *Antioxidants from Plant Sources and Free Radicals. In (Ed.), Reactive Oxygen Species.* https://doi.org/IntechOpen. https://doi.org/10.5772/intechopen.100350 2. Choose citation style Download citation IntechOpen Publish with IntechOpen Next

Das S, Mondal P, Z. K. (2013). Curcuma caesia Roxb and its medicinal uses: A Review. *Int J Res Pharm Chem*, *3(2)*, 370–375.

Flieger J, Flieger W, Baj J, M. R. (2021). Antioxidants: Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles. Materials (Basel). *Materials (Basel)*, *14*(15), 4135.

Jelita Rahma Hidayati, Ervia Yudiati., et. a. (2019). Antioxidant Activities, Total Phenolic Compound And Pigment Contents of Tropical Sargassum sp. Extract, Macerated In Different Solvents Polarity. *Jurnal Kelautan Tropis*, *22*(1), 73–80.

Juliana Isis Araújo Pereira, et. a. (2022). Phytochemical investigation and biological activities of extracts obtained from Montrichardia linifera (Arruda) Schott leaves collected in different localities from Piauí coast. *Research, Society and Development, 11*(4). https://doi.org/DOI: http://dx.doi.org/10.33448/rsd-v11i4.27231

Kristina von Rintelen, Evy Arida, C. H. (2017). *A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries expand article info*. https://doi.org/https://doi.org/10.3897/rio.3.e20860

Lourenço SC, Moldão-Martins M, A. V. (2019). Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules.*, *24*(22), 4132. https://doi.org/10.3390/molecules24224132. PMID: 31731614; PMCID: PMC6891691.

Mamta Yadav, K. K. S. (2019). Yadav M, Saravanan KK. Phytochemical Analysis and Antioxidant Potential of Rhizome Extracts of Curcuma amada Roxb and Curcuma caesia Roxb. *Joutnal Og Frug Delivery & Therapeutic*, *9*(5).

Melannisa, Rosita. Muhammad Da'I, R. T. R. (2011). Uji Aktivitas Penangkap Radikal Bebas dan Penetapan Kadar Fenolik Total Ekstrak Etanol Tiga RIMPANG Genus Curcuma Dan Rimpang Temu Kunci (Boesenbergia pandurata). *Pharmacon*, *12(1)*, 40–43.

Mina K.C. (2021). Antioxidant And Cytotoxic Properties Of Black Turmeric (Curcuma Caesia Roxb.) Rhizome Extract. *A Dissertation Of Central Department Of Botany Institute Of Science And Technology Tribhuvan University Kirtipur, Kathmandu, Nepal*.

Munteanu, I.G.; Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int. J. Mol. Sci.*, *22*, 3380.

Om Prakash Arya., et al. (2022). Health-promoting bioactive phenolic compounds in different solvent extracts of Curcuma caesia Roxb. rhizome from North-East India. *Journal of Food Processing And Preservation*.

Pakkirisamy M, Kalakandan SK, R. K. (2017). Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of Curcuma caesia Roxb (Black Turmeric). *Pharmacognosy Journal.*, *9*(6), 952–956.

Setiawan, Vendra, S. P. (2021). Rapid Screening Analysis of Antioxidant Activities in Green Tea Products Using DPPH and FRAP. *PHARMACEUTICAL JOURNAL OF INDONESIA*, *7*(1), 9–14.

Shalaby, E. (2019). *Antioxidants. IntechOpen.* https://doi.org/https://doi.org/10.5772/intechopen.77838

Sudarshana Borah, P. S., & Sharma, H. K. (2020). Chemical Profiling, Free Radical Scavenging and

Anti-acetylcholinesterase Activities of Essential Oil from Curcuma caesia of Arunachal Pradesh, India. *Pharmacogn. Res*, *12*, 76–84.

Tilaar, Martha dan Widjaja, B. T. (2014). *The Power of Jamu*. PT Gramedia Pustaka Utama.

Tutik, R. P. dan S. M. (2015). Validasi Metode Analisis Siklamat Secara Spektrofotometri dan Turbidmetri. *J.Sains Dasar*, *4(1)*, 23–29.

UTAMI, M. D. P. (2020). Aktivitas Antioksidan Dari Ekstrak Kunyit Hitam (Curcuma Caesia Roxb.) Dan Ekstrak Temu Ireng (Curcuma Aeruginosae) Menggunakan Metode Dpph (2,2-Difenil -1Pikril hydrazyl). *Skripsi Program Studi Farmasi Fakultas Kesehatan Universitas Ngudi Waluyo*.