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Bioactive Compounds in Tombili Seeds and Tubile Roots as the Alternative for Synthetic Pesticide to Protect Wheats from Insects and Pests

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Abstract: This study was intended to isolate and characterize the pesticide property of secondary metabolite compounds in tombili (*Caesalpinia bonduc* (L.) Roxb) seed and tubile (*Derris elliptica* (Roxb) Benth) root. The pure compound isolated from 1.5 kgs tombili seeds and 500 grams tubile roots were macerated using methanol to get methanol extracts of 85.79 and 30 grams, respectively. Phytochemical testing showed positive results for flavonoid, terpenoid, alkaloids, tannin and saponin test. An insecticide bioactivity testing for both plants showed that the most effective result was achieved for ethyl acetate fraction in concentration of 0.05%. IR spectrophotometer for tombili seeds showed functional groups O-H, aliphatic stretching C-H, stretching C=O, aromatic stretching C=C, bending C-H, and stretching C-OH that marked the presence of terpenoid compounds. This result was supported by a UV-Vis spectrophotometer data with 236.00 nm wavelength absorption, possibly correlated with the transition of electrons that not bound to the anti-bonding orbital ($n \rightarrow \pi^*$) caused by the presence of the chromophore group C=O. Purity test for tubile roots only resulted in a single spot, therefore the phytochemical test was conducted and showed positive result for flavonoid. This result was supported by two bands resulted from UV-Vis spectrophotometer data with wavelength 280 nm and 235.50 nm for tape-1 and tape-2, respectively. The IR spectrophotometer also showed the functional groups O-H, stretching C-H, stretching C=O, aromatic stretching C=C, bending O-H, bending C-H, and alcoholic stretching C-O, indicating that the isolate was a flavonoid compound.
Keywords: Isolation, characterization, secondary-metabolites pesticide, *Caesalpinia bonduc*, *Derris elliptica.*

Introduction

Indonesia has numerous species of flora that can be used to produce a wide range of beneficial compounds. This biodiversity offers various chemical resources to support human needs like pharmacological substances, insecticides and cosmetics[1].

The bioactivity of plants exists in their secondary metabolites, and the secret inside these metabolites always rise interesting challenges for the scientists to isolate, characterize and synthesize them [2]. Studies to characterize beneficial properties on these metabolites are highly recommended in order to convert this magnitude of biodiversity into the continuous innovation profitable for human living.

In Indonesia, particularly in Gorontalo, there are many plants known to exhibit certain advantageous bioactivities. Two of them are tombili seeds (*Caesalpinia bonduc* (L.) Roxb) and tubile roots (*Derris elliptica* (Roxb) Benth). Unfortunately, the chemical properties of these plants are barely documented despite the popularity of its traditional usage. Mixed with another plants, tombili seeds and tubile roots are popularly used by farmers as botanical pesticide to protect wheat from insects and pests.

Tombili or *Caesalpinia bonduc* (L.) Roxb is a *Caesalpiniaceae* family, can be found worldwide, especially in the tropical area of India, Sri Lanka, Andaman and Nicobar. Indian people also used it to cure some diseases[3]. Yadav demonstrated that *C. bonduc* contains cassanediterpenes, a terpenoid compound, using NMR spectroscopy along with IR and UV-Vis spectroscopy[4]. Kinoshita also found similar compound, cassanefuranoditerpen, using similar method[5]. Mobasher et.al., also reported another terpenoid, 6β,7β-dibenzodyloxyvouacapen-5α-ol[6].

Tubile or tuba roots can be found almost everywhere in Nusantara. Widely used to kill fish (as poison), this plants is also effective as biopesticide at farms[7]. Gorontalo farmers use this plant to fight insects and pests[8].

This study was aimed to isolate and characterize the secondary metabolites in tombili seeds and tubile roots, and to test their effectiveness against pests in farm.

Methods

Tools and materials. Plant materials used are tombili seeds and tubile roots. Chemicals used include: methanol, n-hexane, ethyl acetate, MTC, Mayer reagent, Wagner reagent, Hager reagent, distilled water, concentrated HCl, Mg powder, NaOH, concentrated H_2SO_4, chloroform, acetone, silica gel and GF_{254} TLC plate. Tools used are: blender, analytical balance, UV lamp, microwave, tools to visualize H_2SO_4 spots, filter papers, glass set, rotary vacuum evaporator, thin-layer chromatography and column chromatography equipments, cutter, test tubes, and test tube rack.

Extraction. Fresh samples of tombili seeds and tubile roots were dried by aeration in the open air, direct sunlight avoided. Dry samples were macerated using methanol for 3 × 24 hours. The solvent was replaced every 24 hours until the filtrate is colorless. The filtrate then evaporated, resulting in a thick methanol extract. This condensed extract was mixed with distilled water with ratio of methanol in water was 1: 2, then partitioned with n-hexane and ethyl acetate, successively, to obtain each partition of both fractions. Each partition was evaporated at 30 – 40 °C to obtain the extract from n-hexane, ethyl acetate and water.

Flavonoid test. About 0.1 gram of condensed extract was dissolved in 10 ml of methanol and distributed into four test tubes. The first tube was used as the control tube, and solution in three other tubes were mixed with NaOH, concentrated H_2SO_4, and Mg powder plus concentrated HCl, respectively. This test yield positive result (and flavonoid compound detected) if there were a marked differences between the color of solution in the three last tubes compared with the color of solution in the first tube[9].

Alkaloid test. About 0.1 gram of condensed extract was dissolved with 10 ml of ammoniac chloroform and distributed into two test tubes. The first tube was tested with Hager reagent, while the second was mixed with 0.5 ml of H_2SO_4 2 N. The acid layer was separated then distributed into three test tubes. Each tube was tested with Mayer, Dragendorff, and Wagner reagent, respectively. The test yield positive result (or the solution is an alkaloid solution) if sedimentation was detected.

Steroid,terpenoid, and saponintest. About 0.1 gram of condensed extract dissolved with 10 ml of diethyl ether. The dissolved part was dripped on a drop plate, with additional 2 drops of anhydride acetic acid and 1 drop of H_2SO_4. The remaining undissolved part was mixed with a small volume of hot distilled water, moved to a test tube, mixed with hot distilled water again, and then shaken vigorously for 15 minutes. The filtrate under the foam was taken and placed on a vaporizer cup, mixed with HCl and evaporated until it dried as a crust. A drop of diethyl ether was dripped into the crust, plus 2 drops of anhydride acetic acid and 1 drop of strong H_2SO_4. The presence of steroid substance in the crust would change its color into bluish green; any terpenoid would change its color into brownish red, while saponin would produce foams above the crust.

Tanin test. A condensed extract as much as 0.1 gram was dissolved with alcohol in a test tube. $FeCl_3$ 1% then was dropped into the tube. This test yield positive result,in the presence of tanin, if the color changed into dark blue or dark green.

Insecticide bioactivity test. Eight groups of wheat plants chosen randomly were treated with different dose of tombili and tubile extracts to observe their survival against insects and/or pests. The type of treatments and dosage are listed in Table 1. This treatment and observation was done periodically during the whole season.

Table 1. Type of extract and dosage used in insecticide bioactivity test.

Group	Type of extract	Concentration(%)
	Tombili	
1	Methanol	0.25
2	n-Hexane	0.05
3	Ethyl acetate	0.05
4	H_2O	0.25
	Tubile	
5	Methanol	0.25
6	n- Hexane	0.05
7	Ethyl acetate	0.05
8	H_2O	0.25

Purification and Separation

The purification and separation procedures were done using thin-layer and columnar chromatography. TLC was first used to choose the best extract to be used for separation. With a mix of n-hexane and ethyl acetate in ratio 8:2 as the mobile phase, and the silica gel as the static phase, all fractions were tested for their purity and the best would underwent the separation process. Subsequent purity tests then were conducted by means of TLC.

The separation was run using columnar chromatography. First, a cotton filter was used to suspend the silica from dropping down with the solvent. The preparation of column was done with dry method using n-hexane as solvent and hot silica gel previously prepared with microwave for about 20 minutes. The best extract previously selected from TLC mixed with silica gel and then dropped into the column in open state. The gravitation forced the formation of certain number of fractions to be analyzed individually using TLC with a mixed of n-hexane, MTC and acetone as eluent in ratio 6:2:2. Nodes were grouped and symbolized as fractions. The purity of fractions then was analyzed by examining node patterns and calculating their Rf values.

Result and Discussion

Phytochemical tests for tombili seeds.

Phytochemical test was a qualitative chemical test for a plant to determine the presence of certain secondary metabolite inside its extract. This study involved some routine phytochemical tests such as flavonoid test, alkaloid test, steroid, terpenoid, and saponin test, and also a tanin test.

This study demonstrated that all extracts (in methanol, ethyl acetate, n-hexane and distilled water) contain flavonoid substance marked with certain color changes when mixed with flavonoid reagents.

The alkaloid test for methanol, ethyl acetate and distilled water extract yield positive results, marked with a sedimentation formed when the extracts were mixed with alkaloid reagents, while such sedimentation was not seen in n-hexane fraction.

Steroid and terpenoid tests for tombili seeds done using Lieberman-Bouchard reagent resulted in brownish red coloration indicating the presence of terpenoid and the absence of steroid substance. The saponin test yielded negative results for n-hexane and ethyl acetate fractions, but positive for methanol and distilled

water extracts, marked with foam formation caused by the glycoside ability to form foam in water . The addition of hot distilled water into saponin test was ended in hydrolysis reaction.

The tanin test yielded positive results for all extracts, marked with dark blue or dark green coloration because of the polifenolic property in tanin (Harborne, ; in Darmawijaya and Yudha)[10].

Phytochemical tests for tubile roots.

Phytochemical test for tubile root extract demonstrated that all fractions (methanol, n-hexane, ethyl acetate and distilled water) contain flavonoid substance.

Insecticide bioactivity test.

As seen in Table 1, the observation for insecticide bioactivity was conducted by dividing a wheat farm into eight areas or groups, while each group received different extract fraction and dosage of tombili seeds or tubile roots.There are two type of concentration used: 0.05 and 0.25%. Both were created by dissolving 0.5 and 2.5 grams (respectively) of extract in 1,000 ml distilled water.

By a serial observation, this study demonstrated that the most effective treatment was achieved using the ethyl acetate fraction at concentration 0.05%. This was proven by the total volume of rice produced from that area, 24 sacks, compared with only 10 sacks produced from the control area. Other 7 areas only produced 18 – 20 sacks of rice, but still superior compared with the production from control area.

Separation and purification of tombili seeds.

TLC was used to choose which of four extracts to be used for separation with columnar chromatography. This selection demonstrated that the methanol extract showed the best node pattern and separation, therefore it would be used for the separation phase.

About 10.6 grams of methanol extract mixed with silica gel and poured into the column in open state. Elution with ethyl acetate forced the separation of extract from the eluent and formed a gradient of concentration from 100% ethyl acetate to 100% methanol extract. This then was analyzed with TLC technique again using eluent of n-hexane : MTC : acetone in order to separate the nodes. Similar nodes grouped as fractions and this process produced 14 fractions with 58 node groups(Figure 1).The most crystallized fraction suitable for next purification was A_1.

The A_1 fraction then was separated again with columnar chromatography using the same eluent and produced 62 node groups. Tested with TLC, this resulted in five fractions ($A1_1$through $A1_5$) as shown in Figure 2. The fraction $A1_2$ was found to be the best subject for the next purification because it was the most crystallized fraction and has a single node pattern.

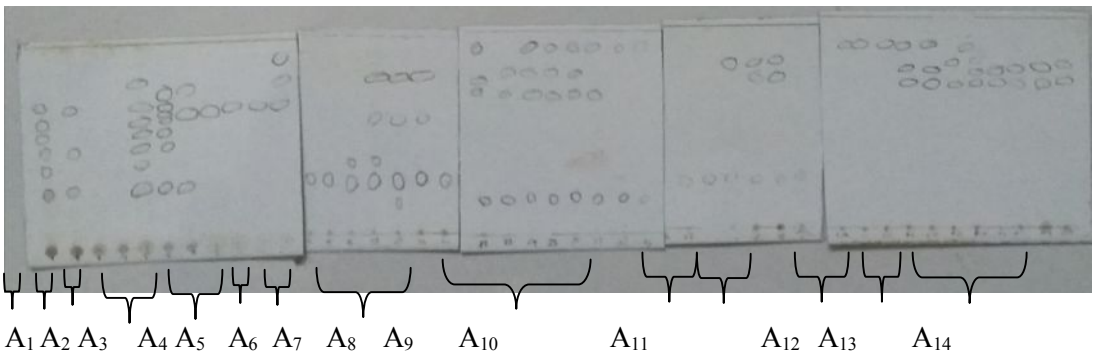

Figure 1. The result of columnar chromatography and TLC using eluent n-hexane : MTC : acetone (6:2:2).

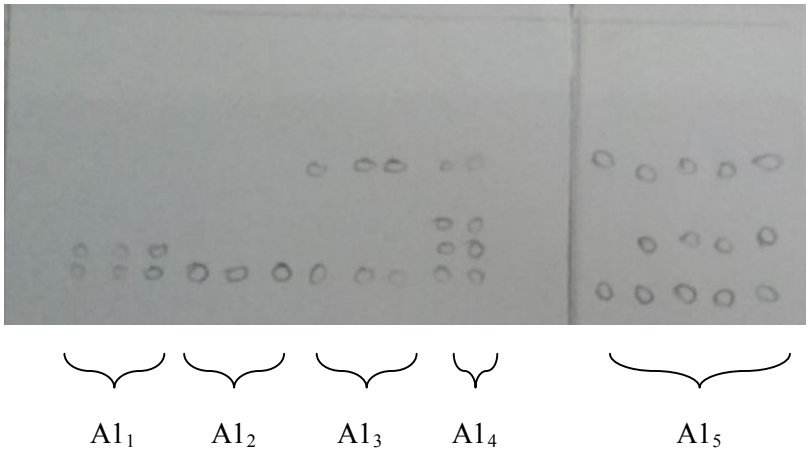

Figure 2. The result of columnar chromatography and TLC for fraction A_1 using n-hexane :ethyl acetate (9:1).

Table 2. The result of TLC and Rf values for fraction $A1_2$ using three different eluents.

Node pattern			
Eluent	n-hexane : ethyl acetate (9:1)	n-hexane : MTC : acetone (7:1:2)	chloroform : methanol (9:1)
Rf value	0.325	0.727	0.909

Tested with TLC using three different eluents, the purification of the $A1_2$ fraction resulted in node patterns and Rf values as shown in Table 2.

A two-dimension TLC was used to confirm the purity of the last fraction. This was done by standing the plate in two directions with the second was 90° from the first direction. Each direction used different eluent. First eluent (E1) was n-hexane : ethyl acetate in ratio 9:1, and the second eluent (E2) was n-hexane : MTC : acetone in ratio 7:1:2. The result was shown in Figure 3.

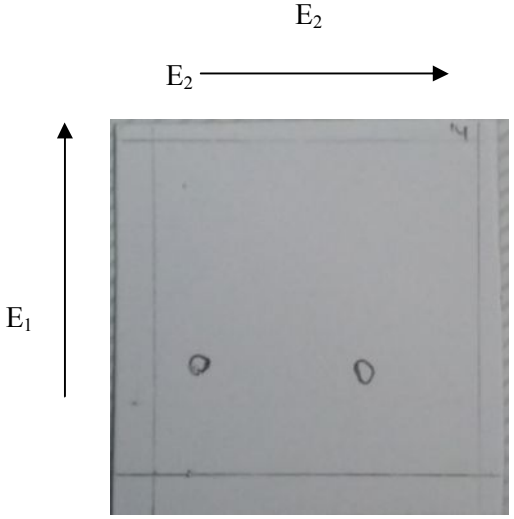

Figure 3. The result of two-dimensional TLC using two different eluents.

Separation and purification of tubile roots.

Columnar chromatography (20 × 2.5 cm) was used for separation of 1 gram of methanol extract. The mobile phase was ten gradients of ethyl acetate : methanol (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10), with the last gradient was methanol 100%. This resulted in 34 fractions to be analyzed using TLC.

The best node from each fraction was tested using TLC GF_{254} with ethyl acetate : methanol (7:3) as the mobile phase. By calculating the Rf values, this test was successfully differentiated three groups with similar Rf values as shown in Figure 4 and Table 3.

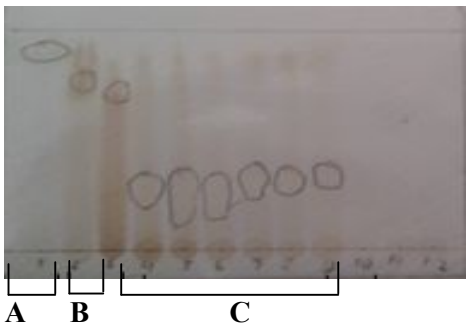

Figure 4. The result of TLC using eluent ethyl acetate : methanol (7:3).

Table 3. The grouping of isolates based on Rf values.

Group	Distance traveled by solvent	Distance traveled by stain	Rf value
A	4.0 cm	3.6 cm	0.90
B	4.0 cm	3.0 cm	0.75
C	4.0 cm	1.0 cm	0.25

The best isolate, the fraction B, tested again with TLC using the same eluent. Based on the result as shown in Figure 5, despite there was only a single node created by this elution, some stain still present in the baseline, suggesting that this isolates was not pure yet. Therefore, a subsequent columnar chromatography still needed to purify the isolate.

Figure 5. The result of TLC for fraction B using eluent ethyl acetate : methanol (7:3) showing a single node and some stain in the base line.

Using silica gel as the static phase and gradients of ethyl acetate :metanol as the mobile phase, the second columnar chromatography produced 18 fractions of isolates. The most crystallized isolate was fraction 1, therefore it was used in the subsequent TLC using ethyl acetate : methanol (7:1) as eluent. The result was shown in Figure 6. The calculation of Rf values separated these isolates into two fractions, B_1 and B_2, as shown in Table 4.

A B

Figure 6.TLC profile for fraction 1 using eluent ethyl acetate : methanol (7:3).

Table4. The grouping of isolates for fraction B by their Rf values.

Group	Distance traveled by solvent	Distance traveled by stain	Rf value
B_1	4.4 cm	3.7 cm	0.84
B_2	4.4 cm	2.3 cm	0.52

Based on this TLC profile, the first isolate in group B_1 was the most crystallized isolate, therefore it was chosen for the subsequent TLC. Using two eluents, ethyl acetate : methanol (8.5:1.5) and chloroform : methanol (9:1), the last TLC produced two profiles as shown in Figure 7a and 7b.

Both profiles were single-node profiles, suggesting that they were taken from a pure isolate. To confirm this, a 2D TLC was conducted using ethyl acetate : methanol (8.5:1.5) as E1 and chloroform : methanol (9:1) as E2. The resulting profile showed a single node pattern (Figure 8), suggesting that the purity of this isolate was confirmed.

a b

Figure 7.TLC profile for the first isolate in group B_1 using two eluents, E1 and E2.

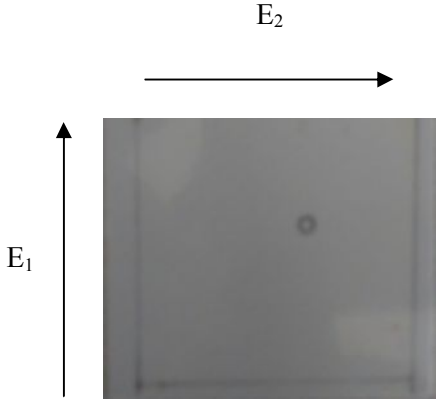

Figure 8. The 2D TLC profile using two different eluents.

Phytochemical tests for the pure isolate of tombili seeds.

As shown in Table 5, all phytochemical tests for the pure isolate of tombili seeds yielded negative results, except the test using Lieberman-Bouchard reagent that resulted in brownish red coloration. This result suggested that the tombili seed contains terpenoid substance.

Phytochemical tests for the pure isolate of tubile roots.

As shown in Table 6, phytochemical tests for the pure isolate of tubile roots ended in negative results, except in all reactions using reagents to detect flavonoid. This result suggested that the tubile root contains flavonoid substance.

Table 5. Phytochemical tests result for the pure isolate of tombili seed.

Second methabolite	Reagent	Result	Description
Flavonoid	NaOH H_2SO_4 Mg-HCl	(-) (-) (-)	No color change No color change No color change
Steroid	Lieberman-Bouchard	(-)	No bluish green coloration
Terpenoid	Lieberman-Bouchard	(+)	Brownish red coloration
Alkaloid	Mayer Wagner Dragendorff Hager	(-) (-) (-) (-)	No precipitation No precipitation No precipitation No precipitation

Table 6. Phytochemical tests result for the pure isolate of tubile root.

Second methabolite	Reagent	Result	Description
Flavonoid	NaOH H_2SO_4 Mg-HCl	(+) (+) (+)	Bright orange to red coloration Dark red to orange coloration Orange to chocolate coloration
Steroid	Lieberman-Bouchard	(-)	No bluish green coloration
Terpenoid	Lieberman-Bouchard	(-)	No brownish red coloration
Alkaloid	Mayer Wagner Dragendorff Hager	(-) (-) (-) (-)	No precipitation No precipitation No precipitation No precipitation

Infrared and UV-Vis spectrophotometer identification for tombili seed.

Based on analysis to the spectrum produced by infrared spectrophotometer for the pure isolate of tombili seed, several absorptions were noted in certain wavelength intervals (Figure 9 and Table 7).

The first was absorption found at 3,479.58 cm^{-1} probably due to the presence of the stretching O-H. Similar results also reported by Mobasher, *et.al* ., (3,400 cm^{-1})[6], Yadav *et al*, (3,420 cm^{-1})[4], Kinoshita, (3,584 cm^{-1})[5] and Silverstein, (3,550 – 3,200 cm^{-1})[11]. The second was probably the stretching C-H absorbed at 2,926 cm^{-1}. Creswell et al also reported similar finding at 3,000 – 2,700 cm^{-1} wavelength[12]. The next absorption at 1,743 cm^{-1} was similar with other findings reported by Mobasher,*et al*., (1,730 cm^{-1})[6], Yadav *et al*, (1,725 cm^{-1})[4], Kinoshita, (1,720 cm^{-1})[5] and Silverstein, (1,870 – 1,540 cm^{-1})[11]. This absorption probably due to a strong C=O functional group. Other absorptions found at 1,649.14 and 1,510.26 cm^{-1} were similar with those reported by Silverstein et al, (1,667 – 1,500 cm^{-1})[11] and Creswell *et al*, (1,675 – 1,500 cm^{-1})[12]. This was probably a stretching aromatic C=C. The bended C-H with narrow band was absorbed at 1,460.11 and 1,438.9 cm^{-1}; similar with a report from Silverstein, (1,475 – 1,300 cm^{-1})[11]. A group of absorption at 1,031.92 and 997.2 cm^{-1}

indicated that this was probably a stretching C-OH similar with 1,100 – 990 cm^{-1} wavelengths reported by Creswell *et al*,[12].

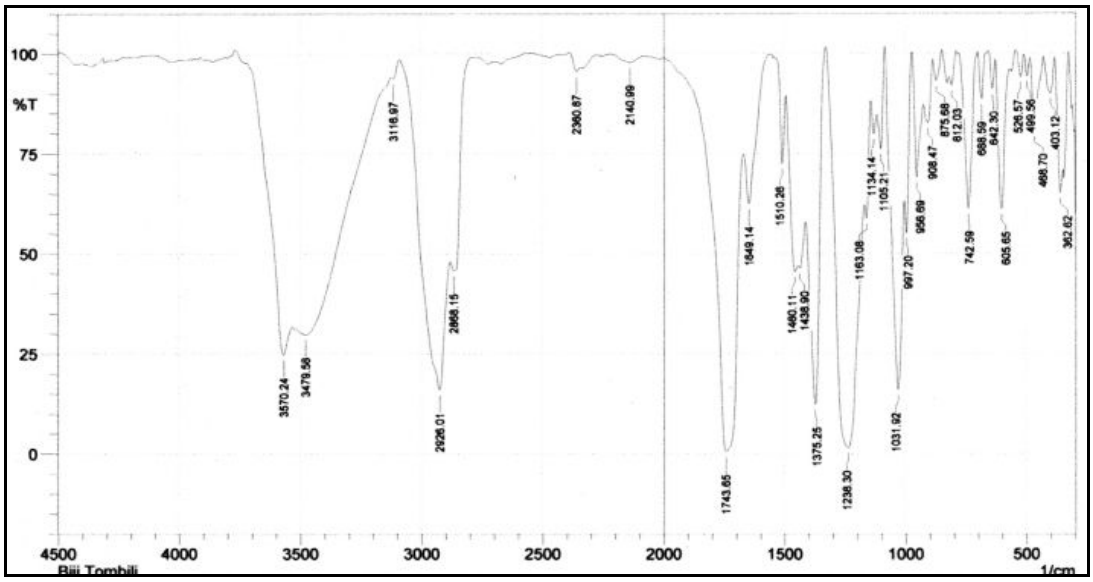

Figure 9. Infrared spectrophotometer spectrum for the pure isolate of tombili seeds.

Table 7. Infrared spectrophotometer data for the pure isolate of tombili seeds compared with reports from other studies.

Wavelength (cm^{-1})						Band	Intensity	Suggested group
Current Isolate	**Mobasher 2014**	**Yadav et al 2009**	**Kinoshita 2000**	**Silverstein 1984**	**Creswell 2005**			
3,570 – 3,479	3,400	3,420	3,584	3,550 – 3,200		Wide	Strong	Stretched O-H
2,926 – 2,868					3,000 – 2,700	Narrow	Strong	Stretched C-H
1,743	1,730	1,725	1,720	1,870 – 1,540		Narrow	Strong	Stretched C=O
1,649 – 1,510				1,667 – 1,640	1,675 – 1,500	Narrow	Weak	Stretched C=C
1,460 – 1,438				1,475 – 1,300		Narrow	Intermed	Bended C-H
1,031 – 997					1,100 – 990	Narrow	Strong	Bended C-OH

The pure isolate of tombili seed was also identified using UV-Vis spectrophotometer and produced a spectrum as shown in Figure 10. This spectrum clearly illustrates an absorption band at a low wavelength, 236 nm, probably due to the transition of electrons that are not bound to anti-binding orbital ($n \rightarrow \pi^*$) in the presence of chromophore functional group C=O. Astuti *et al*., suggested that any absorption with low wavelength like this is related to the terpenoid compounds characterized by chromophore with unconjugated double bindings[13]. The spectrum from IR spectrophotometer confirmed this suggestion with the C=O functional groups found at 1,743.65 cm^{-1} wavelength.

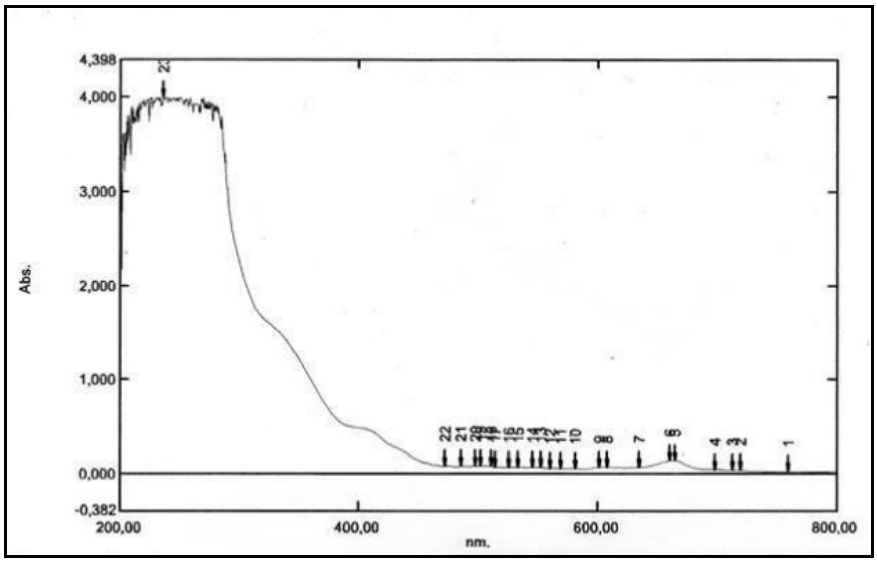

Figure 10. UV-Vis spectrum for the pure isolate of tombili seed in methanol extract.

UV-Vis and infrared spectrophotometer identification for tubile root.

The spectrum from UV-Vis spectrophotometer showed two bands at wavelength 280.00 and 235.50 nm as shown in Table 8.

Table 8. Spectrum data from UV-Vis spectrophotometer for pure isolate of tubile root.

Band	Wavelength (nm)	Absorption (cm^{-1})
I	280.00	3,623
II	235.50	4,000

This spectrum was specific for flavonoid, especially catechin, as Tempesta and Michael suggested that catechin has two bands with the first band has maximum wavelength of 275 – 280 nm and the second has maximum wavelength of 225 – 235 nm[14].Neldawati and Gusnedi also found two peaks of UV-Vis spectrum that were identified as the absorption of an aromatic functional groups from flavonoid[15]. Markham also reported that flavonoid was a poliphenolic acid characterized by two bands on UV-Vis spectrophotometer[16]. The strong absorption in UV area was due to functional group C=C conjugated at flavonoid aromatic system. This resulted in energy transition capable of absorbing the 200–400 nm wavelength in the spectrum of UV-Vis spectrophotometer[12].

The IR spectrum, as illustrated in Table 9, showed an absorption at wavelength 3,387 cm^{-1} due to the presence of a stretched O-H with wide band characteristic. Compared with existing literatures, this type of absorption was similar with those reported by Daniel, (3,387 cm^{-1})[17], Sudjadi, (3,350 cm^{-1})[18] and Silverstein, (3,550 – 3,200 cm^{-1})[11]. Stretching C-H produced two bands with wavelength 2,926.01 and 2,856.58 cm^{-1}. This finding was similar with reports from Shukla et al, (2,920 cm^{-1})[19], Daniel, (2,924.09 and 2,854.65 cm^{-1})[17] and Sudjadi, (2,926 and 2,853 cm^{-1})[18]. The next absorption was found at 1,869.02 cm^{-1} due to a strong functional group C=O, similar to another finding of 1,870 – 1,540 cm^{-1} wavelength documented by Silvestein,[11]. An absorption at wavelength area of 1,612.49 cm^{-1} suggested the presence of aromatic stretched C=C as supported by data from Shukla[19] , Than *et al*.,[20], Daniel[17], Silverstein[11] and Sudjadi[18] noting absorptions at 1,660 cm^{-1}, 1,649 cm^{-1}, 1,604.77 cm^{-1}, 1,667 – 1,640 cm^{-1} and 1,610 – 1,650 cm^{-1}, respectively. A bended O-H with strong intensity produced an absorption at 1,448.54 cm^{-1}, similar with reports from Than [20], Daniel[17] , Silverstein[11] and Sudjadi[18] with absorption at 1,465 cm^{-1}, 1,458.18 cm^{-1}, 1,420 cm^{-1} and 1,465 cm^{-1}, respectively. An absorption at 1,382.96 was related to bended C-H with narrow band. This was similar to what have been reported by Shukla *et al*., (1,360 cm^{-1})[19], Than, (1,361 cm^{-1})[20], Daniel, (1,373.32 cm^{-1})[17], Silverstein, (1,330 cm^{-1})[11] and Sudjadi, (1,370 cm^{-1})[18]. The last absorption was found at 1,282.66 cm^{-1} indicating the presence of alcoholic

stretching C-O as supported by data from Shukla[19], Than[20], Daniel[17] and Silverstein[11] with absorption at wavelength 1,295 cm^{-1}, 1,262 cm^{-1}, 1,265.30 cm^{-1} and 1,260 – 1,000 cm^{-1}, respectively.

Table 9. Infrared spectrum data from the pure isolate of tubile root.

Wavelength (cm^{-1})						Band	Intensity	Suggested group
Current isolate	Shukla et al 2012	Than et al 2005	Daniel 2010	Silverstein et al 1984	Sudjadi 1983			
3,387.00	3,330	3,414	3,387.00	3,550 – 3,200	3,350	Wide	Weak	Stretched O-H
2,926.01	2,920	2,928	2,924.09	-	2,926	Narrow	Strong	Stretched C-H
2,856.58	-	2,853	2,854.65	2,830 – 2,695	2,853	Wide	Strong	Stretched C-H
1,869.02	-	1,717	1,728.22	1,870 – 1,540	-	Wide	Strong	Stretched C=O
1,612.49	1,660	1,649	1,604.77	1,667 – 1,640	1,610 – 1,650	Narrow	Weak	Aromatic stretched C=C
1,448.54	-	1,465	1,458.18	1,420	1,465	Narrow	Weak	Bended O-H
1,382.96	1,360	1,361	1,373.32	1,330	1,370	Narrow	Weak	Bended C-H
1,282.66	1,295	1,262	1,265.30	1,260 – 1,000	-	Narrow	Strong	Alcoholic stretched C-O

Analyzing this spectrum, it was therefore concluded that the pure isolate of tubile root contains a flavonoid compound, marked with the presence stretching O-H, stretching C-H, stretching C=O, aromatic stretching C=C, bended O-H, bended C-H and alcoholic stretching C-O.

Conclusion

1. The extract of tombili seed contains flavanoid, alkaloid, terpenoid, saponin and tanin, while the extract of tubile root contains flavonoid.
2. The phytochemical tests for pure isolate of tombili seed detected the presence of terpenoid, while for the pure isolate of tubile root revealed a flavonoid compound.
3. Insecticide bioactivity test demonstrated that the most effective formula of tombili seed and tubile root extract was the ethyl acetate fraction at concentration 0.05%.
4. The IR and UV-Vis spectrum for the pure isolate of tombili root suggested the presence of terpenoid compound, while for the pure isolate of tubile root suggested a flavonoid compound.

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