HAYATI
Journal of Biosciences

Vol. 31 No. 6, November 2024 1144-1153
DOI:10.4308/hjb.31.6.1144-1153
ISSN: 1978-3019
EISSN: 2086-4094

Chemicals Identification in The Ethyl Acetate Fraction and The Antioxidant Activity from Calabash Seed (*Cresentia cujete*) Extract

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ARTICLE INFO

Article history:
Received March 6, 2024
Received in revised form June 10, 2024
Accepted July 3, 2024

KEYWORDS:
Antioxidants,
Crescentia cujete,
DPPH,
Extraction,
LC-MS

ABSTRACT

Calabash (*Crescentia cujete*) is a plant that grows in tropical climates and is scattered and easily found in Indonesia. Calabash is also known to contain several secondary metabolite compounds that have the potential as an antioxidant. This research is purposed to identify phytochemical compounds in calabash fruit seed extract, identify compounds based on the result of LC-MS, and know the antioxidant activity of calabash fruit seeds. The samples were extracted using the maceration method, and then phytochemical screening, LC-MS tests, and antioxidant tests were continued using the DPPH method. This research showed that the ethanol extract of calabash seed positive contains saponin, flavonoids, and triterpenoid compounds. Ethyl acetate extract of calabash seed contains flavonoids, alkaloids, and triterpenoids. The *n*-hexane extracts positively contain tannins, alkaloids, and steroids. The values of antioxidant activity of ethanol, ethyl acetate, and *n*-hexane extracts were IC_{50} = 29.7883, 7.219, and 848.712 ppm (µg/ml), respectively. Ethyl acetate extract as the best antioxidant activity was then tested with LC-MS, and results obtained from 26 compounds with the composition of the largest % area is convolvidine (11.277%), coumaroylquinic acid (8.290%) and taxchinin G (8,233%). They showed that calabash fruit seed extract has very strong antioxidant activity, with the best activity being the ethyl acetate extract of calabash fruit seed.

1. Introduction

Calabash is a plant that grows in tropical climates, so calabash fruit is spread and easily found in Indonesia (Atmodjo 2019). In Indonesia, the calabash fruit is known as the Maja fruit. There are two types of maja, namely maja bael (*Aegle marmelos*) and maja berenuk (*Crescentia cujete*). Maja bael and maja berenuk have quite clear differences in their morphological systems. Maja berenuk belongs to the genus Bignoniaceae, the Lamiales, while the maja bael belongs to the family Rutaceae, the Rutales (Atmodjo 2019). Maja berenuk or calabash (*Crescentia cujete*) is widely found in Maluku, so it is easy to find and be used for further research (Figure 1).

Calabash cannot be consumed and is considered poisonous, with sticky flesh and an unpleasant aroma that gives the impression of a bitter taste (Atmodjo 2019). In Maluku, the local community uses calabash as a fence plant, and the fruit's skin is used to make handicraft items and kitchen utensils like diapers, spice storage, etc. Calabash is still limited to the fruit's skin, while the seeds and flesh are no longer used, so it's thrown as waste. Calabash can be used for more efficient utilization because almost all parts of calabash (*Cresentia cujete*) have important biological activities such as anticontralytic, antimicrophilariae, antiinfalmatic, analgesic, antiulcer, anticonvulsant, antidepressant, antifertility, antifungal, antibacterial, and insecticidal activity (Parvin *et al.* 2015).

Calabash also contains several secondary metabolite compounds. Secondary metabolite compounds are active compounds found in plants that keep them from disease, and each plant has specific, non-essential secondary metabolites. According to Bhar *et al.* (2019), calabash contains several secondary metabolite compounds such as

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A B

Figure 1. (A) calabash fruit and (B) calabash fruit seed

alkaloids, terpenoids, tannins, steroids, coumarins, vitamins, and fatty acids. The content of secondary metabolites, especially flavonoids and phenolics, can have potential as antioxidants because they have hydroxyl groups that can capture free radicals (Putri *et al.* 2021).

Antioxidants are compounds that can inhibit or neutralize free radicals. Free radicals are compound molecules with one or more unpaired electrons, highly reactive and radical. Natural antioxidants from plant parts such as roots, stems, leaves, fruits, flowers, seeds, and pollen contain vitamins A, C, E and phenolic compounds that can inhibit free radicals. The determination of antioxidant activity value can be tested using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). DPPH is a free radical compound that is quite stable and can absorb visible light at a wavelength of 517 nm (Almey *et al.* 2014).

Das *et al.* (2014) have researched testing the antioxidant activity of ethyl acetate extract of calabash's leaves and bark. The IC_{50} values obtained sequentially are 11.25 µg/ml and 8.78 µg/ml. Testing of the antioxidant activity of calabash has also been researched (Helmi *et al.* 2021). The study's results have obtained the value of calabash's antioxidant activity of IC_{50} of 158.46 µg/ml. Testing the antioxidant activity of calabash's methanol leaves extract has also been researched by (Balogun & Sabiu 2023), which obtained the inhibition activity value to DPPH free radical with IC_{50} of 34.01 µg/ml. (Syaefudin *et al.* 2018) researched determining the antioxidant activity of calabash's bark ethyl acetate fraction and obtained free radical inhibition activity with an IC_{50} value of 174.56 µg/ml. The research that has been done on calabash so far has only been on the bark, leaves, and fruits of calabash. Research on calabash fruit seeds is still not carried out. Therefore, it is necessary to research calabash fruit seeds to identify the compound content of calabash fruit seed extract using liquid chromatography with tandem mass spectrophotometry (LC-MS/MS) and also determine the antioxidant activity with using the DPPH method to see the inhibition value.

2. Materials and Methods

2.1. Materials and Instruments

The seeds of the Calabash Fruit (*Cresentia cujete*) are taken in Waiheru, Ambon, Maluku, Indonesia. For the extraction process, solvents 96% Ethanol (E.mecrk), Ethyl Acetate (E.Merck), and *n*-hexane (E.Merck) are used. Chemicals used for phytochemical screening are glacial acetic acid (E.Merck), hydrochloric acid (E.Merck), sulfuric acid (E.Merck), Wagner reagents

(Technical), chloroform (E.Merck), Magnesium (E.Merck), sodium chloride (E.Merck), $FeCl_3$ (E.Merck), and Aquades. The chemicals used for antioxidant activity tests are Methanol (E.Merck) and DPPH (E.Merck).

Analytical balances (Adventurer Pro analytical, Ohaous), Dimples, Glassware set (Pyrex), Oven (Shel lab), Desiccator (Pyrex), UV-Vis Spectrophotometer (Apple PD-303 UV), and Liquid Chromatography-Mass Spectrophotometry/LC-MS (Thermo scientific).

2.2. Sample Preparation and Moisture Content Determination

The sample preparation process is carried out using samples of calabash fruit seeds that have been separated from the fruit flesh, washed, and dried until they are not sticky. Then, the sample is mashed. After that, the crushed calabash fruit seeds are put as much as 2 g into a porcelain dish and dried in the oven at 105°C for 3 hours, then removed and put in a desiccator for 30 minutes, done 3 repetitions. The moisture content was calculated using the following equation:

$$\% \text{ water content} = \frac{W}{Ws} \times 100$$

Where,
Ws : wet sample weight (g)
W : difference in sample weight (Ws - Wi) (g)

2.3. Calabash Seed Extraction

The finely refined calabash fruit seeds were taken 50 g, macerated with 500 ml of ethanol, ethyl acetate, and *n*-hexane, respectively, soaked in a dark bottle and stirred using a magnetic stirrer for 5 day (Harborne 1998). The maserat obtained was collected and concentrated by cold drying in a room with a temperature of 16°C. Then, the dry extract was obtained to determine its weight. The yield was calculated by the following equation:

$$\text{Yield } (\%) = \frac{\text{extract weight}}{\text{sample weight}} \times 100$$

2.4. Phytochemical Screening

2.4.1. Saponin Test

2 ml of calabash seed extract was inserted into a test tube, and 2 ml of distilled water and shaken. If there is a foam that is stable and persistent for 10 minutes, the extract contains saponins (Dasgupta and Mehta 2023).

2.4.2. Tannin Test

0.5 g of the dried extract was boiled in 20 ml of distilled water in a test tube and then filtered. A few drops of 0.1 % $FeCl_3$ were added and observed for brownish green-black (Evans 2009).

2.4.3. Flavonoid Test

Calabash seed extract as much as 2 ml, added 0.1 mg of Mg powder, then added HCl until color changes. The formation of orange-yellow indicates the presence of Flavanoid content in the extract (Evans 2009).

2.4.4. Alkaloid Test

2 ml of calabash seed extract was added to HCl 2 M, heated stirred chilli sauce, after which it cooled to room temperature. Then NaCl powder is added, stirred, and reacted with 1-3 drops of Wargner reagent. The formation of a brownish-red precipitate indicates that the test sample contains alkaloid compounds (Jones and Kinghorn 2006).

2.4.5. Steroid and Triterpenoid Test

2 ml of calabash seed extract, 1 ml chloroform, 3 ml of anhydrous acetic acid, heated for 5 minutes, cooled solution, and then added 1-3 drops of concentrated sulfuric acid. A red or purple color change indicates the presence of steroids, and a green or blue color indicates the presence of triterpenoids in the test sample (Kinam *et al.* 2021).

2.5. Antioxidant Activity Test

The antioxidant activity of calabash fruit seed extract was determined using the DPPH method. First, the viscous extract of calabash fruit seeds was dissolved in methanol with concentration variations of 10, 20, 30, 40, and 50 ppm (µg/ml). The extract solution that has been made is taken as much as 1 ml and added with 2 ml DPPH 40 ppm, then incubated in a dark room for 30 minutes at room temperature. After incubation, absorbance measurements were measured at a wavelength of 517 nm using a UV-Vis spectrophotometer with a blank of 2 ml DPPH 40 ppm (µg/ml) dissolved with 1 ml methanol, and 3 measurements were made (Utomo 2021). The antioxidant activity of the seed extract of calabash fruit is expressed by the percentage of inhibition, which is calculated by the formula:

$$\% \text{ inhibiion} = \frac{(\text{A blank - A sample})}{\text{A blank}} \times 100$$

After getting the percentage of inhibition, IC_{50} is calculated to determine the sample concentration that can reduce free radicals by as much as 50%. The results of calabash fruit seed extract, which has the best antioxidant activity among ethanol, ethyl acetate, and *n*-hexane extracts, will then be analyzed for the composition of the compound using LC-MS.

3. Results

3.1. Water Content and % Ethanol Extract Bath, Ethyl Acetate, *N*-Hexane Calabash Fruit Seeds (*Cresentia cujete*)

Determination of water content using gravimetric method obtained the water content of calabash fruit seeds is 9.44%, while for % soaking of ethanol, ethyl acetate, and *n*-hexane extracts can be seen in Table 1.

3.2. Phytochemical Screening of Ethanol, Ethyl Acetate, and *n*-hexane Extracts of Calabash Fruit Seeds (*Cresentia cujete*)

Calabash fruit seed extract is tested phytochemically qualitatively by adding reagents to each extract to be tested and paying attention to changes in the color and shape of the sample. The results of phytochemical screening showed that the positive calabash fruit seed extract contained saponins, flavonoids, and triterpenoid compounds in ethanol extract. Positive ethyl acetate extract contains flavonoid, alkaloid, and triterpenoid compounds, while positive *n*-hexane extract contains tannins, alkaloids, and steroids. Screening can be seen in Table 2.

Table 1. Result of calabash fruit seed extract (*Cresentia cujete*) by various solvents

Types of solvents	Sample (g)	Extract (g)	Yield (%)
Ethanol	50.9110	15.2391	29.93
Ethyl acetate	50.0293	12.2375	24.46
n-Hexane	50.1590	12.7422	25.40

3.3. Antioxidant Activity Test of Ethanol, Ethyl Acetate, and *n*-hexane Extracts of Calabash Fruit Seeds (*Cresentia cujete*)

The antioxidant activity of calabash fruit seed extract was tested using the DPPH method. The DPPH test is an effective method that can be used to determine free radical activity. The measurement results using a UV-Vis spectrophotometer at a wavelength of 517 nm obtained the absorbance value of each concentration. The absorbance value obtained is used to calculate % inhibition, which can then be calculated as IC_{50}. The calculation results obtained data as in Table 3 and Figure 2.

3.4. Determination of Chemical Compound Content with LC-MS from Ethyl Acetate Extract of Calabash Fruit Seeds (*Cresentia cujete*)

The determination of the compound composition in calabash fruit seed extract was analyzed using Liquid Chromatography-Mass Spectrometry. Identification of compounds from ethyl acetate extract of calabash fruit seeds using LC-MS in chromatograms obtained data on retention time (tR), percentage of area (%area), molecular weight (m/z), and type of compound. On chromatogram data, there are 26 compounds contained in the ethyl acetate extract of calabash fruit seeds, consisting of secondary metabolite groups of flavonoids, alkaloids, steroids, glycosides, and beta carotene. Possible compounds contained in ethyl acetate extract of calabash fruit seeds (*Cresentia cujete*) identified based on the results of LC-MS analysis can be seen in Table 4. Identification of compounds from LC-MS analysis is carried out using direct libraries from instruments, or based on literature studies, and based on mass spectra or molecular mass values of compounds that have the highest similarity from

Table 2. Phytochemical screening results of calabash fruit seeds (*Cresentia cujete*)

Parameters	Results			Observation of positive results
	Ethanol extract	Ethyl acetate extract	*n*-hexane extract	
Saponins	+	-	-	Foam
Tannins	-	-	+	Green color
Flavanoids	+	+	-	Green/blue-black color
Alkaloids	-	+	+	Brick-red color
Triterpenoids	-	-	+	Purple ring
Steroids	+	+	-	Blue color

Table 3. Absorbance, %Inhibition, and IC_{50} value of calabash fruit seed extract

Extract	Concentration	Absorbance value			% inhibition	IC_{50}
		P1	P2	P3		
Ethanol	10	0.484	0.472	0.467	45.759	29.788
	20	0.449	0.457	0.467	47.531	
	30	0.449	0.430	0.441	49.484	
	40	0.424	0.422	0.403	52.238	
	50	0.410	0.380	0.380	55.223	
	Blanks	0.871				
Ethyl acetate	10	0.464	0.514	0.317	51.573	7.219
	20	0.483	0.485	0.316	51.910	
	30	0.394	0.383	0.345	57.977	
	40	0.394	0.329	0.345	59.213	
	50	0.310	0.345	0.348	62.471	
	Blanks	0.890				
N-Hexane	10	0.752	0.751	0.756	1.181	848.712
	20	0.740	0.745	0.745	2.493	
	30	0.739	0.742	0.742	2.756	
	40	0.736	0.737	0.739	3.281	
	50	0.737	0.729	0.736	3.675	
	Blanks	0.762				

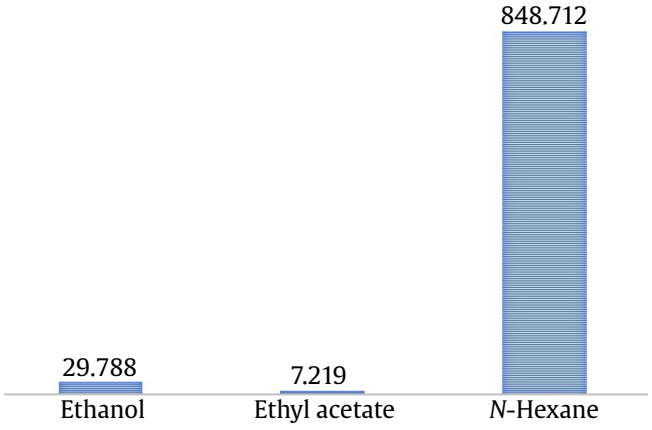

Figure 2. Free radical inhibition diagram DPPH of calabash fruit seed extract

the database of natural compound products in NCBI PubChem and Massbank.

4. Discussion

The water content and % yield of calabash fruit seed extract have been tested using gravimetry and maceration methods. Based on the % yield data in Table 1, it can be seen that the largest marinade extract is ethanol extract at 29.93%. The water content contained in the calabash fruit seeds (*Cresentia cujete*) is only a little bit. This is very helpful in the extraction process and also against sample damage because the greater the water content, the greater the possibility of the sample being damaged, and the more it affects the extraction results. The difference in the % yield in Table 1 is due to the different types of polarity of the three types of solvents used, so the amount of chemical compounds that can be extracted from each solvent is also different; this affects the difference in % yield.

Calabash fruit seed extract is also tested phytochemically qualitatively by adding reagents to each extract to be tested and paying attention to changes in the color and shape of the sample. The Saponin test is used to know the presence or absence of saponins in the extract, the test results are positive when producing foam. Based on Table 2, it is known that the ethanol extract of calabash fruit seeds contains saponin compounds expressed by the formation of foam on the extract after being given test treatment. The formation of foam in the test treatment results is caused by the presence of glycoside content in the extract, which can produce foam in hydrolysed water (Karlina *et al.* 2023).

For tannin, tests are carried out using a reagent in the form of $FeCl_3$, which can form green or yellow complexes. Based on the data in Table 2, the three fractions of calabash fruit seed extract, it is known that n-hexane extract hangs tannin compounds, indicated by the change in the color of the extract from colorless to greenish-yellow. The color change occurs due to the complex bond between Fe and tannin compounds or polyphenols that have hydroxyl groups, where Fe will bond to nucleophile O atoms (Parbuntari *et al.* 2018).

Table 4. Compound Identification by LC-MS Analysis

Rt (Min)	% Area	Observed	M/Z	Proposed compound	Ref
0.373	5.872	303.365	303.3615	Evodiamine	Tsugawa *et al.* 2019
3.671	1.669	490.2249	491.4342	Glimepiride	MSBNK-Athens_Univ-AU235501
4.278	1.784	604.869	604.7256	Ginsenosides Rh_3	Tsugawa *et al.* 2019
4.712	5.584	717.56724	717.7721	Phosphatidylcholine alkenyl	Taguchi *et al.* 2010
5.007	3.396		830.8221	1,2-di-O-arsidonolicin-glycerol-3-phosphocholine	PubChem
5.250	8.834	927.135	928.0008	Salcosaponin C	Tsugawa *et al.* 2019
6.222	0.921	288.255	288.3044	Erydiktiol	Tsugawa *et al.* 2019
6.361	1.036	537.37944	537.3624	phosphatidylcholine lyso 19:0	Taguchi *et al.* 2010
7.177	3.622	302.282	302.2994	3',5,7-Trihydroxy-4'-methoxy flavanone	Sawada *et al.* 2008
8.704	4.145	319.3606	319.6059	Konvolin	MSBNK- NGA01972
8.878	1.784	362.4703	362.5297	Poststeron	MSBNK- NGA02089
9.520	1.381	425.433	425.3248	p-Hydroxyironlglucocyanates	Tsugawa *et al.* 2019
10.839	1.266	304.2587	304.5486	Taksivolin	MSBNK-RIKEN_NPDepo-NGA03059
13.616	1.525	301.383	301.3622	Trachelatin	Tsugawaet *et al.* 2019
14.623	0.863	556.652	556.6713	Brevifoliol	Madhusudanan *et al.* 2002
15.907	8.290	338.31	337.4751	Commaroylquinic acid	PubChem
16.984	3.094	594.566	594.7272	2',6'-Dihydroxy-4-metoxicalkon-4'-O-neohesperit	Tsugawa *et al.* 2019
17.140	3.483	512.7364	512.7423	acetyl oxandrolone acid	MSBNK-RIKEN_NPDepo-NGA01302
17.331	3.396	379.389	379.3997	S-Pyrufoylglutathione	MSBNKRIKEN_ReSpect-PT206830
18.372	4.174	395. 631	395.3952	Solasodin	MSBNK- RIKEN-PR310695
18.580	7.980	380.48884	380.4619	Konferon	MSBNK-RIKEN_NPDepo-NGA01099
19.084	4.637	680.1588	680.7874	Genistein 4',7-O-diglucoside malonic acid	MSBNK-IPB_Halle-PN000033
19.292	8.233	552.617	552.7345	Taksikinin G	MSBNK-RIKEN_ReSpect-PM000811
20.143	11.227	608.7384	608.8499	Convolidin	MSBNK-RIKEN_NPDepo-NGA01558
21.115	5.458	441.4825	441.5082	Floridiamine	MSBNK-RIKEN_NPDepo-NGA02239
22.121	4.829	277.18305	277.1835	Amitriptyline	MSBNK-Fiocruz-FIO00475

Then, the flavonoid test is carried out with the addition of HCl and magnesium powder (Mg). The addition of concentrated HCl is used to hydrolyze flavonoids into their aglycone, namely by hydrolyzing O-glycosyl. H+ will replace Glycosyl from acid because of its electrophilic nature. This reduction with concentrated Mg and HCl produces complex compounds that are flavonol, flavanone, and flavanonol (Harborne 1998). Flavonoid tests generally show positive results in the presence of brick red or yellow discoloration. Based on the results of phytochemical screening in Table 2, it is known that ethanol extract and ethyl acetate extract of calabash fruit seeds contain flavonoid compounds. Ethanol extract of calabash fruit seeds after being treated with a flavonoid test resulted in a color change to blue. The blue color produced by ethanol extract indicates the presence of genistein or anthocyanins group at flavonoid compounds which are known to have a purple or blue that kwon as genistein's color and the blue color that kwon as anthocyanins colors at a neutral pH. Flavonoid tests on ethyl acetate extract tested positive with a change in color to green; the color produced by ethyl acetate extract showed the presence flavonoid group, which produces yellow or green pigments (Barnes *et al.* 2013).

The test for the presence of alkaloids is carried out using Wagner reagents; the formation of orange or brown deposits characterizes positive results.

Wagner reagent was prepared from bismuth nitrate and dissolved in HCl. The addition of acid aims to prevent hydrolysis reactions because bismuth salts are easily hydrolyzed to form bismuth ions (Harborne 1998). Based on the results of phytochemical screening in Table 2, it is known that ethanol extract does not contain alkaloids. In contrast, ethyl acetate extract and *n*-hexane extract contain alkaloid compounds with color changes resulting from both extracts after being given a test treatment, which is formed in brownish-red color. The color formed in the alkaloid test is caused by the covalent coordinate bond between the N atom in the alkaloid with K+ from the reagent to form a potassium alkaloid (Parbuntari *et al.* 2018).

Identification of triterpenoid and steroid group compounds was carried out using the Liebermen-Burchard reagent, which is a mixture of glacial acetic acid with concentrated H_2SO_4. A positive result is marked in blue or green if it is positive for steroids, and there is a red or purple ring if it contains terpenoids. The color change that shows positive results for steroids is caused by the acetylation reaction of the -OH group on steroids after adding glacial acetic acid. In contrast, the formation of blue color in extracts containing terpenoids is caused by the oxidation of terpenoid compounds through the formation of conjugated double bonds (Parbuntari *et al.* 2018). Based on the results of phytochemical screening in Table 2, it is known that ethanol extract and ethyl acetate extract of k calabash fruit seeds contain steroid compounds with a color change to blue after being given test treatment. In contrast, the *n*-hexane extract of calabash fruit seeds contains terpenoid compounds with a color change to purple. Besides the phytochemical analysis, calabash extract was also used to test the antioxidant activity using the DPPH method. The DPPH test is an effective method that can be used to determine free radical activity. DPPH contains unstable nitrogen with strong absorbance at a wavelength of 517 nm and is dark purple. Antioxidant testing with the DPPH method is seen from the presence or absence of antioxidant compound activity that can reduce the intensity of free radicals from DPPH so that it can produce color changes as a result of antioxidant activity that can reduce color intensity in DPPH. The measurement of antioxidant activity is carried out using different concentrations. The higher the concentration of the extract, the greater the antioxidant activity, which is characterized by the formation of color changes in DPPH from purple to yellow. Antioxidant activity can be known from the IC_{50} value calculated based on the absorbance value of the sample with DPPH as the control.

The measurement results using a UV-Vis spectrophotometer at a wavelength of 517 nm obtained the absorbance value of each concentration. The absorbance value obtained is used to calculate % inhibition, which can then be calculated as IC_{50}. The calculation results obtained data as in Table 3. The determination of the IC_{50} value can be calculated using the linear regression equation of the relationship between extract concentration and % inhibition. From the results of % inhibition, a diagram of DPPH free radical inhibition activity is made. Based on linear regression, an equation was obtained from each extract, which was then calculated, as well as the results of DPPH free radical inhibition activity from the three extracts. Ethanol extract with regression equation y = 0.2364x + 42.957 with IC_{50} value of 29.7883 ppm (µg/ml), ethyl acetate extract with regression equation y = 0.291x + 47.899 obtained IC_{50} value of 7.219 ppm (µg/ml), while for *n*-hexane extract with regression equation y = 0.0578x + 0.9444 and IC_{50} value is 848.712 ppm (µg/ml). The IC_{50} results of the three extracts showed that the composition of ethyl acetate extract and ethanol extract of calabash fruit seeds has a very strong antioxidant activity ability (IC_{50}<50 ppm), with the smallest IC_{50} value in ethyl acetate extract of calabash fruit seeds which is 7.219 ppm (µg/ml) which indicates that with an ethyl acetate extract concentration of 7.219 ppm (µg/ml) can inhibit 50% DPPH. The *n*-hexane extract has very small antioxidant activity with an IC_{50} value of 848.712 ppm (µg/ml), so it can be concluded that n-hexane extract does not have antioxidant activity.

Compared to previous research conducted by Syaefudin *et al.* (2018) obtained an IC value of 50 calabash bark extract is 174.56 µg/ml, Das *et al.* (2014) ethyl acetate extract of calabash leaves and bark showed strong DPPH radical inhibition activity (IC_{50} of 11.25 µg/ml and 8.78 µg/ml). It can be concluded that the antioxidant activity of calabash fruit seed extract obtained from this study is stronger.

Calabash fruit seed extract that has greater antioxidant activity from the three extracts, which is ethyl acetate extract, then continued to determine compound composition by analyzing it using liquid

chromatography-mas spectrometry. Identification of compounds from the ethyl acetate extract of calabash fruit seeds using LC-MS in chromatogram data shows that there are 26 compounds contained in the ethyl acetate extract of calabash fruit seeds, consisting of secondary metabolite groups of flavonoids, alkaloids, steroids, glycosides, and beta carotene (seen in Table 4).

Flavonoid compounds contained in ethyl acetate extract of calabash seeds based on LC-MS results can be classified into various classes, namely: 1) Flavonols obtained erydiktiol compounds at a retention time of 6.222 minutes. Eriditiol has been widely researched and is known to have potential as an antimicrobial (Lee *et al.* 2011). 2) Flavones obtained at a retention time of 10.839 minutes, namely taxivolin compounds, and 3) Flavanones obtained 3',5,7-Trihydroxy-4'-methoxy flavanone with a retention time of 7.177 minutes. 4) Flavonoid glycosides obtained 2',6'-Dihydroxy-4-metoxicalkon-4'-o-neohesperid at a retention time of 16.984 minutes, have biological activity as anti-inflammatory (Tiwari & Husain 2017). 5) Isoflavones at a retention time of 19.084 minutes obtained Genistein 4',7-o-diglucoside malonilate, this compound is known to have activity as an antioxidant and anti-inflammatory (Unnikrishnan *et al.* 2014; Wang 2014).

Alkaloids are also one of the groups of secondary metabolite compounds present in the ethyl acetate extract of calabash fruit seeds. The results of the identification of groups of alkaloid secondary metabolite compounds are: 1) Tropane alkaloid class alkaloid compounds, namely Konvolin obtained at a retention time of 8.704 minutes, are known to have activity as antioxidants, antiaging and neurosis abilities (Balaji *et al.* 2014), and also Convolidin with a retention time of 20.143 minutes which has the ability as an antioxidant (Khan 2021). 2) The pyrocilialid class of alkaloids, Trachelatin, with a retention time of 13.616 minutes, is known to have biological activity as an antioxidant (Azimova *et al.* 2013), and Floridamin appeared at a retention time of 21.115 minutes to have activity as an antifungal (Dascalu *et al.* 2020).

Terpenoid compounds were also obtained based on the results of LC-MS. The results of the identification of terpenoid compounds contained in ethyl acetate extract of calabash fruit seeds include Ginoside Rh_3 at a retention time of 4.278 minutes, which is a class of triterpenoid saponins and is known to have anti-inflammatory activity (Xu *et al.* 2019). Saicosaponin C, at a retention time of 5.250 minutes, is also one of the compounds of the triterpenoid class of saponins and has anti-inflammatory activity (Shyu *et al.* 2004). Brevifoliol, with a retention time of 14.623 minutes, is a diterpenoid group compound that has activity as an antioxidant (Ahmed *et al.* 2022). Conferon, at a retention time of 18.580 minutes, is a group of coumarin sesquiterpenes known to have anticancer activity (Housang *et al.* 2023). In addition, it was also found that Taxikinin G compounds, which are diterpenoid groups with a retention time of 19.292 minutes, have the potential to capture free radicals and also have anticancer and anti-inflammatory activities (Lei *et al.* 2018). At a retention time of 17.140 minutes, Lialosidate compounds were obtained, which are terpenoid glycosides that have biological activity that can help improve memory (Bhalerao *et al.* 2016).

Based on the results of LC-MS, it is known that the extract also contains several steroid compounds. These, namely Solasodin compounds, appear at a retention time of 18.732 minutes and Progesterone compounds at a retention time of 8.878 minutes. Glycoside compounds contained in ethyl acetate extract, namely alkenyl phosphatidylcholine at a retention time of 4.712 minutes, 1,2-di-o-arsidonolicin-glycero-3-phosphocholine retention time of 5.007 minutes, phosphatidylcholine liso 19:0 appeared at a retention time of 6.361 minutes, and at a barrage time of 9.520 minutes p-hydroxybesilglucosinolate. In addition to glycoside compounds, amino acids were obtained, namely coumaroylquinic acid at a retention time of 15.907 minutes and S-Pyrufoylglutathione at a retention time of 17.311 minutes. The group of beta carotene compounds is also present in ethyl acetate extract, namely Avodiamine, at a retention time of 0.373 minutes. There are two natural compounds, namely Glimperid at a retention time of 3.671 minutes and Amitriptyline at a retention time of 22.121 minutes. The compound components obtained from the LC-MS results can be analyzed quantitatively using the value of the % area of each component to obtain the sum of the composition of each compound. Based on the % area of the 26 peaks that appeared, the compounds with the largest % area were Convolidin (11.277%), Komaroolquinic Acid (8.290%), and Taxikinin G (8.233%). Convolidin is

an alkaloid compound that has antioxidant activity (Khan 2021). Koumarolquinic acid has antioxidant, antithrombotic, anticancer, and anti-inflammatory activities (Aldaba-Muruato *et al.* 2021). Taxikinin G has the potential to capture free radicals and also has anticancer and anti-inflammatory activities (Lei *et al.* 2018).

In this study, calabash fruit seeds had phytochemical compounds, with the compounds contained in ethanol extract being a group of phytochemical compounds of saponins, flavonoids, and triterpenoids. Ethyl acetate extract of calabash fruit seeds positively contains a group of phytochemical compounds, flavonoids, alkaloids, and triterpenoids. *n*-hexane extract contains phytochemical compounds of tannin groups, alkaloids, and steroids. The antioxidant activity of calabash fruit seed extract from ethanol, ethyl acetate, and *n*-hexane solvents obtained IC_{50} values of 29.7883 ppm (µg/ml), 7.219 ppm (µg/ml), and 848.712 ppm (µg/ml) respectively. The best antioxidant activity is in ethyl acetate extract of kalabsa fruit seeds, so it is included as a very strong antioxidant. As the greatest antioxidant activity, ethyl acetate extract can then be used for LC-MS analysis. The results showed that there were 26 compound components contained in the ethyl acetate extract of calabash fruit seeds, with compounds with the largest composition, namely Convolidin (11.277%), Koumarolquinic Acid (8.290%) and Taxikinin G (8.233%). From this research, it can be recommended for the next research to combined the ethyl acetate extract of kalabsa fruit seeds for lotion application, followed by analysis of antioxidant and anti-inflammatory activity on skin.

Acknowledgements

The author would like to thank the dean of the Mathematics and Natural Sciences Faculty, Pattimura University, for the financial support and the head of the Biochemistry Laboratory for the laboratory facilities.

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