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Identification of Active Compounds of Tantrum Leaves (*Indigofera tinctoria*) as Natural Textile Dyes

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Abstract. The use of natural dyes as textile dyes is increasing due to environmentally friendly standards and does not require the use of synthetic dyes. By banning the use of synthetic dyes containing azo groups, this is the right moment to reintroduce natural dyes that have been abandoned for a long time. This study aims to identify the active compound of tantrum leaves (*Indigofera tinctoria*) as a natural textile dye by maceration extraction method with variations in maceration time and material treatment on wet and dry tantrum leaves. This experimental research method was carried out using several stages of analysis, namely the phytochemical test, FTIR (Fourier Transform Infrared Spectroscopy), and UV-Vis (Ultraviolet-Visible Light) spectrophotometer. The results of the phytochemical tests showed that the compounds contained in the *Indigofera tinctoria* plant were indigo, flavonoids, steroids, and alkaloids. The FTIR test indicated that the dye content in the extract was from the indigo group. The results of the UV-Vis spectrum showed that there was maximum absorption in wet tantrum leaf extract, dry tantrum leaf extract, and alkaline extract at wavelengths of 406.50 nm, 365.50 nm, and 664.50 nm, respectively. Natural dyes in textiles have a positive impact on the environment, the color waste products are biodegradable and can be decomposed.

Keywords: *Indigofera tinctoria*, Active Compound, Maceration Extraction

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INTRODUCTION

The development of the use of natural dyes as textile dyes has recently increased. This is related to environmental standards and the prohibition on the use of synthetic dyes containing an azo group, such as in Germany and the Netherlands which require the use of environmentally friendly textile dyes and do not require the use of synthetic dyes. With the ban on the use of synthetic dyes containing azo groups, this is the right moment to reintroduce natural dyes that have long been abandoned (Mayun et al., 2016). Dyes have been used in various industries, such as food, beverages, cosmetics, textiles, etc. Dyes according to their origin are divided into two, namely natural dyes and synthetic dyes (Dkhil et al., 2020). However, in its use, synthetic dyes are more widely used in various industries, especially in the textile industry. This is because synthetic dyes have higher stability and their use in small amounts is sufficient to give the desired colour. On the other hand, waste from the use of synthetic dyes can cause side effects that show carcinogenic properties and pollute the environment (Kumbasar, 2016, Kulkarni & Rathod, 2015). Thus, the use of synthetic dyes is better replaced with natural dyes, commonly used natural dyes such as indigo tree leaves, turmeric, noni root, and others. Among the natural dyes used and recognized throughout the world are natural dyes from tantrum plants hich are the oldest known natural dyes (Ariyanti & Asbur, 2018).

Indigofera plants, also known as Indigo based on their protein content, are divided into three classes, including the first quality, only the leaves can be harvested at the age of one month. Harvested every two months, the leaves and stems are taken. This indigofera plant material can be developed and cultivated as a natural dye pigment. The existence of natural colors has been displaced by synthetic coloring due to a large number of consumer demands, being able to simplify and provide economic benefits, time efficiency, and

the resulting colors vary. But the impact caused by liquid and solid waste resulting from the synthetic dyeing process has properties that are difficult to dissolve or are difficult to decipher. This is indicated by the production of liquid waste that is cloudy and thick in color.

The dye produced by tantrum is a colorless glucoside derivative in the enol form of indoxyl, for example, indicant (indoxyl-D-glucoside). Tantrum plant (*Indigofera tinctoria*) is a plant that produces a naturally blue color from the extraction of leaves. Apart from being a producer of blue or indigo tantrum, it is also used as a producer of green color by combining it with other yellow natural dyes. In the pharmaceutical field, tantrum plants are known as anti-cancer, anti-oxidant, and anti-microbial (Vijayan et al., 2018a, Dkhil et al., 2020) tantrum plants are typical cultivated plants from Boja and are species of the genus Indigofera so that people know tantrum plants as indigo. According to (Muzzazinah et al., 2016) there are 65 types of compounds found in plants of the genus Indigofera including flavonoids, terpenoids, steroids, and various other compounds. In the Boja area, the dye from tantrum/indigo leaves is used by local people as a dye to make natural batik called Linggo Batik. The color is obtained from the soaking of cotton cloth on tantrum leaves which has been added with sugar as a reducing agent.

A dye compound that comes from plants in leaves, stems, fruits and roots can be obtained using the extraction method. There are various extraction methods commonly used, both cold extraction and heating extraction. Extraction of dyes from plants can be done by extraction methods such as maceration, soxhlation and reflux using solvents with different polarities (Nurhasnawati et al., 2017). Extraction is a process carried out to extract the content of compounds contained in the separated sample using a solvent. The resulting extract is a thick sediment from the active compound obtained. Maceration is one way of extraction which is done by soaking the sample that has been in the form of a powder using a certain solvent at room temperature to attract the compounds contained in a sample. Maceration is often used in the extraction process because it has advantages including not using high temperatures so it is safe for various types of compounds that are not heat resistant, can attract many compounds (Safitri et al., 2018).

This study aims to maximize the utilization of tantrum leaves (*Indigofera tinctoria*) as a natural textile dye by maceration extraction method with variations in maceration time and material treatment on wet and dry tantrum leaves. To determine the active compounds, phytochemical analysis was carried out and to determine the class of dyes contained in tantrum leaves, the dyes were characterized using UV-Vis (Ultraviolet-Visible Light) and FTIR (Fourier Transform Infrared Spectroscopy) spectrophotometers. Based on the results of the UV-Vis spectrophotometer, the wavelength of the dye is known, while with FTIR the functional group of the dye is known. It is hoped that this research can provide information that tantrum leaves can be used as a source of natural dyes that are environmentally friendly and can be applied to textiles. In addition, it is expected to be able to provide solutions in reducing the use of synthetic dyes.

METHODS

This research was carried out from September to October 2021. Phytochemical, FTIR and UV-Vis analyses were carried out by the Chemistry Laboratory of the FST State Islamic University Walisongo Semarang and plant identification in Boja Kendal. The tools used in this research are FTIR spectrophotometer, UV-Vis's spectrophoto meter, and other practical tools. The materials used in this study were distilled water (H_2O), 5% iron III chloride ($FeCl_3$), 96% technical ethanol (C_2H_5OH), iodide (I_2) solids, potassium iodide (KI) solids, calcium carbonate ($CaCO_3$) technical, sodium hydroxide (NaOH) 10%.

Compound Extraction

Base Extraction

Extraction of tantrum leaves under alkaline conditions is carried out by soaking tantrum leaves for 24 hours, then adding lime until a pH of 8-9 is obtained, then the soaking results are filtered to separate the filtrate and residue to obtain a thick extract (Hakiim & Sari, 2018).

Wet sample extraction

Wet sample extraction was carried out by cleaning and smoothing tantrum leaves, then weighing 20 grams of the sample and then macerating for 1-24 hours using 96% ethanol (C_2H_5OH) solvent. Filtering the obtained macerate then evaporated to obtain a thick extract and the pH of the extract obtained was measured (Hassen et al., 2007).

Dry sample extraction

Extraction of dry samples was carried out by cleaning the tantrum leaves obtained and then

drying and smoothing the tantrum leaves. Next, weigh the leaves to be macerated. Maceration was done by immersing the sample in ethanol for 1 x 24 hours, then filtered and evaporated to obtain a thick extract and then the pH of the extract was measured (Hassen et al., 2007).

Phytochemical Analysis

Flavonoid Test

The flavonoid test was carried out by diluting the thick extract and pipetting a few drops of the sample into a drip plate then adding two drops of concentrated sulfuric acid (H_2SO_4) and observing the colour changes that occurred. Positive results are shown when the H_2SO_4 reagent produces a dark yellow colour to pink, in NaOH there is a colour change from dark yellow to light yellow and with the addition of 5% $FeCl_3$ there is a yellowish green colour.

Alkaloid test

This test was carried out by diluting the thick extract and pipetting a few drops of the sample into a drip plate and then adding Meyer's reagent to each sample, which was indicated by the presence of an orange or brown precipitate and a positive result of Wagner's reagent indicated a yellowish white precipitate.

Steroid test

This test was carried out by diluting the thick extract and pipetting the sample into a drip plate then adding Lieberman-Burchard reagent and observing the colour changes that occurred. Steroid positive is indicated by the formation of a blue or green colour.

FTIR Analysis

The analysis was carried out by making a sample preparation consisting of a thick extract and KBr then measuring at a wavelength of 4000-400 cm-1.

UV-Vis Spectrophotometer Analysis

The analysis was carried out by pipetting the extract as much as 1 ml into a test tube and then diluting it 100 times, then taking measurements at a wavelength of 300-800 nm.

RESULTS AND DISCUSSION

Tantrum Leaf Extract

Based on the results of research conducted, the yield of tantrum leaf extract obtained can be seen in Table 1.

The extraction process is carried out in three ways, namely alkaline extraction using water and lime, extracting wet and dry samples using ethanol as a solvent. Base extraction is a traditional extraction with the addition of lime to obtain a pH of 8.6. While the extraction with ethanol is a lower condition extraction with a pH of 7.2 and 6.8, respectively. Ethanol is a solvent that is commonly used as an extracting solution because of its ability to attract components of compounds in a sample, whether polar, semi-polar or non-polar. In addition, ethanol is also volatile so that it can be separated easily from the compound to be extracted. The yield obtained for dry tantrum leaf extract was 19.1 %, wet tantrum leaf extract was 47.8% and for base extract was 70.1%. The yield of wet tantrum leaf extract obtained was higher than that of dry tantrum leaf extract because the wet extract still contained a lot of water content compared to the dry extract. Meanwhile, the yield of traditional extracts is quite high, this is due to the addition of lime in the extraction process.

Phytochemical Analysis

Characterization of the content of compounds contained in indigo leaves extracted using ethanol on wet, dry and alkaline extraction tantrum leaves can be seen in Table 2.

Tantrum leaves extracted under alkaline conditions showed positive results when reacted with H_2SO_4 and 5% $FeCl_3$ as reagents in the flavonoid content test. In the alkaloid test, positive results were shown in Mayer and Wagner reagents, while Liebermen-Burchard reagents did not show positive results for steroid tests. This is because alkaline pH extraction is not able to attract these compounds. The compounds contained in the extract of wet tantrum leaves and dried tantrum leaves both showed positive results on $FeCl_3$ and NaOH reagents for flavonoid tests. In the wet sample alkaloid test showed positive results for all

Table 1. Yield of Tantrum Leaf Extract

Sample	Yield (%)	pH
Base Extraction	70.1	8.6
Wet sample extraction	47.8	7.2
Dry sample extraction	19.1	6.8

Table 2. Phytochemical Test Results

Sample	Flavonoid Test			Alkaloid Test		Steroid Test
	H_2SO_4	$FeCl_3$	NaOH	Wagner	Mayer	Liebermen-Burchard
Base Extraction	+	+	-	+	+	-
Wet sample extraction	-	+	+	+	+	+
Dry sample extraction	-	+	+	+	+	+

Table 3. FTIR Results of Tantrum Basa Leaf Extract

Wavenumber (cm^{-1})	Intensity	Functional groups
3453.20	Wide	N-H
1630.50	Currently	N-H
1484.61	Currently	C=C
1461.71	Currently	CH_2
1319.11	Currently	CH
1076.98	Currently	CO

Table 4. FTIR Results of Wet Tantrum Leaf Extract

Wavenumber (cm^{-1})	Intensity	Functional groups
3452.52	Wide	N-H
2927.55	Weak	CH sp^3
1638.30	currently	NH
1464.07	Weak	CH_2
1403.52	Weak	CH_3
1167.36	Weak	CH

Table 5. FTIR Results of Dried Tantrum Leaf Extract

Wavenumber (cm^{-1})	Intensity	Functional groups
3403.19	Wide	N-H
2928.97	currently	CH Sp^3
1622.83	Strong	NH
1516.90	currently	C=C
1456.34	Weak	CH_2
1405.17	Weak	CH_3
1350.73	currently	CH
1235.84	currently	C-O
1071.86	currently	C-O

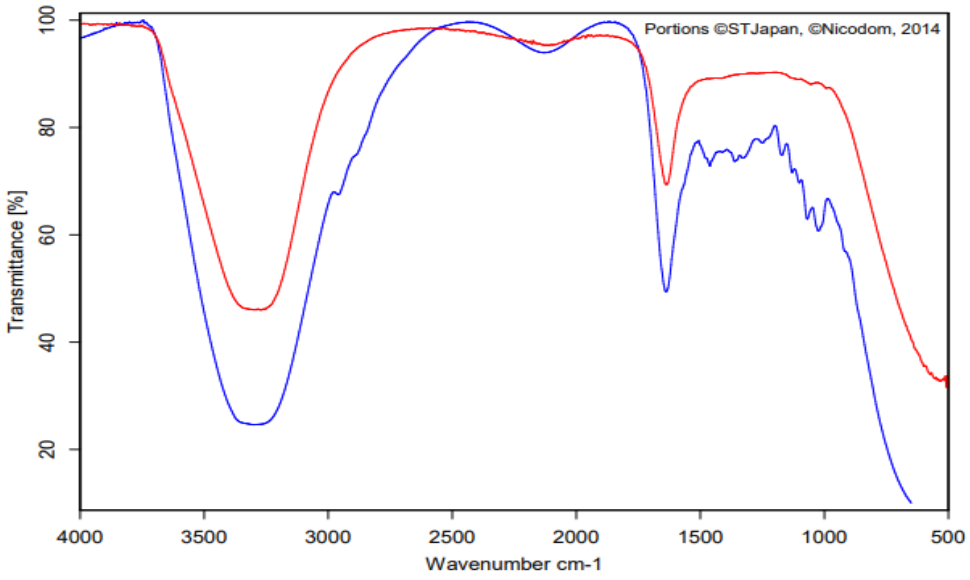

Figure 1. FTIR Measurement Results of Base Extract

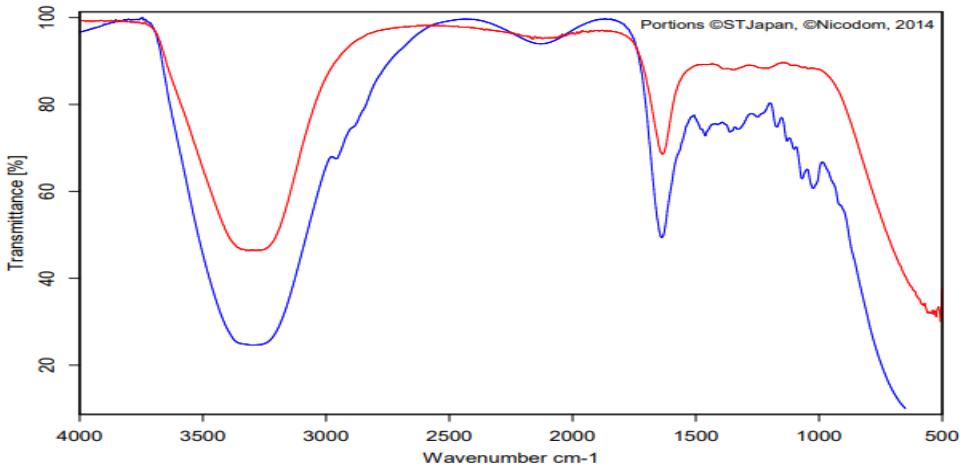

Figure 2. FTIR Measurement Results of Wet Tantrum Leaf Extract

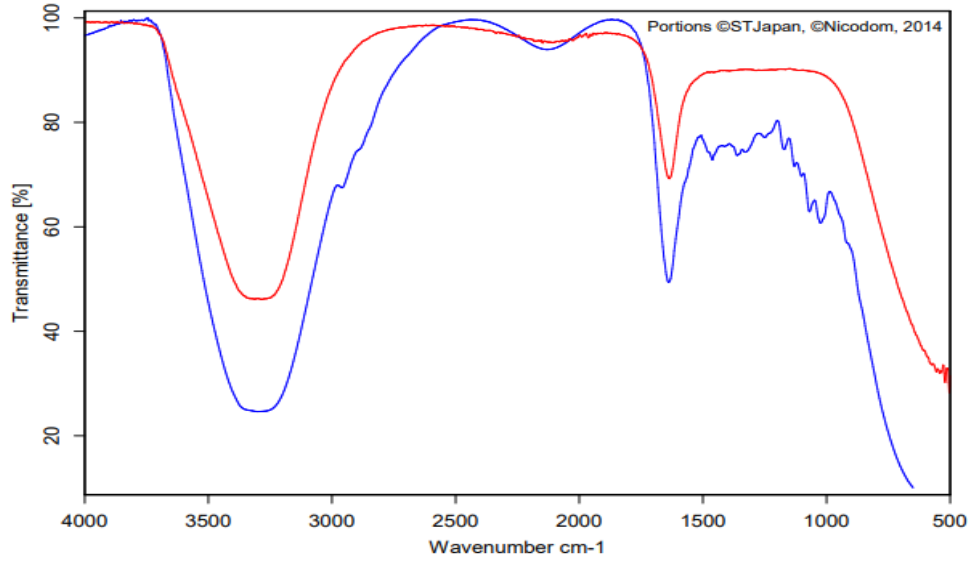

Figure 3. FTIR Measurement Results of Dried Tantrum Leaf Extract

types of reagents used, namely Mayer and Wagner reagents. However, the dried tantrum leaf extract only showed positive results on Mayer and Wagner reagents. In Liebermen-Burchard reagent both extracts showed positive results for steroids.

These results are not much different from previous studies (Hakiim & Sari, 2018) indigo leaves using ethanol using the Sokhlet method, the compounds obtained included flavonoids, alkaloids and steroids.

FTIR Analysis

The FTIR spectrophotometer aims to determine the functional groups of wet and dry tantrum leaf extract compounds using ethanol and alkaline extracts. The results of the analysis can be seen in Table 3.

Identification of functional groups using FTIR on tantrum leaf samples was measured at a wavelength of 4000-400 cm^{-1}. The measurement results for samples under alkaline conditions showed an absorption band at a wavelength of 3453.20 cm^{-1} which indicated the presence of an NH group, 1630.50 cm^{-1} the presence of an NH group supported by absorption at a wavelength of 1319.11cm^{-1} which indicated the presence of a CH group bound to an amine, at wavelengths of 1461.71 cm^{-1} and 1484.61 cm^{-1} indicated the presence of CH_2 bending and C=C aromatic groups. The measurement results can be seen in Figure 1.

For the wet sample, the absorption band is shown at a wavelength of 3452.52 cm^{-1} indicating an NH group, 2927.55 cm^{-1} indicating a CH sp3 group, at an absorption of 1638.30 cm^{-1}, indicating the presence of an NH group supported by an absorption at 1167.36 cm^{-1}, which indicates a cluster. The CH bound to the amine, 1464.07 cm^{-1} and 1403.52 cm^{-1} show CH_2 and CH_3 groups, respectively. The measurement results can be seen in Figure 2.

The dry sample extracted using ethanol solvent showed an absorption band at a wavelength of 3403.19 cm^{-1} indicating the NH functional group, 2928.97 cm^{-1} indicating the CH sp3 group, 1622.83 cm^{-1} indicating the NH functional group and at 1350.73 cm^{-1}, indicating the presence of a CH group bound to the amine, at 1516.98 cm^{-1}, indicates aromatic C=C, at absorptions of 1456.34 cm^{-1} and 1405.17 cm^{-1}, indicating CH_3 and CH_2 functional groups, respectively, and at absorption of 1071.86 cm^{-1}, indicating a functional group. CO function (Chanayath et al., 2002, Nurhasnawati et al., 2017). The measurement results can be seen in Figure 3.

UV-Vis Spectrophotometer Test

Determination of the group of compounds based on the wavelength produced was measured using a UV-Vis spectrophotometer. Analysis of compound content with UV-Vis spectrophotometer was measured at a wavelength of 300-800 nm. The results of the study on alkaline extracts showed absorption in the UV region with a wavelength of 664.50 with an absorbance of 2,141 measured at pH 8.6. The results of the analysis can be seen in Figure 4.

These results lead to a spectrum of plant pigments called *Leuco indigo* (Chanayath et al., 2002),(Leite et al., 2006) *Leuco indigo* is a blue

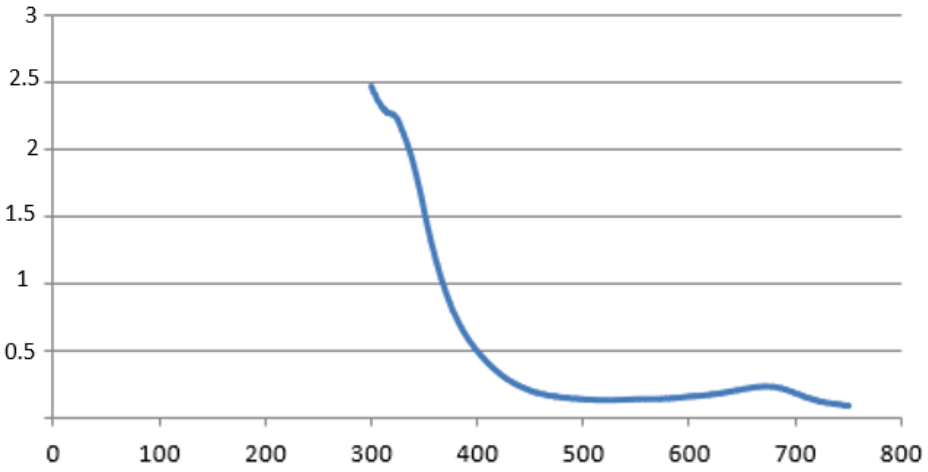

Figure 4. Results of UV-Vis Measurement of Base Extract

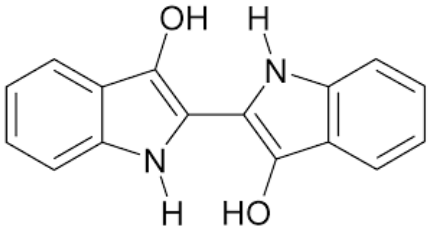

Figure 5. Structure of *Leuco indigo*

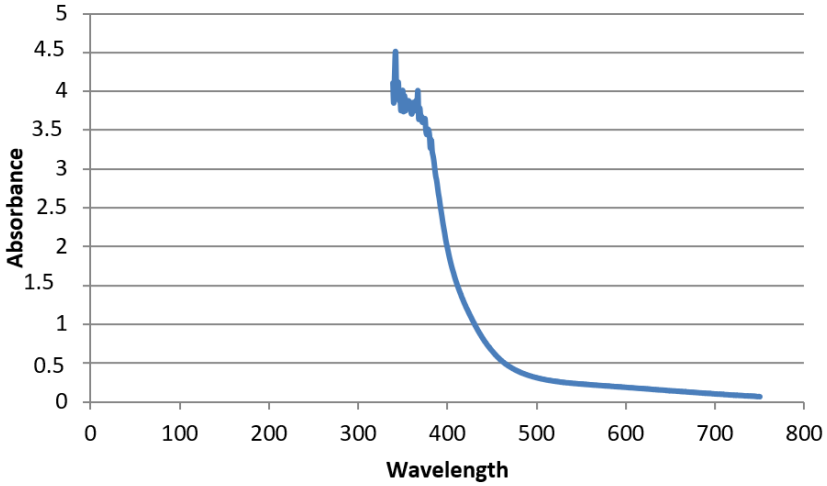

Figure 6. Results of UV-Vis Measurement of Dried Tantrum Leaf Extract

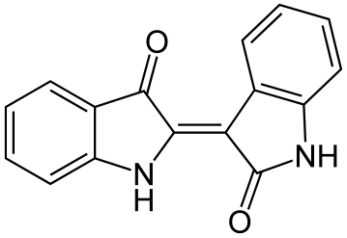

Figure 7. Structure of Indirubin

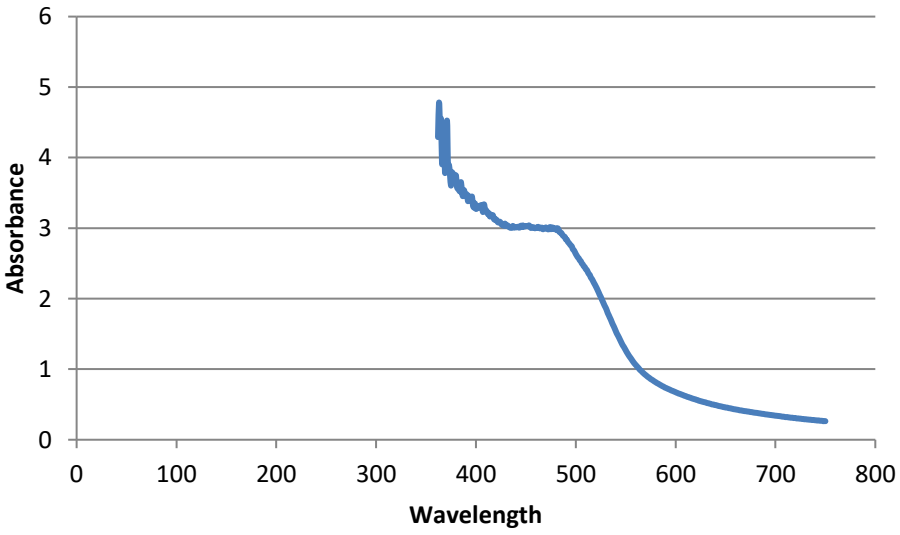

Figure 8. UV-Vis Measurement Results of Wet Tantrum Leaf Extract

dye that can be obtained from the soaking process of tantrum which undergoes fermentation and oxidation to form a blue color which can settle together with lime which is used to make the color darker. The structure of *Leuco indigo* can be seen in Figure 5.

The results of the analysis of dried tantrum leaves show the maximum wavelength absorption in the area of 365.50 with an absorbance of 6.041, measured at pH 6.8 the measurement results can be seen in Figure 6.

The results of measurements of dried tantrum leaf extract showed conformity with the maximum wavelength of indirubin dye. The difference in the solvent used causes the colour of the extract obtained to be reddish green. Indirubin is a group of red indigo compounds soluble in polar solvents such as methanol (Chanayath et al., 2002, Hassen et al., 2007, Oluwafemi R. A et al., 2020). The structure of indirubin can be seen in Figure 7.

The results of the analysis of wet tantrum leaf extract maximum absorption at a wavelength of 406.50 with an absorbance of 1,194 measured at pH 7.1 can be seen in Figure 8.

These results indicate a shift in wavelength when compared with dried tantrum leaves and tantrum leaves extracted under alkaline conditions. This shift is called the bathochromic shift (Sastrohamidjojo, 2013). Bathochromic shift occurs when a sample contains many compounds contained in it, a pile of compounds that have saturated bonds causes the transition energy to decrease so that the absorbed wavelength is greater. This is due to the large number of compounds contained in tantrum leaves that are still wet or fresh compared to dried tantrum leaves which allow denaturation or damage due to heating, besides the solvent used also affects the results. However, the results of this measurement are still consistent with the dye indirubin (Chanayath et al., 2002, Santos et al., 2015).

The three extracts measured showed absorption in the UV-Vis region. This absorption is due to the presence of chromophore and auxochrome groups. The chromophore group is a functional group that can absorb electromagnetic radiation in the UV and visible regions. The chromophore and auxochrome groups can experience electron delocalization when exposed to sunlight, which causes electron transitions→* and → * transitions. The auxochrome group has little effect on absorption in the UV region. However, it is very influential on the absorption of –OH, -OR, -NHR so that this group will cause a higher wavelength shift when it binds to the chromophore group (Wang et al., 2021,Vijayan et al., 2018b). These results were seen in the absorption region of the three extracts containing the –NH group. Overall absorption shifts towards higher wavelengths.

CONCLUSION

A dye compound that comes from plants in leaves, stems, fruits and roots can be obtained using the maceration extraction method. Through phytochemical tests, the compounds contained in the *Indigofera tinctoria* plant are indigo, flavonoids, steroids, and alkaloids. The FTIR spectrum indicated that the dye content in the extract was from the indigo group. The results of the UV-Vis spectrum showed that there was a maximum absorption of wet tantrum leaf extract, dried tantrum leaf extract and alkaline extract at wavelengths of 406.50 nm, 365.50 nm and 664.50 nm, respectively.

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