

## HPLC DETERMINATION OF BENZOXAZOLINONE ON JERUJU PLANT (*ACANTHUS ILICIFOLIUS*): NARRATIVE REVIEW

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### ABSTRACT

Mangrove plants in Indonesia are the largest in the world, both in terms of area quantity (+42,550 km<sup>2</sup>) and number of species (+45 species). Mangroves are a diverse group of salt tolerant plants that grow in intertidal tropical and subtropical forests and represent a rich biodiversity of plants, animals and microorganisms. *Acanthus ilicifolius* is one of the true mangrove plants which is used as medicine. This literature review aims to determine the study of *benzoxazolinone* compounds in jeruju plants (*Acanthus ilicifolius*) from previous studies and to determine the methods, plant parts and solvents used. The literature review method related to the jeruju plant (*Acanthus ilicifolius*) uses an electronic data base *google scholar*. *Nuclear Magnetic Resonance* (NMR) spectroscopy analysis method is one of the spectroscopic methods widely used in previous studies, because NMR spectroscopy is useful in determining the structure of a chemical compound, both proton (1H) and carbon (13C) NMR. Jeruju leaf extract contains alkaloids, flavonoids, polyphenols, phenyletanoid glycosides and coumarin compounds. The methanol fraction of jeruju leaf extract has a pharmacological effect to inhibit edema (inflammation). The extraction method used is maceration, because this method is simple method and most widely used. Meanwhile, the solvents that are more widely used are methanol and ethanol solvents.

**Keywords:** HPLC; *Acanthus ilicifolius*; Benzoxazolinone

## 1. INTRODUCTION

Mangrove plants in Indonesia are the largest in the world, both in terms of area (+42,550 km<sup>2</sup>) and the number of species (+45 species). Mangroves have many benefits that are directly related to human life, ranging from ecological benefits to being a source of food and medicine. Many universities and research institutes have explored various plants as sources of medicine but have not looked at the benefits of mangroves (Suryati et al., 2018). Mangroves are a diverse group of salt-tolerant plants that grow in tropical and subtropical intertidal forests and represent a rich biodiversity of plants, animals and microorganisms (Cai et al., 2017).

*Acanthus ilicifolius* is one of the mangroves used for treatment. Traditionally all parts of this plant are used as a treatment, as in the fruit part it is used for Aphrodisiac and asthma; on the part of the leaves and roots are used for antidiabetic, diuretic, hepatitis, leprosy; the bark of the stem is used for neuralgia, roundworms, rheumatism, skin disease, and abdominal pain. Decoction of jeruju leaves is mostly used for energy recovery after giving birth by boiling and used for bathing water, restoring stamina after giving birth and preventing infection in the uterus (Suryati et al., 2018).

*2-Benzoxazolinone* (BOA) is an allelochemical, derived from plant secondary metabolism, which plays a key role in various biological processes. Its derivatives exhibit various properties important to human health through interactions with a number of cellular targets involved in

various disease conditions. BOA derivatives have been associated with various types of biological properties. Lespagnol et al. was first prepared and reported BOA has hypnotic properties. Many BOA derivatives were tested for various activities including anticonvulsant, antipyretic, analgesic, cardiotoxic, antiulcer, antineoplastic or antibacterial, antimicrobial, and antifungal effects (Verma & Silakari, 2018).

The results of this study will later be obtained discoveries in the form of Benzoxazinone compounds from the jeruju plant extract (*Acanthus ilicifolius*) carried out by several previous studies.

## 2. METHODS

This research method uses a literature review method with a quantitative approach, while the method used is to collect international and national articles and journals from 2001 to 2022. Using *electronic databases*. The *data base* used is *Google Scholar* by using keywords in English, namely *HPLC*, *Acanthus ilicifolius*, *benzoxazinoid glucoside*, and *antibacterial activity*. The article or journal must be accessible in its entirety through the database (not only abstract) and must be written in Indonesian or English.

## 3. RESULTS AND DISCUSSION

Research conducted by Yin et al (2008) used the leaves of the jeruju plant (*Acanthus ilicifolius*) to study the isolation and purification of two *benzoxazinoids*, HBOA Glc and DIBOA-Glc from *A. ilicifolius* using HSCCC with HPLC analysis method and separation was carried out with HSCCC. The extraction method used is by maceration. The results showed that HSCCC was able to be used for isolation and purification of 2-benzoxazinoids, HBOA Glc and DIBOA-Glc from *A. ilicifolius* using HSCCC.

On research Kanchanaphoom et al (2001) using the leaves of the jeruju plant (*Acanthus ilicifolius*) to determine the structure of *benzoxazinoid glucoside* compounds from *Acanthus ilicifolius* by NMR spectrum analysis method, JEOL JNM A-400 spectrometer, and HPLC preparative. The extraction method used is by maceration. The results of the glucoside compound 7-chloro-(2R)-2-Obd-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one were obtained.

On research Zhao et al (2015) using the roots of the jeruju plant (*Acanthus ilicifolius*) to determine the cytotoxic activity of *Acanthus ilicifolius* in vitro from new isolates against HepG2, HeLa, and A-549 cancer cell lines by NMR analysis method and isolation using HPLC. The extraction method used is by maceration. The results of extracts derived from *Acanthus ilicifolius* and 2-benzoxazinone have been shown to have antitumor effects with a range of IC50 values of 7.8-26.6 M.

On research Bhattarai et al (2022) using the roots of the jeruju plant (*Acanthus ilicifolius*) to develop *Data Dependent Acquisition* (DDA) and *liquid chromatography mass spectrometry* (LC MS) techniques on the isolation of *benzoxazinoid* compounds with the analysis method of *Data Dependent Acquisition* (DDA) and *liquid chromatography mass spectrometry* (LC MS). The extraction method used is by maceration. It was found that the discovery of DIBOA-glucoside acetate and HBOA-glucoside acetate in the roots of *Acanthus ilicifolius*.

On research Tian et al (2021) using the leaves of the jeruju plant (*Acanthus ilicifolius*) to determine the inhibitory effect of BOABB compounds on the proliferation and apoptosis of C-33A cervical cancer cells with the NMR analysis method and using the CCK-8 test. It was found that BOABB had a pronounced inhibitory effect on C-33A cells, with an IC50 of 32.3 M. Apoptosis assays showed that BOABB had induced apoptosis in C-33A cells (from 10.86% to 34.70%).

**Table 1.** Journal Synthesis Matrix

No	Writer & Year	Destination	Analysis Method	Sample (Plant Part)	Finding	Extraction Method	Solvent
1	(Yin et al., 2008)	To study the isolation and purification of 2-benzoxazinoids, HBOA-Glc and DIBOA-Glc from <i>A. ilicifolius</i> using HSCCC.	It was analyzed with HPLC and separation was performed with HSCCC.	Leaves of <i>Acanthus ilicifolius</i> L.	This study proved that HSCCC is capable of being used for isolation and purification of 2-benzoxazinoid, HBOA-Glc and DIBOA-Glc from <i>A. ilicifolius</i> using HSCCC.	Maceration	Ethanol
2.	(Kanchanapoom et al., 2001)	Determination of the structure of the compound <i>benzoxazinoid glucoside</i> of <i>Acanthus ilicifolius</i> .	NMR spectrum, JEOL JNM A-400 Spectrometer, and preparative HPLC.	Leaves of <i>Acanthus ilicifolius</i> L.	Obtained glucoside compound 7-chloro-(2R)-2-Obd-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one.	Maceration	Methanol
3.	(Zhao et al., 2015)	Knowing the cytotoxic activity of the <i>Acanthus ilicifolius</i> plant in vitro from new isolates against HepG2, HeLa, and A-549 cancer cell lines.	NMR and isolation using HPLC.	Roots of <i>A. ilicifolius</i> .	Extracts derived from <i>A. ilicifolius</i> and 2-benzoxazolinone have been shown to have antitumor effects with an IC50 value range of 7,8-26,6 M.	Maceration	Ethanol
4.	(Bhattarai et al., 2022)	Developed the Data Dependent Acquisition (DDA) and liquid chromatography mass spectrometry (LC MS) technique on the isolation of <i>benzoxazinoid</i> compounds.	Using Data Dependent Acquisition (DDA) and liquid chromatography mass spectrometry (LC MS).	Roots of <i>A. ilicifolius</i> .	Found DIBOA-glucoside acetate and HBOA-glucoside acetate in the roots of <i>Acanthus ilicifolius</i> .	Maceration	Methanol
5.	(Tian et al., 2021)	Knowing the inhibitory effect of BOABB compounds on the proliferation and apoptosis of C-33A cervical cancer cells.	Using NMR and using the CCK-8 test.	Leaves of <i>Acanthus ilicifolius</i> L.	BOABB has a pronounced inhibitory effect on C-33A cells, with an IC50 of 32,2 M. Apoptosis assays show that BOABB has induced apoptosis in C-33A cells (from 10,86% to 34,70%).	-	-

### **3.1. Analysis Method**

Based on **Table 1**, most experts choose analysis methods using Nuclear Magnetic Resonance (NMR) Spectroscopy, among others, namely ([Bhattarai et al., 2022](#); [Kanchanapoom et al., 2001](#); [Tian et al., 2021](#); [Zhao et al., 2015](#)) Nuclear Magnetic Resonance (NMR) Spectroscopy is a spectroscopic method that is very useful in determining the structure of a chemical compound, both proton (<sup>1</sup>H) and carbon (<sup>13</sup>C) NMR. This method is based on the magnetic moment of the atomic nucleus. Certain nuclei exhibit behavior as if they were spinning. If a nucleus with spin is placed between the poles of a very strong magnet, the nucleus will align its magnetic field parallel (parallel) or against (antiparallel) the magnetic field ([Utami et al., 2020](#)). In addition, NMR spectroscopy is useful for identifying the structure of compounds or the molecular formula of organic compounds.

### **3.2. Plan Part (Leaf)**

Most of the experts shown in **Table 1** use the leaf part in the jeruju plant (*Acanthus ilicifolius*) which is used as extraction, among others. ([Kanchanapoom et al., 2001](#); [Tian et al., 2021](#); [Yin et al., 2008](#)) Jeruju leaves can be used for energy recovery after giving birth by boiling and used for bathing water, restoring stamina after giving birth and preventing infection in the uterus. In addition, jeruju leaf extract contains alkaloids compounds, flavonoids, polyphenols, phenyletanoid glycosides and coumarins. The methanol part of thistle leaf extract has a pharmacological effect to inhibit edema (inflammation) ([Suryati et al., 2018](#)).

### **3.3. Extraction Method (Maceration)**

Extraction is a method of separating substances based on the difference in the solubility of two different insoluble liquids, usually water and the other in the form of an organic solvent. There are several methods that can be used in extraction, one of which is the maceration method ([Badaring et al., 2020](#)).

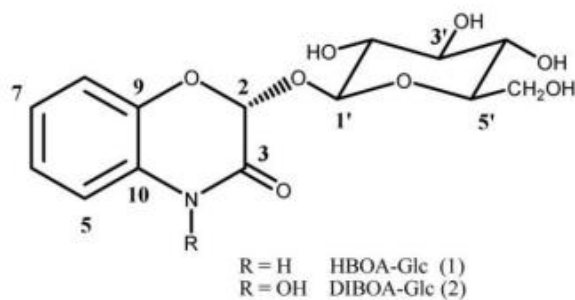
Most experts use the maceration extraction method, including: ([Bhattarai et al., 2022](#); [Kanchanapoom et al., 2001](#); [Yin et al., 2008](#); [Zhao et al., 2015](#)). This method was chosen because it is the simplest method that is most widely used. This method is suitable, both for small scale and industrial scale. This method is carried out by placing the plant powder and suitable solvent in an inert container that is tightly closed at room temperature. The extraction process was stopped when an equilibrium was reached between the concentration of the compound in the solvent and the concentration in the plant cells. After the extraction process, the solvent is separated from the sample by filtration. In addition, the maceration method can avoid the destruction of thermolabile compounds ([Mukhriani, 2014](#)).

### **3.4. Solvent (Ethanol and Methanol)**

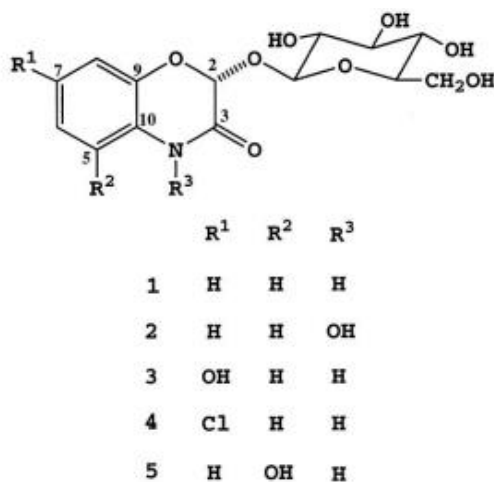
Solvent extraction is the most commonly used technique for the isolation of plant antioxidant compounds. The yield of the extract to produce the antioxidant activity of the plant material is highly dependent on the nature of the extractor solvent. The presence of different antioxidant compounds varies in chemical characteristics and polarity which may or may not be soluble in certain solvents. The most suitable polar solvents for the recovery of polyphenols from plant matrices are aqueous mixtures (hot or cold) containing ethanol, methanol, acetone, and ethyl acetate. Methanol and ethanol have been widely used to extract antioxidant compounds from various plants and plant foods (fruits, vegetables and others) such as strawberry, pomegranate, broccoli, rice, wheat germ, orange peel, and many other fruit peels ([Apriasari, 2015](#)).

Methanol is a solvent that can dissolve almost all organic compounds, both polar and non-polar because methanol has a polar group (-OH) and a nonpolar group (-CH<sub>3</sub>). While ethanol is a solvent that is universal, polar, and easy to obtain. In addition, ethanol is relatively non-toxic compared to methanol ([Sayuti, 2017](#)).

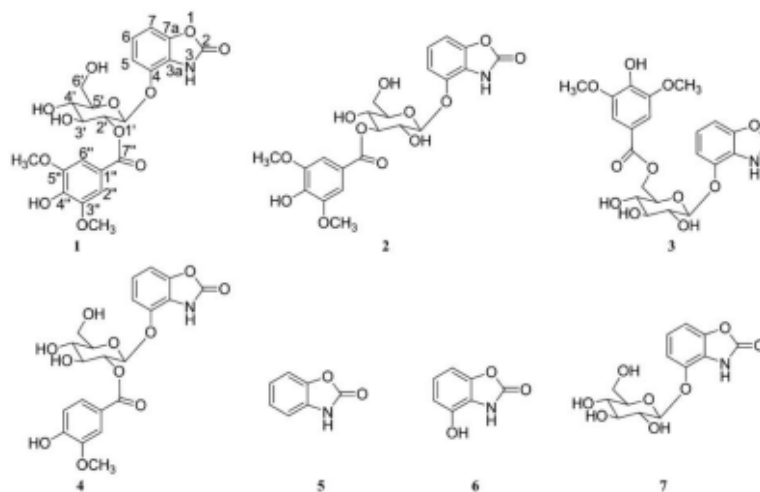
### 3.5. Chemical Structure of Benzoxazolinone



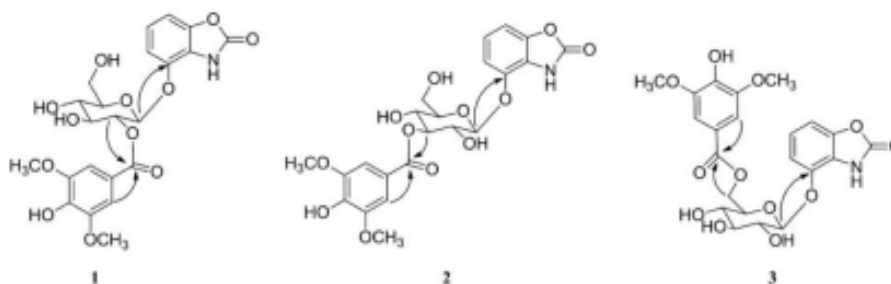
**Figure 1.** Chemical structure of HBOA-Glc and DIMBOA-Glc (Yin et al., 2008).



**Figure 2.** Chemical structure of BOA-Glc, BOA-Glc, and DOA-Glc (Kanchanapoom et al., 2001).



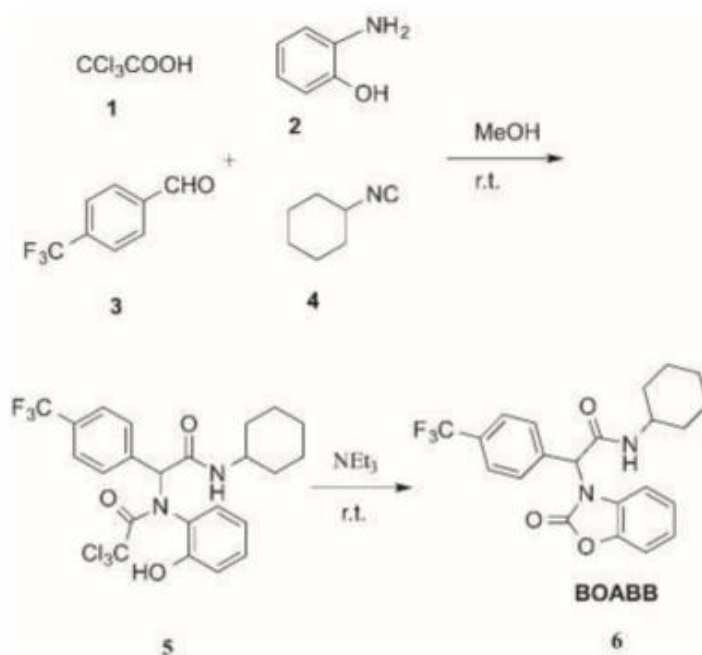
**Figure 3.** Chemical structure of compounds 1-7 (Zhao et al., 2015).



**Figure 4.** Key HMBC correlation ( $\gamma$ ) of 1-3 compounds (Zhao et al., 2015).



**Figure 5.** Chemical structure of DIBOA (Bhattarai et al., 2022).



**Figure 6.** Synthesis of N-cyclohexyl-2-(2-benzoxazolone-3-yl)-2-p-trifluoromethylphenylacetamide (Tian et al., 2021).

From **Figure 1** to **Figure 6** above, experts use the chemical structure of benzoxazolinone including HBOA-Glc, DIMBOA-Glc and DHBOA-Glc. The chemical structure is a derivative based on BOA or another name for *2-Benzoxazolinone*. Structural modifications in the core scaffold are affected by the diverse incorporation of a number of related building blocks, which further results in final compounds with different structural features and pharmacological properties. This has become a major focus of the medicinal chemistry research community, to develop new agents against various disease conditions (Verma & Silakari, 2018). The limitations of this study are in the form of limited research on the study of *benzoxazolinone* compounds in the jeruju plants (*Acanthus ilicifolius*) using HPLC so that the reference to the article in this narrative review is small.

#### 4. CONCLUSION

From the results of the research literature review, the results obtained are: The benzoxazolinone compound of the jeruju plant (*Acanthus ilicifolius*) can be used as a traditional medicine, but this compound has chemical resistance to insects, fungi, bacteria, and viruses in many Gramineae family plants, as well as mutagenic activity. In addition, extracts derived from *A. ilicifolius* and *2-benzoxazolinone* have been shown to have antitumor effects. The analytical method used for the *benzoxazolinone* compound is Nuclear Magnetic Resonance (NMR) spectroscopy, because this method is a spectroscopic method that is very useful in determining the structure of a chemical compound, both proton (<sup>1</sup>H) and carbon (<sup>13</sup>C) NMR. The jeruju plant used for research is the leaf part, because the jeruju leaf can be used for energy recovery after



giving birth by boiling and used for bathing water, restoring stamina after giving birth and preventing infection in the uterus. In addition, jeruju leaf extract contains alkaloids, flavonoids, polyphenols, phenyletanoid glycosides and coumarin compounds. The extraction method used is maceration, because maceration is a simple method that is most widely used. This method is suitable, both for small scale and industrial scale. The solvents used are methanol and ethanol because methanol and ethanol have been widely used to extract antioxidant compounds from various plants and plant foods (fruits, vegetables, etc.) orange peel, and many other fruit peels. Identification of *Benzoxazolinone* compounds is better if the extraction is carried out using the maceration method with methanol solvents, since the methanol solvent is slightly more polar compared to ethanol solvents and for the identification of compounds can be carried out using the KLT (Thin Layer Chromatography) method.

## 5. ACKNOWLEDGMENT

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