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Solvent Optimization for Extraction of Mesua ferrea l. Leaves as An Antioxidant Using Simplex Lattice Design Method

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ABSTRACT

Mesua ferrea L. leaves methanol extract has the highest antioxidant activity than the other part of the plant. However methanol is toxic solvent, so the using of methanol for extraction may harmful for health and environment. The purpose of this study was to optimize the solvent combination of ethanol and water for extraction of Mesua ferrea L. leaves by using Simplex Lattice Design (SLD) method. The samples were extracted by Ultrasonic Assisted Extraction (UAE) using variation of solvents ethanol 96%, ethanol 50% and water. The solvent combination was optimized by using SLD method based on the Total Phenolic Content of all crude extract. The result showed that the TPC of ethanol 96%, ethanol 50% and water crude extracts were 574.7±66.97 mgGAE/g, 657.8±37.02 mgGAE/g and 524.23±14.48 mgGAE/g respectively. SLD was calculated and the optimal solvent obtained was ethanol 55% with TPC of 626.235± 23 mgGAE/g. The ethanol 55% extract of Mesua ferrea L. leaves has strong antioxidant activity (IC50 57.77) ppm using DPPH method...

Keywords*: Antioxidant, Mesua ferrea L., Simplex Lattice Design, Total Phenolic Content.*

INTRODUCTION

Mesua ferrea L., belongs to family *Clusiaceae*, is known for its biological activity such as antioxidant, anti-inflammatory, anti-tumor, antimicrobial, anti-asthmatic and other activities (Sharma et al., 2016). *Mesua ferrea* is rich in secondary metabolites such as tannin coumarin, xanthone, phenolic, flavonoid, metabolites such as mesuaxanthones A and mesuaxanthones B (Hartanti et al., 2012, 2019; Sharma et al., 2016). These metabolites give *Mesua ferrea* its antioxidant activity (Marwah et al., 2007). It is reported that methanol extract of *Mesua ferrea* leaves has the highest antioxidant activity (IC_{50} 89.94±0.65 ppm) than the other part of the plant (flowers, twig, and fruit). This antioxidant activity is synergy with its total phenolic content, which is mostly extracted by methanol (TPC = 348.36±38.53 mg GAE/g) (Chaithada et al., 2018). Those data showed that *Mesua ferrea* has high potency as a new source of antioxidant that can be formulated as therapeutic agents in the treatment of various diseases.

The first step in the utilization secondary metabolites as antioxidants in the manufacture of pharmaceutical products is the extraction of bioactive substances from plant materials. The most commonly used method for producing extracts from plant materials is solvent extraction. Depends on the type of solvent used during extraction, as well as the polarity of solvent, the metabolites extracted may varied (Dai & Mumper, 2010). It is important to choose the right solvent as it can be affects the amount and rate of metabolites extracted. Solvents such as methanol, ethanol, propanol, acetone, methyl acetate, and combination have been used to extract phenol, often with different proportions of water (Garcia-Salas et al., 2010). Methanol, a polar solvent, has generally been found to be more effective in extraction low molecular weight polyphenols including those found in *Mesua ferrea* leaves (Dai & Mumper, 2010). Methanol, however, is a toxic alcohol and its exposure (due to ingestion, skin absorption and inhalation) is extremely dangerous and can cause significant morbidity and mortality if left untreated (Holt & Nickson, 2018; Kraut & Mullins, 2018). According to health department of Indonesia, the only solvent that can be used for medicinal purposed is ethanol, water, combination water and ethanol or ether (BPOM RI, 2010). Ethanol, a polar solvent with lower polarity than methanol, is a suitable solvent for polyphenol extraction and is safe for human consumption (Dai & Mumper, 2010). The polarity of ethanol depends on its concentration. Combination of ethanol and water can increase the polarity of ethanol, so it will increase the rate of extraction of higher polar metabolites (Setiawansyah et al., 2017).

This experiment using Simplex Lattice Design (SLD) method based on phenolic content of extract with variation combination of ethanol and water to obtain the optimal combination of ethanol and water as solvent. The objectives in this research were to determine the optimal solvent for extraction total phenolic content of M*esua ferrea* L. and its antioxidant activity using DPPH method.

MATERIAL AND METHODS

Chemical and Instrument

Ethanol 96%, water, methanol p.a., ethanol p.a., were purchased from Merck Ltd. Samples were extracted using sonicator Elmasonic®. Standards of phenolic content (gallic acid), Follin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Merck Ltd. Ferric Chloride ($FeCl_3$), Sodium Chloride (NaCl), Magnesium (Mg), Hydrochloric Acid (HCl), gelatin 1%, isopropyl alcohol, ammonia 25% for screening test were obtained from Merck Ltd. The absorbance of samples was measured with UV-Vis Spectrophotometer (Specord® 200).

Plant material

The leaves of *Mesua ferrea* L. were collected in January 2020 from Lingsar, sub-district Lingsar, West Lombok Regency, West Nusa Tenggara Province (8^{o}34'30.0"S 116^{o}10'49.8"E). the samples determination was conducted in Biology Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University with the identification herbarium specimen number of SKI 63/UN18.7/LB/2019.

Preparation of Extract

Freshly collected *Mesua ferrea* L. leaves was washed thoroughly under running tap water, sortated from other part of the plant, and dried under shade for two weeks. Dried plant material was ground into coarse powder using an electric blender and stored in closed air tight container until further use. Each the 100 grams of powder material was extracted separately using Ultrasonic Assisted Extraction (UAE) for 3 x 30 minutes, using 800 mL each of ethanol 96%, ethanol 50% and water. The respective extracts were concentrated in water bath at a temperature below 50^{o}C.

Determination of total phenolic content

The total Phenolic Content (TPC) was determined using the Follin-Ciocalteu method. Briefly, 0.3 mL of crude extract (1000 ppm) was mixed with 1.5 mL Follin-Ciocalteu reagent (1:10). After 8 minutes, 1.2 mL of 7.5% sodium carbonate was added. The tubes were mixed and left for 45 minutes, and the absorbance was measured at 752 nm using UV-Vis spectrophotometry. All measurements were made 3 times. The TPC was expressed as mg / g of gallic acid equivalent (GAE) per gram of extract.

Optimization solvent using simplex lattice design

A Simplex Lattice Design (SLD) was adopted to optimize the solvent based on the TPC of each extract with different types of solvent. In this experiment, 3 types of solvent were used, ethanol 96%, ethanol50%, and water Table 3. Based on these data, the optimal equation was obtained by using the following formula:

$$Y = a\,(A) + b\,(B) + ab\,(A.B)$$

Where Y is the respond of the experiment (TPC), A is the ratio of ethanol, B is the ratio of the water and a, b and ab are the coefficient of the equation. Based on the equation we can predict the TPC of each ratio of ethanol. The optimal solvent is the solvent which has the highest TPC from the equation (teoritical TPC). The TPC of each sample were determined after it was extracted with the optimal solvent (actual TPC). The equation was then validated by using statistical analysis One Sample T-test SPSS 16.0. The equation is valid if there is no significant difference between the data ($P > 0.05$).

Phytochemical screening

The presence of polyphenols, tannins and flavonoids of Messua ferrea L. leaves extract with optimal solvent were analyzed with the standard qualitative phytochemical screening method using Braymer's test, Shinoda's test and gelatin test respectively (Pooja & Gm, 2016). (Pooja, 2016). The presence of xanthones and coumarin were analyzed using

NaOH reagent (Mendonça et al., 2015; Savithramma et al., 2011). Meanwhile, for the presence of lignan, the extract were analyzed using Thin Layer Chromatography (TLC) with water: propan-2-ol: ammonia 25% (1:8:1) eluent (Stasevich et al., 2009). For comparison, a blank test was run for each test using only water and reagents.

Evaluation of antioxidant activity

Antioxidant activity was determined using the method proposed by Yen and Chen (1995) with minor modifications. Briefly, to 1 mL extract at different concentrations with 3 mL ethanol, 1 ml of 0,4 mM DPPH were added. Radical stock solution (DPPH) was freshly prepared daily. Controls containing methanol and DPPH solutions were also performed. All solutions were incubated in the dark at room temperature for 30 minutes. The absorbance of the solution was subsequently measured at 516 nm by UV-Vis spectroscopy. The results were the analyzed using statistical analysis One Way ANOVA SPSS 16.0.

RESULTS AND DISCUSSION

Extraction yield

In this study, *M. ferrea* leaves were extracted with 3 different solvents, ethanol 96%, ethanol 50% and water using Ultrasonic-Assisted Extraction (UAE) method. This technique was used because it does not require complex instruments, can be rapidly operated with a wide range of solvents, and can be used on a small or large scale in the phytopharmaceutical extraction industry (Dai & Mumper, 2010; Khoddami et al., 2013). In addition, this extraction technique has been shown to reduce phenol degradation and improve extraction yields by 6 to 35% (Dai & Mumper, 2010; Garcia-Salas et al., 2010). The yields of each extract in different solvent are given in the Table 1.

Based on the table 1, the highest yield of the extract (16.09%) was obtained by ethanol 96% extract. This percentage of yield shows the amount of metabolite that were extracted by the solvent (Hasnaei et al., 2019). This result was similar to the study that reported that the higher the concentration of ethanol used, the higher its capability to destruct the outer cell, resulted in the higher amount of metabolite extracted (Mardaningsih,2012).

Total phenolic content (TPC) of extract

The total phenolic content of the extract was determined using the Follin-Ciocalteu (F-C) method. This method has been found more preferable than the other method. This assay relies on the transfer of electrons in the alkaline medium from the phenolic compound to the phosmolibdic / phosphotungstic acid complex to form blue complexes, which is measured spectroscopically at about 760 nm

Table 1. The yields of the extracts obtained by the different solvents.

Extract	% yield
Ethanol 96% extract	16.09
Ethanol 50% extract	12.28
Water extract	8.75

(Dai & Mumper, 2010). In this study, the TPC values were obtained from the gallic acid calibration curve Y = 0.0093x + 0.1702, R2 = 0.996, where x is the absorbance and y is the concentration of gallic acid solution expressed as mg GAE / g extract. Results of TPC were showed in Table 2.

Based on the Table 2, the highest TPC was found in samples extracted with ethanol 50%. It is shown that *M. ferrea* leaves extracted by using ethanol 50% (657.8 ± 37.02 mg GAE/g extract) has the higher TPC than *M. ferrea* leaves extracted by methanol as reported by Chaitada, (2018) (348.36 ± 38.53 mg GAE/g extract). These findings were in line with a result reported previously in UAE extraction of polyphenols from *P. Atrosanguinea* which stated that 50% aqueous concentrations of each solvent were the most effective for extracting polyphenols when compared to other concentration and 50% aqueous ethanol was the most effective solvent when compared to MeOH, 50% MeOH, EtOH, acetone and 50% acetone (Kalia et al., 2008) and other findings which is stated in table 3. Due to water soluble nature of phenolics, which is enhanced by the presence of solvent which facilitate solubilization through penetration in plant cell structure, Kelia (2008) claimed that the addition of some water improves the extraction efficiency.

Table 2. Total phenolic content of *Mesua ferrea* L. leaves extracts

Extract	TPC (mg GAE/g extract) (Mean ± SD)
Ethanol 96% extract	574.7 ± 66.97
Ethanol 50% extract	657.8 ± 37.02
Water extract	524.23 ± 14.48

Solvent optimization using Simplex Lattice Design (SLD)

Based on the TPC of each extract, equation of Simplex Lattice Design was determined. The teoritical TPC of each concentration of ethanol was predicted by the equation of Y = 5.5837(A) + 5.2423(B) + 0.0466(A.B) that were obtained from SLD method. As results in the Figure 1, the highest teoritical TPC were obtained by ethanol 55% of 658.342 mg GAE/g extract, which is mean that ethanol 55% is the optimal solvent for extraction of phenolic from *Mesua ferrea* leaves.

These results were in line with some researches that showed ethanol 50% was the optimal solvent for phenolic compound targeted extraction (Table 3). Using various solvents and extraction temperature, Rostagno et al., (2003) study the efficiency of extracting four isoflavone derivatives from freeze-dried soybeans for UAE extraction. The result was ethanol 50% appear to be the ideal option of solvent for extracting isoflavones from ground soybeans, due to its great efficiency, cheap cost, low toxicity and environmental friendliness. Different types of extraction also resulted that ethanol 50% is the most effective solvent for extraction phenolic content of *Phaseolus vulgaris* L. (Microwave-Assisted Extraction (MAE)) (Sutivisedsak et al., 2010), *Melilotus officinalis* L. Pallas (MAE) (Martino

Table 3. The optimal solvent for extraction of phenolic compound using Ultrasonic Assisted Extraction method

Sample	Optimal solvent	Other solvent	Phenolic content	Reference
Potentilla atrosanguinea L.	Ethanol 50%	MeOH, 50% MeOH, EtOH, acetone, 50% acetone	27.80[a]	(Kalia et al., 2008)
Phaseolus vulgaris L.	Ethanol 50%	Water and EtOH 100%	70,57 ± 1,36[a]	(Sutivisedsak et al., 2010)
Melilotus officinalis L. Pallas	Ethanol 50%	MeOH 50%, MeOH, boiling water	3,620 ± 0,062[b]	(Martino et al., 2006)
Soy bean	Ethanol 50%	EtOH, MeOH, and MeCN (30-70 %)	1154.31[c]	(Rostagno et al., 2003)
Fagopyrum essculentum M.	Ethanol 50%	Water and EtOH 100%	18,52 ± 0,2[a]	(Inglett et al., 2010)

[a] TPC (mg GAE/g), [b] Coumarin (mg/g), [c] Isoflavon (µg/g)

et al., 2006) and *Fagopyrum essculentum* M. (microwave irradiated) (Inglett et al., 2010).

Equation was validated by comparing the teoritical TPC and the actual TPC of ethanol 55% by using statistical analysis One Sample T-test. Table 4 shows that there is no significant difference (P = 0.137, P > 0.05) between the data, and it can be concluded that the equation was valid.

Phytochemical screening

Analysis of *Mesua ferrea* leaves extract with ethaol 55% revealed the presence of polyphenol, tannin, flavonoid, xanthones, coumarin, and phenolic Table 5. The presence of these compound may contribute to its antioxidant activity. These phenolic compounds have redox characteristic due to their chemical structure, which can play an important role in adsorbing and neutralizing reactive oxygen species (ROS), quenching singlet and triplet oxygen, or decomposing peroxides (Cartea et al., 2011). Phenolic compounds have extended conjugates aromatic system to delocalize an unpaired electron and hydroxyl groups that are susceptible to donate a hydrogen atom or an electron to free radicals (Dai & Mumper, 2010).

Antioxidant activity

The antioxidant activity of *M. ferrea* leaves extract by ethanol 55% was compared with Anonna muricata extract and ascorbic acid as standard solutions. The results are shown in Table 6. It is shown that *M. ferrea*

Table 4. Equation validation using One Sample T-test SPSS

M. ferrea **leaves ethanol 55% extract**	**Total phenolic content (mg GAE/g extract)**	**Sig.**	**Conclusion**
Teoritical	658.342	0.137	There is no statistical difference
Actual	626.235		

Table 5. Phytochemical screening of *Mesua ferrea* leaves extract

Phytochemical Screening	Reagent	Result
Polyphenols	FeCl3	Black-ish green (+)
Tannin	Gelatin 1% + NaCl 10%	Presence of white percipitate (+)
Flavonoid	Concentrated HCl + Mg	Red (+)
Xanton	NaOH (pH = 11)	Yellow (+)
Lignan	KLT (air:propan-2-ol: amonia 25%)	Blue flourescence at UV 254 nm
Kumarin	NaOH 10%	Yellow (+)

(+) : positive, presence of metabolite

Table 6. Antioxidant activity of *M.ferrea* leaves extract by ethanol 55%

Extract	IC50 (ppm) (Mean ± SD)	IC50 M.ferrea leaves extract in methanol (ppm) (Chaitada, 2018)
M. ferrea leaves EtOH 55% extract	57.77 ± 1.55	89.94 ± 0.65
A. muricata EtOH 50% extract	97.70 ± 1.71	
Ascorbic acid	9.83 ± 0.51	

leaves extract with ethanol 55% (57.77 ± 1.55 ppm) have the higher antioxidant activity than M.ferrea leaves extract with methanol (89.94 ± 0.65 ppm). The methanol extract may either have higher concentration of non-phenolic compounds or possess phenolic compounds with fewer active groups than the ethanol solvent. Methanol itself has the higher polarity than ethanol and have been proven to be more effective at extracting non polar compounds and polyphenols with lower molecular weight (Dai & Mumper, 2010). But due to its toxic properties, it can't be used for extraction in medicinal purposes. Ethanol, can increase its polarity by combinate it with some water, so the polarity will closed to phenolics in *M.ferrea* leaves extract. This finding is in line with Chew et al, 2011 showed that combination of solvent gave higher TPC than one pure solvent.

The result then analyze statistically to see the difference of antioxidant activity of *Mesua ferrea* and the standard solutions. Samples were analyze using One Way ANOVA with rejection rate of 5%. Figure 2 shows that the

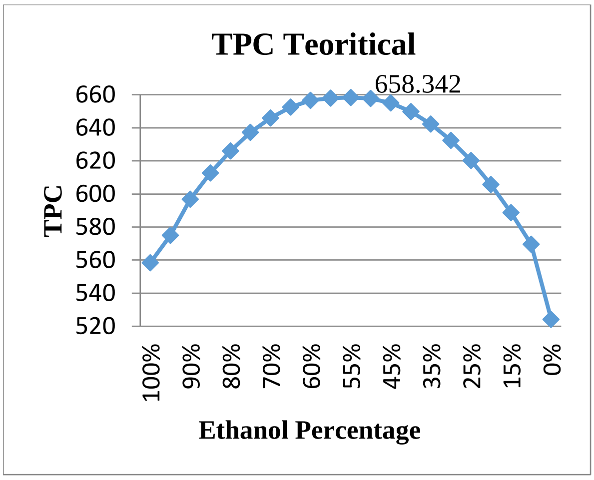

Figure 1. Total phenolic content teoritical obtained from equation SLD method

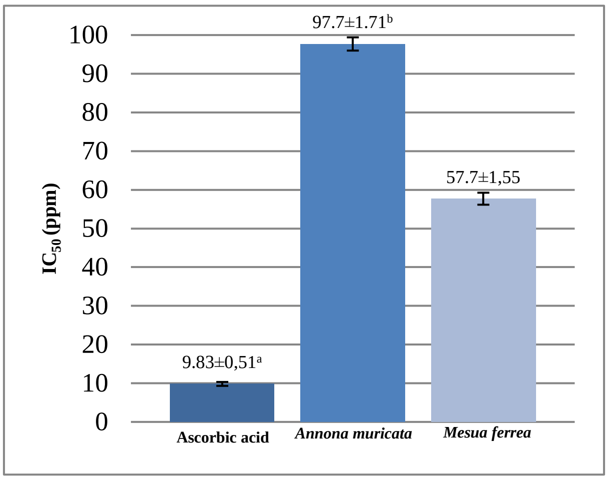

Figure 2. Statistical difference antioxidant activity of samples, [a]P < 0.05 (between ascorbic acid and *M.ferra*), [b]P < 0.05 (between *A. muricata* and *M.ferra*).

significance between *Mesua ferrea* and the two standard solutions P = 0.000, P < 0.005, which we can conclude that sample was significantly different with the standard (*Anonna muricata* and ascorbic acid).

CONCLUSION

The optimal solvent for extraction *Mesua ferrea* L. leaves as antioxidant were obtained by using simplex lattice design method based on total phenolic content of extract. In this study sample were extracted using ethanol 96%, ethanol 50% and water and TPC was determined using follin-ciocalteu method. The result showed the total phenolic content of ethanol 96%, ethanol 50% and water crude extracts were 574.7±66.97, 657.8±37.02 and 524.23±14.48 mg GAE/g extract, respectively. The equation of simplex lattice design that were obtained is Y = 5.5837(A) + 5.2423(B) + 0.0466(A.B). From this equation, the optimal solvent for extraction phenolic of *M.ferrea* leaves is ethanol 55%. *M.ferrea* leaves extract with ethanol 55% revealed the presence of polyphenols, flavonoid, phenolic, coumarins, xanthones and lignans. The antioxidant activity of this extract was evaluated using DPPH radical scavenging assay and it is shows that *M. ferrea* extract with ethanol 55% (IC_{50} = 57.77 ± 1.55 ppm) has higher antioxidant activity than previous study with methanol solvent.

REFERENCES

BPOM RI. (2010). *Parameter Standar Umum Ekstrak Tumbuhan Obat*. Departemen Kesehatan RI, Direktorat Jenderal Pengawasan Obat dan Makanan .

Cartea, M. E., Francisco, M., Soengas, P., & Velasco, P. (2011). Phenolic compounds in Brassica vegetables. *Molecules*, *16*(1), 251–280. https://doi.org/10.3390/molecules16010251

Chaithada, P., Supapan, J., Rodthuk, P., & Chainarong, S. (2018). Total Flavonoids, Total Phenolic Content and Antioxidant Activity From Fruits, Leaves, Twigs and Flowers of Mesua ferrea L. *Walailak Journal of Science and Technology*, *15*(4), 295–304. https://doi.org/10.14456/vol15iss1pp

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313–7352. https://doi.org/10.3390/molecules15107313

Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Phenolic-compound-extraction systems for fruit and vegetable samples. *Molecules*, *15*(12), 8813–8826. https://doi.org/10.3390/molecules15128813

Hartanti, D., Arafani, zahra muftia, Nurlativah, A., & Hakim, Z. R. (2012). Constituents and Antibacterial Activity of Extract of Nagasari (Mesua ferra) Leaves. *Proceeding of The 2nd UMP-PIC & 8th ISCC*, *66*(October 2017), 37–39.

Hartanti, D., Rahman Hakim, Z., Nurlativah, A., Nur Cahyani, A., Zahra Nadia, F., & Djaliasrin Djalil, A. (2019). Phytochemical analysis, FTIR fingerprint and bioactivity of crude extract and fractions of Mesua ferrea L. *Research Journal of Life Science*, *6*(1), 54–65. https://doi.org/10.21776/ub.rjls.2019.006.01.7

Hasnaei, Wisdawati, & Usman, S. (2019). Pengaruh Metode ekstraksi Terhadap Rendemen dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (Lunasia amara Blanco). *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *5*(2), 175–182. https://doi.org/10.22487/j24428744.2019.v5.i2.13149

Holt, N. R., & Nickson, C. P. (2018). Severe methanol poisoning with neurological sequelae: implications for diagnosis and management. *Internal Medicine Journal*, *48*(3), 335–339. https://doi.org/10.1111/imj.13725

Inglett, G. E., Rose, D. J., Chen, D., Stevenson, D. G., & Biswas, A. (2010). Phenolic content and antioxidant activity of extracts from whole buckwheat (Fagopyrum esculentum Möench) with or without microwave irradiation. *Food Chemistry*, *119*(3), 1216–1219. https://doi.org/10.1016/j.foodchem.2009.07.041

Kalia, K., Sharma, K., Singh, H. P., & Singh, B. (2008). Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of Potentilla atrosanguinea. *Journal of Agricultural and Food Chemistry*, *56*(21), 10129–10134.

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

Kraut, J. A., & Mullins, M. E. (2018). Toxic Alcohol : Review Article. *The New England Journal of Medicine*, *378*(378:3), 278–280.

Mardaningsih, F., M.A.M. Andriani dan Kawiji. (2012). The influence of ethanol concentration and temperature of spray dryer for chlorophyll powder characteristic of alfafa (Medicago sativa L) by using binder maltodekstrin. *Jurnal Teknosains Pangan*. 1(1), 110-117

Martino, E., Ramaiola, I., Urbano, M., Bracco, F., & Collina, S. (2006). Microwave-assisted extraction of coumarin and related compounds from Melilotus officinalis (L.) Pallas as an alternative to Soxhlet and ultrasound-assisted extraction. *Journal of Chromatography A*, *1125*(2), 147–151. https://doi.org/10.1016/j.chroma.2006.05.032

Marwah, R. G., Fatope, M. O., Mahrooqi, R. al, Varma, G. B., Abadi, H. al, & Al-Burtamani, S. K. S. (2007). Antioxidant capacity of some edible and wound healing plants in Oman. *Food Chemistry*, *101*(2), 465–470. https://doi.org/10.1016/j.foodchem.2006.02.001

Mendonça, I. C. G. de, Porto, I. C. C. de M., do Nascimento, T. G., de Souza, N. S., Oliveira, J. M. dos S., Arruda, R. E. dos S., Mousinho, K. C., dos Santos, A. F., Basílio-Júnior, I. D., Parolia, A., & Barreto, F. S. (2015). Brazilian red propolis: Phytochemical screening, antioxidant activity and effect against cancer cells. *BMC Complementary and Alternative Medicine*, *15*(1), 1–12. https://doi.org/10.1186/s12906-015-0888-9

Pooja, S., & Gm, V. (2016). Phytochemical screening for secondary metabolites of Opuntia dillenii Haw. *Journal of Medicinal Plants Studies JMPS*, *39*(45), 39–43. http://www.plantsjournal.com/archives/2016/vol4issue5/PartA/4-4-38-365.pdf

Rostagno, M. A., Palma, M., & Barroso, C. G. (2003). Ultrasound-Assisted Extraction of Soy Isoflavones. *Journal of Chromatography A*, *1012*, 119–128.

Savithramma, N., Rao, M. L., & Suhrulatha, D. (2011). Screening of Medicinal Plants for Secondary Metabolites. *Middle-East Journal of Scientific Research*, *8*(3), 579–584.

Setiawansyah, A., W., S. Y., Turisia, N. A., Rosnani, & Aprianti, Y. (2017). Optimasi Metode dan Pelarut Ekstraksi terhadap kadar Kurkumin Pada Temulawak (Curcuma xanthorrhiza). *Prosiding Seminar Nasional Kesehatan*, *01*, 1–7. http://www.albayan.ae

Sharma, A., Sharma, S., Rohit, Naresh, & Parashar, B. (2016). Mesua ferrae linn:- A review of the Indian medical herb. *Systematic Reviews in Pharmacy*, *8*(1), 19–23. https://doi.org/10.5530/srp.2017.1.5

Stasevich, O. v., Mikhalenok, S. G., & Kurchenko, V. P. (2009). Selection of optimal conditions for separating lignan-containing extract from oil flax seed by thin-layer chromatography. *Pharmaceutical Chemistry Journal*, *43*(7), 415–417. https://doi.org/10.1007/s11094-009-0313-0

Sutivisedsak, N., Cheng, H. N., Willett, J. L., Lesch, W. C., Tangsrud, R. R., & Biswas,

A. (2010). Microwave-assisted extraction of phenolics from bean (Phaseolus vulgaris L.). *Food Research International*, *43*(2), 516–519. https://doi.org/10.1016/j.foodres.2009.09.014

Yen, G. C. dan Chen, H. Y. (1995). Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. *Journal of Agricultural and Food Chemistry*. 43(1), 27–32. DOI: 10.1021/jf00049a007.