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**RESEARCH ARTICLE**

# ANTIOXIDANT ACTIVITY OF FABACEAE FAMILY PLANTS BASED ON MACERATION EXTRACTION WITH DPPH METHOD

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## ABSTRACT

In Antioxidants are compounds possessing the capability to neutralize or stabilize free radicals. In general, plants from the Fabaceae family have antioxidant activity. The research aimed to determine the antioxidant activity of the Fabaceae family plant based on maceration extraction using the DPPH method as seen from $IC_{50}$. The method used was the narrative review method, where the selected articles were based on inclusion and exclusion criteria and obtained from Google Scholar, PubMed and ScienceDirect databases published between 2011-2021. The results indicated that the Fabaceae family plants having the strong antioxidant activity based on $IC_{50}$ were methanol extract of *Indigofera cassioides* leaf of 13.97 g/mL, methanol extract of Gogon Sirih (*Albizia richardiana*) bark of 17.8 g/mL, ethanol extract of vegetable hummingbird (*Sesbania grandiflora*) stem of 24.30 g/mL and ethanol extract of butterfly pea (*Clitoriaternatea*) of 41.36 g/mL. The strong antioxidants were found in methanol extract of blackbead (*Pithecellobium jiringa*) peel of 51.19 g/mL and acetone extract of vegetable hummingbird leaves of 56.57 g/mL; the medium antioxidant was in methanol extract of dadap (*Erythrina subumbrans*) bark of 189.57 g/mL; the weak antioxidants were in ethanol extract of old acacia (*Acacia auriculiformis*) leaves of 433.63 g/mL and ethanol extract of young acacia leaves of 464.23 µg/mL; the very weak antioxidant was found in ethanol extract of flame tree (*Delonix regia*) leaves of 4.030 g/mL.

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# INTRODUCTION

Free radicals are atoms or groups that have one or more unpaired electrons (Dungir, Katja & Kamu 2012, 11). These radicals tend to organize chain reactions that will cause continuous damage (Wahdaningsih, Setyowati&Wahyuono 2011, 157). As a solution to overcome the dangers of free radicals, antioxidants are needed (Cahyaningsih, Sandhi & Santoso 2019, 51). Antioxidants are substances that can neutralize free radicals, or a material that functions to prevent the body's biological system from adverse effects arising from processes or reactions that cause excessive oxidation (Rohmah *et al*. 2020, 68). Compounds that have potential as antioxidants are generally flavonoids, phenolic compounds and alkaloids (Surya 2017, 88). Plants of the Fabaceae family are a large plant family, with 650 genera and over 18,000 species. Most fabaceae plants contain flavonoid compounds that have been reported to effectively inhibit free radicals (Syukur *et al*. 2011, 62). The maceration extraction method is the process of soaking the sample to attract the desired components under discontinuous cold conditions. This method is more practical, the solvent used is less and does not require heating (Putra *et al.* 2014, 114). One of the methods used in testing antioxidant activity is the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) method.

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This test method is a conventional method and has long been used to determine the activity of antioxidant compounds. According to Widyastuti (2010), the DPPH method is easy to use, fast, quite thorough and well used in organic solvents (Sastrawan, Sangi & Kamu 2013, 111).

# RESEARCH METHODS

***Determination of Literature:*** Researchers conducted a search for published articles using Google scholar, Pubmed and ScienceDirect database sources with search keywords namely "*antioxidant of family fabaceae*", "antioxidant family fabaceae", "*radical scavenging of family fabaceae*", "antiradical free family fabaceae".

***Literature Review Criteria:*** Inclusion criteria in this study are the time span of research in the article for a maximum of the last 10 years (2011-2021). Articles using Indonesian and English. Original research articles and have ISSN/ISBN/Doi. *Full text is* available. The article discusses the antioxidant activity of fabaceae family plants based on maceration extraction with the DPPH method. The article includes the IC50 value$_{50}$. Exclusion criteria in this study are articles that are preliminary journals or review articles. The article does not clearly state the extraction method used. Articles using maceration extraction methods followed by fractionation.

# LITERATURE REVIEW RESULTS

**Table 1. Results of antioxidant activity study of plants of the fabaceae family**

| No. | Plant Parts | Genus | Family | Extraction Method | Extract Type | Antioxidant Test Method | $IC_{50}$ (µg/mL) | Category | Author and year of publication |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Turi Stems (*Sesbania grandiflora*) | Sesbania | Fabaceae | Maceration | Ethanol | DPPH | 24,30 | Very strong | Rohmah*et al. 2020* |
| 2. | Turi Leaf (*Sesbania grandiflora*) | Sesbania | Fabaceae | Maceration | Acetone | DPPH | 56,5707 | Strong | Rohmah, Rachmawati&Nisak 2018 |
| 3. | Bark of Dadap Serep(*Erythrina subumbrans*) | Erythrina | Fabaceae | Maceration | Methanol | DPPH | 189,57 | Medium | Herlina*et al*. 2019 |
| 4. | Jengkol Peel (*Pithecellobium jiringa*) | Pithecellobium | Fabaceae | Maceration | Methanol | DPPH | 51,1979 | Strong | Surya 2017 |
| 5. | Flamboyant Leaf (*Delonix regia*) | Delonix | Fabaceae | Maceration | 70% Ethanol | DPPH | 4.030 | Very weak | Syukur *et al*. 2011 |
| 6. | Acacia young leaf(*Acacia auriculiformis*) | Acacia | Fabaceae | Maceration | 96% Ethanol | DPPH | 464,2361 | Weak | Sari & Putra 2018 |
| 7. | Acacia Old Leaf (*Acacia auriculiformis*) | Acacia | Fabaceae | Maceration | 96% Ethanol | DPPH | 433,6332 | Weak | |
| 8. | Butterfly pea flower(*Clitoriaternatea*) | Clitoria | Fabaceae | Maceration | 70% Ethanol | DPPH | 41,36 | Very strong | Andriani &Murtisiwi 2020 |
| 9. | *Indigofera cassioides*leaf | Indigofera | Fabaceae | Maceration | Methanol 80% | DPPH | 13,97 | Very strong | Kumar, Rajkapoor&Peruma 2012 |
| 10. | Gogon Sirih (*Albizia richardiana*) Bark | Albizia | Fabaceae | Maceration | Methanol | DPPH | 17,8 | Very strong | Rahman *et al.* 2015 |

***Literature Review Stages:*** The article search used Google scholar database, Pubmed and Science direct with the keywords "antioxidant of family fabaceae", "antioxidant of family fabaceae", "radical scavenging of family fabaceae", "antiradical free family fabaceae". Based on these keywords, researchers found 52 articles which were then screened based on the inclusion criteria so that 26 articles did not meet the inclusion criteria where as many as 8 articles published under 2011, 8 articles that did not have ISSN/ISBN/Doi, as many as 10 articles that used extraction methods other than maceration. A total of 26 articles that met the inclusion criteria were screened again based on the exclusion criteria so that 17 articles were excluded where 1 article was a review article, 8 articles that did not clearly state the extraction method used and 8 articles that used fractionation results. So the articles that will be reviewed are 9 articles.

# DISCUSSION

Free radicals are unstable compounds that enter the body and can damage the immune system. To reduce the activity of free radicals, antioxidants are needed. Antioxidants are compounds that are able to neutralize or stabilize free radicals by completing the lack of electrons in the free radicals (Sari & Putra 2018, 22). The side effects of synthetic antioxidants make it necessary to look for natural antioxidants derived from plants (Saidi *et al.* 2020, 68). Compounds that have potential as antioxidants are generally flavonoid, phenolic and alkaloid compounds (Surya 2017, 88). Most fabaceae plants contain flavonoid compounds that have been reported to effectively inhibit free radicals (Syukur *et al*. 2011, 62). The preparation done before testing is extraction. Extraction is the process of withdrawing a component (solute) from its solution in water by another solvent that is not mixed using a suitable solvent (Hasrianti, Nururrahmah&Nurasia 2016, 20). Most plants of the Fabaceae family contain flavonoid compounds, so cold extraction is used, namely maceration because flavonoid compounds are a class of compounds that cannot withstand heat and are easily oxidized at high temperatures (Setiani*et al*. 2017, 16). This maceration method is more practical, the solvent used is less and does not require heating (Putra *et al.* 2014, 114). Antioxidant activity test method with 2*,2-diphenyl-1-pyridohydrazyl* (DPPH). This method was chosen because it is a simple, easy, fast, sensitive method and requires few samples for the evaluation of antioxidant activity of natural compounds so it is often used to test the ability of compounds that act as electron donors (Al Ridho 2013). Based on the literature search that has been conducted, 9 articles that meet the inclusion criteria and exclusion criteria will be reviewed. The search results showed several plants from the fabaceae family that have antioxidant activity, namely white turi (*Sesbania grandiflora*), dadapserep (*Erythrina subumbrans),* flamboyant (*Delonix regia),* jengkol (*Pithecellobium jiringa),* acacia (*Acacia auriculiformis)*, Butterfly pea (*Clitoriaternatea*), *Indigofera cassioides*and Gogon Sirih (*Albizia richardiana)*. $_{50}$Some studies prove that plant extracts of the fabaceae family have antioxidant activity including in the research of Kumar, Rajkapoor&Peruma (2012) where *Indigofera cassioides*leaves were extracted by maceration using 80% methanol solvent for 72 hours to obtain methanol extracts which were then tested for antioxidant activity with the DPPH method so that the IC50 value was 13.97 µg/mL. This shows that the methanol extract of *Indigofera cassioides*leaves is included in the very strong category as an antioxidant because according to Ratnawati, Riyanti& Fitriani (2013). This antioxidant effect is due to the presence of phenolic compounds and flavonoids present in the methanol extract of *Indigofera cassioides leaves*.

In the research of Rahman *et al. (*2015) showed that the bark of Gogon Sirih (*Albizia richardiana*) has antioxidant activity.The bark was extracted by maceration using methanol solvent for 2 weeks with the DPPH antioxidant activity test method so that the IC of 17.8 µg/mL was obtained. In the research of Rohmah*et al*(2020) showed that the white turi stem (*Sesbania grandiflora*) which is a family of fabaceae with maceration extraction method using ethanol solvent for 24 hours to obtain ethanol extract which was then tested for antioxidant activity with the DPPH method so that the IC50 value was 24.30 µg/mL. This shows that the white turi stem (*Sesbania grandiflora*) has very strong antioxidant activity. $_{50}$Other parts of this species, namely white turi leaves (*Sesbania grandiflora*) also have antioxidant activity shown in the research of Rohmah, Rachmawati&Nisak (2018) acetone extract of maceration extraction method with DPPH method antioxidant activity test has an IC of 56.5707 µg/mL which is included in the strong category as an antioxidant. Based on the TLC test results, the ethanol extract of white turi stem contains triterpenoids and tannins (Rohmah*et al.* 2020) while the TLC test results of acetone extract of white turi leaves contain tannin compounds (Rohmah, Rachmawati&Nisak 2018). The antioxidant activity of ethanol extract of white turi stem is stronger than the antioxidant activity of white turi stem leaves, this is due to the distribution of secondary metabolite compounds in white turi stems and leaves with different levels. The same compound or the same group of compounds are synthesized or deposited in different organs. The solvent used is also different, the extraction of white turi stems using ethanol solvent which is polar and the extraction of white turi leaves using acetone solvent which is semi-polar.

The compound that is thought to play a role as an antioxidant in white turi is a polar tannin compound so it is recommended to use a polar solvent for extraction. In Andriani &Murtisiwi's research (2020) showed that Butterfly pea flowers (*Clitoriaternatea*) which are a family of fabaceae have antioxidant activity. Butterfly pea flowers (*Clitoriaternatea*) were extracted by maceration with 70% ethanol solvent to obtain a concentrated extract which was then tested for antioxidant activity using the DPPH method to obtain an IC50 value of 41.36 µg/mL. In Surya's research (2017) showed that the skin of jengkol (*Pithecellobium jiringa*) extracted by maceration with methanol solvent which was then tested for antioxidant activity with the DPPH method so that the IC50 value was obtained$_{50}$ of 51.1979 µg/mL. In the research of Herlina*et al*(2019) showed that the bark of Dadap Serep (*Erythrina subumbrans*which was then extracted by maceration method for 24 hours using methanol solvent which was then tested for antioxidant activity with the DPPH method so that the IC was 189.57 µg/mL. Sari & Putra (2018) showed that *acacia* leaves (*Acacia auriculiformis*) have antioxidant activity. In his research using parts of old leaves and young leaves extracted maceration using ethanol solvent for 3 days which was then tested for antioxidant activity with the DPPH method so that the IC50 value$_{50}$ of young leaves was 464.2361 µg/mL and old leaves were 433.6332 µg/mL which included a weak category as an antioxidant because the IC$_{50}$value was between 250-500 ppm. Flamboyant leaves (*Delonix regia*) have activity as a very weak antioxidant category because it has an IC$_{50}$ value of 4,030 µg/mL in the study of Syukur *et al.* (2011).

From some of the data that has been reviewed regarding the antioxidant activity of plants of the fabaceae family based on maceration extraction with the DPPH test method, it is found that plants that have very strong antioxidant activity are the leaves of *Indigofera cassioides*based on phytochemical screening of these plants, presumably the potential antioxidants are flavonoid and phenolic compound groups. Phenolic and flavonoid compounds have hydroxyl groups in their structure that can donate hydrogen atoms to free radicals so that both have potential as antioxidants (Al Ridho 2013). Flavonoid compounds have antioxidant activity by inhibiting oxidation reactions by capturing free radicals (Marliani et al., 2017). The phenol group in flavonoids will donate 1 electron to unpaired free radicals so that free radicals become stable (Do et al. 2014, 297). Phenolic compounds have a form of chemical groups that contain phenol groups (hydroxyl groups) in their basic structure. The antioxidant mechanism of phenolic compounds is based on oxidation-reduction reactions, where phenolics act as reducers that will reduce free radicals (reactive) to unreactive (Andriani &Murtisiwi 2020, 73). Another compound contained in plants of the Fabaceae family that has very strong and strong antioxidant activity is tannin compounds. Tannin compounds are included in the flavonoid group which has strong antioxidant power due to the stability of its structure due to the position of the hydroxy group in the ortho position, namely the OH group bound to C atom number 3 and number 4 in ring B. Tannin compounds will donate H atoms as DPPH free radical absorbers, resulting in radical stabilization of tannin compounds (Rohmah, Rachmawati & Nisak 2018, 674). *Acacia* leaves (*Acacia auriculiformis*) and flamboyant leaves (*Delonix regia*) have weak and very weak antioxidant activity. This can be caused by several factors, namely the type of solvent, extraction method and purity of the compounds produced in the extract.

The type of solvent used for the extraction of acacia leaves (*Acacia auriculiformis*) and flamboyant leaves (*Delonix regia*) is ethanol. The effectiveness of compound extraction depends on the solubility of the compound in the solvent according to the principle of *like dissolve like,* namely the compound will dissolve in a solvent with the same properties. Flavonoid compounds are polar compounds and solvents that are polar are ethanol, methanol, acetone and water (Verdiana, Widarta&Permana 2018, 214). It is recommended for further extraction using other solvents that are polar. In the research of Urmi *et al.* $_{50}$(2013) used methanol solvent to extract acacia leaves (*Acacia auriculiformis*) with a modified maceration extraction method which was then tested for antioxidant activity using the DPPH method so that the IC50 value was 7.95 µg/mL which showed a very strong category as an antioxidant. Flavonoid compounds in ethanol extracts of acacia leaves (*Acacia auriculiformis*) and flamboyant leaves (*Delonix regia*) are in the form of impure extracts so that flavonoid compounds in the extracts may still bind to glycoside groups. According to Harborne (1987) in Al Ridho's research (2013) that flavonoids in plants often exist as glycosides (flavonoid glycosides) and are rarely found in the single form of flavonoids. For this reason, it is recommended to use fractionation in order to separate the main compound content from other compound groups.

# CONCLUSIONS

Based on the data obtained from the review results, it can be concluded that:

1. IC50 value of methanol extract of *Indigofera cassioides*leaves 13.97 µg/mL, methanol extract of sirihgogon bark 17.8 µg/mL, ethanol extract of white turi stem 24.30 µg/mL, and ethanol extract of Butterfly pea flower 41.36 µg/mL, methanol extract of jengkol skin 51,19 µg/mL, acetone extract of white turi leaves 56.57 µg/mL, methanol extract of leaf bark 189.57 µg/mL, ethanol extract of old acacia leaves 433.63 µg/mL, ethanol extract of young acacia leaves 464.23 µg/mL, and ethanol extract of flamboyant leaves 4.030 µg/mL.
2. Very strong antioxidant activity was found in methanol extract of *Indigofera cassioides*leaves, methanol extract of Gogon Sirih bark (*Albizia richardiana*), ethanol extract of white turi stem (*Sesbania grandiflora*), and ethanol extract of Butterfly pea flower (*Clitoriaternatea)*.

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