In Vitro Evaluation Of Antioxidant Activity Of Flavonoid Compounds From Terong Belanda (*Solanum Betaceum, cav*.)

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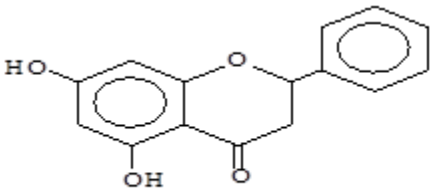
Abstract
Terong belanda (Solanum betaceum, cav), is a fruit that has nutrients and vitamins which are essential for health of human body and it is believed to have a potent antioxidant activity. This research aimed to evaluate *in vitro* antioxidant activity of flavonoid compounds isolated from Terong belanda. *In vitro* antioxidant activity was evaluated using DPPH method. Identification of flavonoids was done using Infrared and UV–Vis Spectrophotometers. Extraction of ten (10) kg terong belanda fruits with ethanol gave of 126.17 g peels extract, 178.44 g flesh, and 253.11 g seed extracts respectively. Phytochemical test showed both peels, flesh, and seed extracts of Terong belanda contained flavonoids. Antioxidant activity test showed peels, flesh, and seeds extracts exhibited antioxidant activity with IC_{50} of 68,14 ppm, 621.45 ppm, and 1162.608 ppm respectively. Separation and purification of these three active extracts using column chromatography gave each one isolate which positive flavonoid on phytochemical test. Infrared spectra of each isolate from peels, flesh, and seed were similar and each revelaed to contain the same functional groups (OH alcohol, CH aromatic, C=O, C=C, and CH aliphatic). Analysis of UV-Vis spectra and its spectra with shifting reagents of the peels isolates gave absorption at the wavelength range of 310-330 nm (Bans I) and 245-275 nm (bands II) due to isoflavones group with a hydroxy group at C6, C7 or C7, C8 and C3', C 4 ' while flesh and seed gave absorption at 300-330 nm (bands I) and 275-295 nm (bands II) that indicated the class of flavanones with hydroxyl groups at C-2', C-5', C-6' and *O*glycoside at C7 for flessh and hydroxyl group at C6, C7 or C7, C8 for the seed.

Keywords: **Antioxidant, DPPH, Isolation, Terong Belanda**

I. INTRODUCTION

chemical compounds produced by plants has been more exploited and investigated as a material for both drugs and pharmaceuticals for the benefit of agriculture (insecticides, fungicides, etc.), as well as the diversity of chemical structures generated will also reduce the side effects residue left behind. For example, morphine is a semisynthetic drug that was originally isolated from the poppy plant (1,2). Searching new compound having antitumor or anticancer activity was intensively conducted by NCI (National Cancer Institute) and have been isolated several compounds such as camptothecin, catharanthine, taxol, and so on. The results of a recent study found pinocembrin (flavonoid) isolated from Eriodictyon californicum can inhibit the growth of cancer cells (1,2).

The structure of the isolated pinocembrin are:



Pinocembrin

Terong belanda is believed to be a source of natural antioxidants, because it contains vitamin E, vitamin A, vitamin C, vitamin B6, carotenoids, anthocyanins, and fiber (3). Anthocyanins are flavonoid glycosides such as red dye found in many flowers, fruits, and leaves. These compounds have been shown to be an antioxidant then protect liver cells (4,5). Therefore it is worthwhile to investigate the antioxidant active and characteristic of flavonoid compound in Terong belanda extract.

II. METHODS AND PROCEDURES

Materials

The materials used in this study were fresh ripe and redish of fruit Terong belanda (Solanum betaceum, Syn.) brought from the District village Batur Kintamani Bangli, Bali. The chemicals used in this study were ethanol, n-hexane, chloroform, ethyl acetate, magnesium metal, hydrochloric acid, sodium hydroxide, DPPH (difenilpikril hidrazil), n-butanol, chloroform, acetic acid, aluminum chloride, sodium acetate, boric acid, ammonia, distilled water, silica gel GF 254, and silica gel 60.

Instrumentation

The equipment used in this study is the blender, balance, a knife, a set of glassware, vacuum rotary evaporator, thin-layer chromatography and column chromatography, UV light, Ultraviolet-visible spectrophotometer (UV-Vis) and infrared

Procedure

Ten kilograms Terong belanda are separated between the peels, flesh and seeds, subsequent repeatedly macerated using 70% ethanol solvent until all components extracted. Ethanol extract (Peels, flesh and seeds) was concentrated using a rotary vacuum evaporator to obtain extracts condensed ethanol. Ethanol extract and then partitioned with n-hexane solvent, chloroform and n-butanol. All three extracts were further tested phytochemicals. The positive extract of flavonoids tested antioxidant activity with DPPH method. Active and positive flavonoid extracts were then separated and purified by column chromatography technique using silica gel as the stationary phase. The next active isolates were identified using infrared spectrophotometer and UV-vis.

III. RESULTS AND DISCUSSION

Isolation of antioxidant compounds from Terong belanda

Terong belanda fruit used in this study is fresh, ripe and red fruit. Ten kilograms of fruit Terong belanda was extracted with ethanol 70% and each extract obtained were shown in Table 1.

Table 1. Crude ethanol extract of peels, flesh and seeds

Samples	**Sample weight (grams)**	**Weight of concentrated extract (grams)**
peels	1.045,89	126,17
flesh	3.434,95	178,44
seeds	2.480,42	253,11

Phytochemical test results indicated that all three extracts containing flavonoids,as seen from a given color change after adding the reagent flavonoids.

Antioxidant activity testing of the crude ethanol extract of peels, flesh, and seeds Terong belanda

Measurement of antioxidant activity by DPPH method. DPPH method is a quantitative way to determine how much activity the ethanol extract of Terong belanda fruit as an antioxidant. Measurements were taken at different concentrations of extracts for the presence of antioxidant compounds that can reduce the intensity of the purple color of DPPH. Decrease in DPPH absorbance value indicates increased activity of antioxidant isolates. Data antioxidant activity of ethanol extracts presented in Table 2.

Table 2. IC_{50} values of crude ethanol extract of the peels, flesh, seeds Terong belanda, and gallic acid

Samples	linear regression	**IC50 (ppm)**
Crude ethanol extrac of peels	y = 0,0213x + 1,537	200,84
Crude ethanol extrac of flesh	y = 0,009x + 0,8581	1365,55
Crude ethanol extrac of seeds	y = 0,0118x – 0,2746	4214,017
Standards of gallic acid	y = 6,6985x + 0,1791	7,438

Partition of crude ethanol extract of Terong Belanda

Terong belanda ethanol extract (peels, flesh and seeds) partitioned with n-hexane solvent, chloroform and Butanol. Phytochemical test for all three fractions, indicating that the n-butanol fraction containing flavonoids. n-butanol fractions further separated and purified and tested the antioxidant activity. Data antioxidant activity of n-butanol fraction of Terong belanda fruit shown in Table 3.

Tabel 3. IC_{50} values of fraction n- butanol terong belanda and gallic acid

Samples	Linear regression	IC50 (ppm)
n- butanol fraction terong belanda peels	y = 0,0118x + 0,2746	68,14
n- butanol fraction terong belanda flesh	y = 0,0798x +0,4078	621,45
n- butanol fraction terong belanda seeds	y = 0,0429x + 0,1241	1162,608
Standards gallic acid	y = 6,6985x + 0,1791	7,438

Based on the antioxidant activity test results of n- butanol fraction as shown in Table 3, the IC50 values of peels, flesh, and seeds were 68.14 ppm, 621.45 ppm, and 1162.608 ppm respectively. The antioxidant activity of n-butanol fraction classified as active and strong for the peels because it has IC50 values ranging from 50-100 / mL (6), weak for flesh because its IC50 value more than 500 ppm and a very weak for seeds. When compared with gallic acid, antioxidant activity of the ethanol extract and n-butanol fraction weaker because ethanol extract and n-butanol fraction has less antioxidant activity than the positive control gallic acid.

Separation and purification of n-butanol fraction Terong belanda

Separation of n-butanol fraction using column chromatography to obtain six fractions for peels (F1-F6), the seven factions for flesh (F1-F7) three fractions for seeds (F1-F3). Phytochemical and purity test of the fractions showed that each fraction obtained one isolate pure and positive flavonoid. Active isolates was subsequently identified by infrared and UV-Vis spectrophotometer

Identification of n-butanol fraction of active isolates Terong belanda

Identification by infrared spectrophotometry

Infrared analysis performed to determine the functional groups characteristic of an isolate. The results of the data analysis of infrared spectra of the three active isolates n-butanol fraction, presented in Table 4.

Table 4. Wave numbers and Assignment of active isolates n-butanol fraction

Wave number (cm^{-1})				Absorption intensity	assignment
Isolate			Ref*		
Peels	flesh	seeds			
3321,42	3518,16	3217,27	3550-3200	Broad	OH
3126,61	3122,75	3031,40	3150-3050	Sharp	C-H aromatic
2958,80 2933,73 2873,94	2960,73 2935,66 2873,94	2846,93	2950-2800	Sharp	C-H alifatic
1710,86	1726,29	1725,61	1850-1730	Sharp	C=O
1648,30	1608,63 1516,05 1463,97	1456,40	1650-1400	Sharp	C=C aromatic
1465,90 1379,10	1398,30	1396,46	1475-1300	Sharp	C-H alifatic

* source (7,8)

Based on the data in Table 4 isolates suspected to contain functional groups-OH, aromatic CH, aromatic C = C and C = O group which is a characteristic of a flavonoid compound.

Identification by UV-Vis spectrophotometry

The results of the analysis using UV-Vis spectrophotometry to provide information about the presence of chromophore groups and auxochromes of an isolate. Based on the analysis using UV-Vis spectrophotometry, peels isolates n-butanol fraction gave two (2) absorption band typical for the flavonoid compounds with absorption in the wavelength range of 310-330 for the wavelength band I and band II to 245-275. The range of UV-Vis absorption spectra in the wavelength range of absorption showed isoflavone class of flavonoid (9). While the flesh and seeds provide an absorption at 300-330 nm wavelength range bands I, at wavelength range 275-295 nm for bands II. Wavelength range was assumed the absorption of the flavonoid class of flavanones or dihidroflavonol.

Oxygenation pattern of the flavonoid compounds can be determined by the addition of a shifting reagent. The position of hydroxy group (OH) in the framework of isoflavones, flavanones and dihidroksiflavonol obtained from the addition of reagents shift such as NaOH, $AlCl_3$, AlCl3 and HCl mixture, NaOAc, NaOAc and H_3BO_3 and mix. The shift is indicated by the absorption band II affect oxygenation pattern in ring A flavonoid compound, while the shift in the band I affect oxygenation in ring B. Wavelength shift in the peels isolate after the addition of reagent shift presented in Table 5.

Table 5. Data wavelength and wavelength shift in the peels isolate after the addition of reagent shift

Reagent shift	wavelength		Wavelength shift	
	λ_{maks} (nm)		λ_{maks} (nm)	
	Bands I	Bands II	Bands I	Bands II
Ethanol	327,20	245,40		
Ethanol + NaOH	387,20	255,40	+ 60	+ 10
Ethanol + NaOH (5 minutes)	387,20	255,40	+ 60	+ 10
Ethanol + NaOAc	387,20	253,20	+60	+7,8
Ethanol + NaOAc + H_3BO_3	334,40	276,20	+7,2	+ 30,8
Ethanol + $AlCl_3$	325,80	243,00	-1,4	-2,4
Ethanol + $AlCl_3$ + HCl	326,60	245,20	-0,6	-0,2

Based on data in Table 5 shows that after the addition of NaOH shifting reagent bathochromic shift occurs in bands I and bands II indicate the presence of OH groups on ring A and B. This assumption is reinforced by the bathochromic shift in band II after addition of NaOAc which showed a 7-OH group on ring A. this happens because NaOAc can only ionize isoflavones in particular the 7-OH group. Bathochromic shift that occurs in bands I and bands II after addition of NaOH also showed the presence of 3 ', 4'-dihydroxy isoflavone (10). The addition of shifting reagent NaOAc - H_3BO_3 also showed a bathochromic shift in band I and band II, which indicates there are in the ortho-OH group on the A ring exactly at position C-6, C-7 or C-7, C-8 (10). Ortho-dihydroxy group on the B ring can not be detected with NaOAc - H_3BO_3 because of lacking effective conjugation with the main chromophore (10). The addition of $AlCl_3$ and $AlCl_3$ - HCl shifting reagent, the hipsocromic shift occured which indicates no OH group at C-5, C-3 in ring A. The addition of $AlCl_3$ and $AlCl_3$- HCl also can not detect any group 3 ', 4'-dihydroxy isoflavones because of ring B has little or no conjugation with the main chromophore (10).

Based on the analysis of UV-Vis spectrum, shift after addition of shifting reagent shows that butanol fraction isolates contains flavonoids of isoflavon class which have the OH substituent on the basic framework at the atoms C-6, C-7 or C-7 , C-8 (ring A) and at the atoms C-3 ', C-4' (ring B).

Table 6. Data wavelength and wavelength shift in the flesh isolate after the addition of reagent shift

Reagent shift	wavelength		Wavelength shift	
	λ_{maks} (nm)		λ_{maks} (nm)	
	bands I	bands II	bands I	bands II
Ethanol	322	285	-	-
Ethanol + NaOH	373,4	-	+51,4	-
Ethanol + NaOH (5 minutes)	373,4	-	+51,4	-
Ethanol + NaOAc	321,2	285	-0,8	-
Ethanol + NaOAc + H_3BO_3	334,2	284,8	+12,2	-0,2
Ethanol + $AlCl_3$	321,2	281,2	-0,8	-3,8
Ethanol + $AlCl_3$ + HCl	320	283,8	-2	-1,2

Based on data from Table 6, the bathochromic shift in band I after addition of NaOH shifting reagent shear of 51.4 which indicates the possibility of the presence of the hydroxy group at the position of atom C-2 ', C-5' or C-6 '(10). These results are supported by the bathochromic shift in band I of 12.2 nm after the addition of reagent shift NaOAc -H_3BO_3 indicating the presence of ortho-dihydroxy groups on the B ring at atom C-5 ', C-6'.

In the bands II bathochromic shift does not occur after the addition of NaOH shifting reagent which means there is no OH group at C-7 atoms and ortho-OH on atom C-5, C-6 or C-7, C-8. This is also supported by a shift hipsocromic in band II after addition of NaOAc and H_3BO_3 shifting reagent.

Shifting hipsocromic shown on the bands I and bands II after addition of AlCl3 and $AlCl_3$ - HCl shifting reagent showed no presence of the hydroxy group at C-3 and C-5 is capable of forming a complex with the keto group with the help of $AlCl_3$ and absence dihydroxy group on the C atom - 4 ', C-5'. Flavonoid compounds in the isolate suspected is not a class of compounds dihidroflavonol but flavanones because of the absence of the hydroxy group at atom C-3 and C-7. (9,10). Hipsocromic shift that occurred in bands II at the time of addition of shifting reagents NaOAc - H_3BO_3 and $AlCl_3$ - HCl showed a group of O-glycosides are not acid resistant to the atoms C-7.

The data in Table 6. shows that the addition of NaOAc shifting reagent cause bathochromic shift in band II, which indicates the presence of hydroxyl group at C-7 atom. This is confirmed also by the addition of H_3BO_3 which showed bathochromic shift in band I and band II, which indicate the presence of ortho-dihydroxy groups on atom C-6 and C-7 or C-7 and C-8. There was a slight shift after addition of $AlCl_3$ so supposedly no hydroxyl group at atom C-3 and C-5.

IV. CONCLUSION

1. The active antioxidant compound in n-butanol fraction of terong belanda peels with IC_{50} 68.14 ppm was tentatively identified as isoflavone with hydroxyl group substituent on the C-6, C-7 or C-7, C-8 and C - 3' dan C-4 '
2. The active antioxidant compound in n-butanol fraction of terong belanda terong flesh with IC_{50} 621.45 ppm was tentatively identified as flavanones with hydroxyl group substituent on atom C-2 ', C-5', C-6 'and O glycosides at C-7
3. The active antioxidant compound in n-butanol fraction of terong belanda seeds with IC_{50} 1162.608 ppm was tentatively identified as flavanones hydroxyl group substituent at C-7, C-8 or C-6, C-7.

ACKNOWLEDGMENTS

The author would like to thank Directorate General of Higher Education and center of research and comunity service Udayana University for funding this research through *Hibah Bersaing* scheme 2013.

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