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Antioxidant Activity Using DPPH & Frap Method and Their Correlation with The Levels of Phenolic and Flavonoid Compounds from Nemba Plants (Azadirachta Indica A. Juss)

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Abstract

One of the herbal plants, namely neem (*Azadirachta indica A. Juss*) can generally be used as an ingredient for traditional medicine. Neem is known to have the potential to produce antioxidants, where it can play an important role in counteracting free radicals. As a result of the increasing number of free radicals, the number of antioxidants and free radicals becomes unbalanced. If this continues, it can cause problems, namely the emergence of various kinds of serious diseases. The purpose of this literature review is to present articles related to the potential for antioxidant activity; content of phenolic compounds and flavonoids; knowing whether there is a relationship between phenolic compounds and flavonoids on antioxidant activity; as well as providing information related to other potentials of neem (*Azadirachta indica A. Juss*) plants. Several studies have stated that the neem plant has antioxidant activity. The strongest antioxidant activity was found in the neem root. The secondary metabolites produced are alkaloids, flavonoids, steroids, polyphenols, glycosides, phenols, tannins, essential oils, carotenoids, fatty acids, and saponins. Another study also stated that neem has the potential for other pharmacological activities such as antibacterial, immunomodulatory, antidiarrheal, and antifungal activities. polyphenols, glycosides, phenols, tannins, essential oils, carotenoids, fatty acids, and saponins. Another study also stated that neem has the potential for other pharmacological activities such as antibacterial, immunomodulatory, antidiarrheal, and antifungal activities. polyphenols, glycosides, phenols, tannins, essential oils, carotenoids, fatty acids, and saponins. Another study also stated that neem has the potential for other pharmacological activities such as antibacterial, immunomodulatory, antidiarrheal, and antifungal activities.

Keywords: neem, *Azadirachta indica*, Antioxidant, Phenolic, Flavonoid, Correlation

INTRODUCTION

Indonesia is a country that has abundant natural resources such as plant diversity and other resources such as race, ethnicity, and culture. Some community groups often use plants as herbal medicines. In recent years, with the issue of back to nature and the prolonged crisis, the use of natural ingredients for medicine or other purposes has

increased. One of the plants that is well known by the Indonesian people is neem plant (*Azadirachta indica A. Juss*) (Faizah et al., 2016). The neem plant is native to India which is often called neem (Soraya et al., 2019). The neem plant comes from the Meliaceae family where this plant thrives in tropical countries including Indonesia, where the average plant height can reach 15-20 meters (Asif, 2012). In India the neem plant has been used as a traditional medicine in ayurvedic medicine, the medicinal properties are believed to come from the leaves, fruit, and wood, while the seeds can be used as antimalarials, fever medicines, antiseptics, antimicrobials, to anthelmintic (Airaodion et al., 2019).). A study states that almost all parts of the neem plant have efficacy as herbal medicine from roots, leaves, seeds to flowers (Asif, 2012). while the seeds can be used as an antimalarial, fever medicine, antiseptic, antimicrobial, to anthelmintic (Airaodion et al., 2019). A study states that almost all parts of the neem plant have efficacy as herbal medicines from roots, leaves, seeds to flowers (Asif, 2012). while the seeds can be used as an antimalarial, fever medicine, antiseptic, antimicrobial, to anthelmintic (Airaodion et al., 2019). A study states that almost all parts of the neem plant have efficacy as herbal medicine from roots, leaves, seeds to flowers (Asif, 2012).

Neem is known to have the potential to produce strong antioxidant activity (Supriyanto et al., 2017). It is known that antioxidants can counteract free radicals due to an oxidation reaction. Free radicals are molecules that do not have a partner so that the molecule becomes unstable and has high reactivity. As the number continues to increase, the amount of antioxidants and free radicals becomes unbalanced, which can cause oxidative cell stress. If this continues, it is feared that it will trigger serious diseases such as cardiovascular, inflammation, diabetes, and cancer (Fitriana et al., 2015).

A study stated that neem leaves were tested for phytochemicals containing compounds including flavonoids, alkaloids, polyphenols, phenols, steroids, glycosides, tannins, saponins, and essential oils (Bharat et al., 2015). Other studies also mention that the seeds and flowers of neem have phenolic compounds (Nahak and Sahu, 2011). Then in a 2014 study found the content of phenolic compounds in the roots of the neem plant (Hossain et al., 2014). Phenol chemical compounds contained in a plant can function well to prevent damage, inhibit lipid oxidation, and extend the shelf life of food (Dungir et al., 2012). Flavonoid chemical compounds are also known to belong to the water-soluble polyphenol family (Arifin and Ibrahim, 2018). Many plants have been studied and produce flavonoid compounds that have activities such as antioxidants and anti-inflammatory (Wang et al., 2016). Secondary metabolites such as phenolics and flavonoids that are widely distributed in plants are known to play a very important role in antioxidant activity, in a study it was stated that the greater the content of phenolic compounds, the greater the antioxidant activity (Shahwar et al., 2010).

Based on the described background that there is potential for neem to produce antioxidant activity and produce secondary metabolites such as phenolics and flavonoids, it is also known that phenolic and flavonoid compounds are thought to be responsible for antioxidant activity. The purpose of this literature review study is to present articles related to the potential antioxidant activity; content of phenolic compounds and flavonoids; knowing whether there is a relationship between phenolic compounds and flavonoids on antioxidant activity; as well as providing information regarding other potentials of neem (*Azadirachta indica A. Juss*) plants.

METHODS

The literature review study conducted is a type of narrative research. This writing uses a literature review study method where the library sources are used in making the literature review journals articles and several research references. The libraries used are scientific journals published in the last 10 years, namely between 2011 - 2020. Among the journals found are National and International journals published online from various web journals such as Google Scholar and PubMed. The process of searching articles using the keywords "neem or Azadirachta and antioxidant activities". The inclusion criteria used were literature published between 2011 – 2020 relating to the measurement results of antioxidants and levels of phenolic and flavonoid compounds and other potentials of neem plants. The exclusion criteria used were literature published before 2011 and cannot be accessed in full text. Based on the results of the search that has been carried out by looking at the inclusion and exclusion criteria, the final results are determined to be 10 journals analyzed where 6 pieces of literature are from PubMed and 4 kinds of literature are from Google Scholar.

RESULTS AND DISCUSSION

Based on the results of the literature search that has been collected, 10 articles were obtained that met the inclusion and exclusion criteria. The results of research from several journals obtained can be seen in (Table 1).

Table 1. Antioxidant Activities and Chemical Compounds of Neem Plants

No	Writer	Plant Parts	Chemical Compound	Method - Antioxidant Activity
1.	(Supriyanto et al., 2017)	Leaf	Tannins, saponins, flavonoids, and terpenoids.	DPPH - IC50 (ppm) 83.28 (80% methanol) 87.51 (60% methanol) 88.12 (80% ethanol) 88.69 (60% ethanol) 90.39 (water)
2.	(Simon et al., 2018)	Leaf	-	DPPH - IC50 (ppm) 99.25 (Vit C) 106.51 (Hexan) 101.53 (Ethyl Acetate) 394.20 (Water)
3.	(Nahak and Sahu, 2011)	Seed Flower	Phenolic	DPPH - IC50 (µg/mL) 39 < IC50 value (water) 140 (methanol) 102 (ethanol)
4.	(Airaodion et al., 2019)	Leaf	Saponins, tannins, phenols, alkaloids, flavonoids, glycosides	FRAP - IC50: 315.25 ± 23.81 mg/g
5.	(Hossain et al., 2014)	Root	Phenolic	DPPH - IC50: 13.81 ± 0.06 g/mL
6.	(Dhakal et al., 2016)	Leaf	Alkaloids, carbohydrates, glycosides, saponins, steroids, flavonoids, tannins, proteins	DPPH - IC50 (µg/mL) 80.28 (methanol) 439.60 (chloroform)
7.	(Pandey et al., 2014)	Leaf	Phenols, flavonoids, tannins	DPPH - IC50: 110.36 g/mL
8.	(Kiranmai et al., 2011)	Root bark	Flavonoids, quercetin	DPPH - IC50: 27.30 g/mL
9.	(Sithisarn et al., 2005)	Leaf Flower Bark	-	DPPH - IC50 (µg/mL) 26.50 27.90

				30,60
10.	(Bharat et al., 2015)	Leaf	Alkaloids, flavonoids, steroids, polyphenols, glycosides, phenols, tannins, essential oils, carotenoids, fatty acids, saponins	-

Antioxidant Activity

Antioxidants are molecules that can prevent oxidation reactions. The presence of free radicals in cellular macromolecules can cause several pathophysiological conditions such as Alzheimer's, hypertension, liver damage, Parkinson's disease, and Down syndrome (Kumaresan et al., 2015). Antioxidant activity has been widely studied which is contained in the neem plant. The method used to measure the amount of antioxidant activity is the DPPH and FRAP methods. The DPPH method (2,2-diphenyl-1-picrihydrazyl) is a nitrogen radical compound. The principle of this method is the hydrogen capture reaction by DPPH from the antioxidant itself. Meanwhile, the FRAP method was tested based on the ability of antioxidant compounds to reducing iron (III)-tripyrydyl-triazine to iron (II)-tripyrydyl triazine (Widyastuti, 2010). Measurement of antioxidant activity can be expressed by the IC50 value. The IC50 value is the effective concentration of the extract required to reduce 50% of the totally free radicals. The category of antioxidant activity is based on the IC50 value according to Molyneux 2004 in (Supriyanto et al., 2017), namely, if the IC50 value is <50 then the antioxidant activity is very strong, the value range is 50-100, which is strong, the value range is 100-150, which is moderate, and in the range of values, the value ranges from 100 to 150. 150 – 200 is weak, the value is expressed in ppm.

The research was conducted to identify neem plants taken from the leaves using different solvents and then continued with the measurement of antioxidant activity using the DPPH method, the results were expressed by the IC50 value. The values obtained with each solvent, namely 80% methanol solvent = 83.28 ppm; 60% methanol solvent = 87.51 ppm; 80% ethanol solvent = 88.12 ppm; 60% ethanol solvent = 88.69 ppm and water solvent = 90.39 ppm, in this case, it can be said that the smallest IC50 value shows strong antioxidant activity, namely 80% methanol extract in the neem leaves (Supriyanto et al., 2017). Neem leaf methanol extract was fractionated with the results of antioxidant activity expressed by IC50, namely the vitamin C fraction was 99.25 ppm, the hexane fraction was 106.51 ppm, the ethyl acetate fraction was 101.53 ppm, and the water fraction was 394, 20 ppm. In this study it was stated that the ethyl acetate fraction had higher antioxidant activity results than the hexane and water fractions, the cause this was that ethyl acetate is a type of semi-polar solvent that can attract non-polar and polar compounds (Simon et al., 2018)

In contrast to (Nahak and Sahu, 2011) in his research, he took extracts from the seeds and flowers which were then identified by the DPPH method. showed a low IC50 value, with methanol extract of 140 g/mL, ethanol extract of 102 g/mL. This study stated that the seeds showed the strongest free radical activity, while the flower parts had moderate antioxidant activity potential (Nahak and Sahu, 2011). The root part of the neem plant was identified using the DPPH method with an IC50 value of 13.81 ± 0, 06 g/mL so that it can be stated that the root part of the neem plant has the greatest potential in producing very strong antioxidant activity (Hossain et al., 2014). The root bark was also identified by (Kiranmai et al., 2011) by stating the results of antioxidant activity using the DPPH method, the DPPH - IC50 value was 27.30 g/mL.

A study in 2016 identified leaf sections using the DPPH method which was read at an absorbance of 517 nm showing the antioxidant activity value of methanol extract of 80.28 g/mL and chloroform extract = 439.60 g/mL. using methanol solvent has better antioxidant activity than extracts using chloroform solvent (Dhakal et al., 2016). The leaf part was identified for its antioxidant activity using the DPPH method which stated the IC50 result was 110.36 g/mL (Pandey et al., 2014). It is different from previous research that in this study (Airaodion et al., 2019) used the FRAP method to identify parts of neem leaves which showed that its antioxidant activity was weak, namely the IC50 value of 315.25 ± 23.81 mg/g.

Based on the parameter IC50 value, the neem plant has antioxidant activity and shows that the neem root has the strongest antioxidant activity. Different results can be influenced by the yields taken, some of the triggers for these differences include plant age, harvesting process, maintenance process, and others (Zuraida et al., 2017). In this case, further studies are needed to evaluate the antioxidant potential contained in neem plants, as well as the isolation and characterization of active compounds for commercialization in the pharmaceutical field.

Phenolic Compounds and Flavonoids

Phenolic compounds and flavonoids are secondary metabolites found in plants that are known to play an important role in antioxidant activity. This compounds examination can be done by qualitative or quantitative tests. Research that took part of the leaves stated that the chemical compounds contained included tannins, saponins, flavonoids, and terpenoids which were tested qualitatively (Supriyanto et al., 2017). Then research (Airaodion et al., 2019) stated that neem leaves contain saponins, tannins, phenols, alkaloids, flavonoids, and phytic acid compounds. In line with the two studies that (Dhakal et al., 2016) also stated that the extract on the neem leaf contains alkaloids, carbohydrates, glycosides, saponins, steroids, flavonoids, tannins, and proteins. In 2015 based on research conducted (Bharat et al., 2015) he found that neem leaves contain steroids, polyphenols, glycosides, essential oils, carotenoids, and fatty acids. Neem leaves were re-identified by (Pandey et al., 2014) that the compounds contained are phenols, flavonoids, and tannins. So based on the above research, the extract on the neem leaves has the potential to produce phenolic and flavonoid compounds.

In addition to the leaves, (Hossain et al., 2014) in 2014 qualitatively analyzed the roots of neem, he found that the roots of the neem plant contain phenolic compounds where these compounds can play an important role in antioxidant activity. Neem root bark was also identified, this study stated that neem root bark contains flavonoid compounds, and quercetin (Kiranmai et al., 2011). While in research (Nahak and Sahu, 2011) he identified neem plants in the seeds and flowers which were analyzed qualitatively, namely containing phenolic compounds with levels of each, namely seeds (oil) of 132 g/mL; flower parts with water solvent of 15 g/mL; methanol solvent of 34 g/mL; and ethanol solvent of 86 g/mL.

Correlation of Phenol and Flavonoid Compounds with Antioxidant Activity

This correlation analysis was conducted to see whether or not there was a close relationship between phenolic compounds and flavonoids with antioxidant activity. In compiling this literature review, the authors have not found appropriate articles, namely articles relating to the correlation or relationship between antioxidant activity

and levels of phenolic and flavonoid compounds from neem plants, but correlation analysis can be carried out by identifying plants that are still in the same family as neem plants. namely the Meliaceae family which can then be analyzed. In his research in 2013 he used the Surian plant, the plant is known to have a similar family to the neem plant, which is still one family Meliaceae, the Surian plant has been identified which is detected to contain flavonoids, and tannins in ethanol extract from various parts of the Surian tree, so in his research, he stated that the flavonoid content in the Surian tree is known to be the cause that the plant has antioxidant activity. Seeing from this study, there is a close relationship between flavonoid compounds and antioxidant activity (Sari et al., 2013).

Meanwhile, the flavonoid compounds tested quantitatively also obtained a correlation value (R2) of 0.98, which means that 98% of the antioxidant capacity in the analyzed samples was influenced by the content of flavonoid compounds and 2% was influenced by other compounds. So in this case it can be said that antioxidant activity has a synergistic action of various compounds that can work in free radical scavenging, and direct lipid peroxidation inhibition (Lompo et al., 2016). If the correlation value (R2) of phenolic and flavonoid compounds obtained is close to zero, then there is no close relationship between antioxidant activity and phenolic and flavonoid compounds (Widyastuti, 2010). In research (Nur et al., 2019) states that the role of antioxidants can be identified through phenolic compounds such as flavonoids, namely flavonols and flavones. In neutralizing free radicals, the activity of flavonoid compounds can depend on the number and location of the –OH group, so that in this case flavonoids in suppressing free radicals are related to the ability of these compounds to donate electrons. In the description, it is the cause that there is a relationship between total phenol content and antioxidant activity. The high value of total phenol and total flavonoid content can suppress the development of free radicals in the human body (Nur et al., 2019) so that in this case flavonoids in suppressing free radicals are related to the ability of these compounds to donate electrons. In the description, it is the cause that there is a relationship between total phenol content and antioxidant activity. The high value of total phenol and total flavonoid content can suppress the development of free radicals in the human body (Nur et al., 2019) so that in this case flavonoids in suppressing free radicals are related to the ability of these compounds to donate electrons. In the description, it is the cause that there is a relationship between total phenol content and antioxidant activity. The high value of total phenol and total flavonoid content can suppress the development of free radicals in the human body (Nur et al., 2019).

The description of the research above states that if the plant contains phenols and flavonoids, the antioxidant activity can be determined, although several studies have stated that the IC50 value is different. So that in this case it is suspected that there is a close relationship between phenolic compounds and flavonoids on the antioxidant activity contained in the neem plant part, and several studies also state that the part of the plant that has more potential as a large antioxidant agent is the leaf part, because that part There are 2 compounds, namely phenolic compounds and flavonoids which are directly related to their antioxidant activity.

The Potential of Neem Plants

Neem is one of natural ingredients that can be used as basic ingredients to be used as traditional medicine and natural ingredients to produce products in the pharmaceutical

sector because the use of materials derived from nature is thought to have lower side effects compared to modern medicine. Several articles mention that neem leaves, apart from producing antioxidant activity, are known to have other potentials that can be produced. Other pharmacological activities that can be produced are as follows:

a. Antibacterial

In his research (Andhiarto, 2019) tested antibacterial activity using 96% ethanol extract from neem leaves using the percolation method, previously he had carried out phytochemical screening to identify secondary metabolites contained in neem leaves. Screening results showed that neem leaves contain alkaloids, saponins, tannins, steroids, and flavonoids. Then the results of the antibacterial activity test of 96% ethanol extract of neem leave with different concentrations, namely at concentrations of 25%, 50%, and 75% stated that all three were able to inhibit the growth of Staphylococcus aureus bacteria, namely that concentrations of 25% and 50% were classified as moderate in diameter. the average inhibition zone was 6.48 mm and 8.42 mm. while at a concentration of 75% it has a strong inhibition zone, namely the average diameter of 12.06 mm (Andhiarto, 2019). Another article stated that neem leaf extract besides being able to inhibit the growth of Staphylococcus aureus could also inhibit Escherichia coli bacteria, the method used was TLC-Bioautography with an Rf value of 0.40. Then the results of the identification of chemical compounds with spray reagents from bioautography tests revealed that neem leaf extract contains phenolic compounds, alkaloids, flavonoids, saponins, and terpenoids. The compounds responsible for the inhibition of these bacteria are saponins, these compounds work to disrupt the stability of the membrane of bacterial cells so that the bacteria are lysed (Ramadhani et al., 2017). Testing of neem plant as an antibacterial was also investigated by (Susmitha et al.,

b. Immunomodulator

Extracts from neem leaves are known to have the potential as immunomodulators. A study stated that the ethanolic extract of neem leaves at a dose of 200 mg/kg / day which was given in 2 days, 4 days, and 6 days showed a decrease in the number of macrophages in test animals. The longer the days with the administration of ethanol extract from the neem leaves, the lower the number of macrophages in the test animals, namely mice. This shows that the ethanolic extract of neem leaves has an immunomodulatory effect on the number of peritoneal macrophage cells with the test animals used are mice that have been induced by the BCG vaccine (Abror et al., 2018)

c. Antidiarrheal

Through research conducted by Puspitaningrum in 2013, he identified leaf parts as samples for antidiarrheal agents. The positive control used was Loperamide HCl. The results obtained were an infusion of neem leaves had antidiarrheal activity against test animals in the form of male mice with the doses given were 204.36 mg/kg BW and 613.08 mg/kg BW. The feces from the test animal were observed. The dose is known to be effective as an antidiarrheal which is comparable to the positive control, namely Loperamide HCl at a dose of 0.73 mg/kg BW (Puspitaningrum, 2013)

d. Antifungal

The antifungal activity was tested using the good diffusion method. Neem leaf chloroform extract was distinguished by several concentrations. Identification of the active compound from the neem found secondary metabolites, namely the terpenoid group. The results of the antifungal activity test of chloroform neem extract against the fungus Fusarium sp had weak to strong activity from 3 concentrations, namely 50 ppm, 70 ppm, 100 ppm (Wahjuni et al, 2016)

CONCLUSION

Based on the journal articles that have been collected and analyzed, it can be said that the neem plant (*Azadirachta indica A. Juss*) has the potential to produce antioxidant activity. The phenolic and flavonoid compounds found in neem have a close relationship with antioxidant activity. The part of the plant that has very strong antioxidant activity is found in neem roots. Other potentials produced by neem plants are antibacterial, immunomodulatory, antidiarrheal, and antifungal.

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