Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 10(1), 32-37, 2022

Phytochemical Screening and TLC Profiling of Combination Extracts of Avocado (*Persea americana* Mill.) and Papaya (*Carica papaya*) Leaves from Timor Island

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Received: October 2021
Received in revised: December 2021
Accepted: April 2022
Available online: May 2022

Abstract

Traditionally in the village of Alor, East Nusa Tenggara Province, a combination of avocado and papaya leaves is often used to treat malaria. To be able to be developed as an ingredient in traditional medicine, it is necessary to conduct preliminary research to determine the content of secondary metabolites contained in the combination of avocado and papaya leaves. This study aims to determine the secondary metabolites contained in avocado and papaya leaves through phytochemical screening and Thin Layer Chromatography (TLC) analysis. Extraction was carried out using methanol solvent by maceration, and the extract yield was 91.4%. Testing the content of secondary metabolites in avocado and papaya leaf extracts was carried out by phytochemical screening. Furthermore, the TLC test was carried out to confirm the presence of a positive group of compounds on phytochemical screening, and to determine the chromatographic profile of the extract. After being tested by phytochemical and TLC analysis, the extract contains several metabolites, including flavonoids, alkaloids, tannins, saponins, and steroids. The TLC system used in this study can separate the phytochemical content and give positive results that confirm the results of the phytochemical screening.

Keywords: Phytochemical Screening, Secondary Metabolite Compounds, Combination Extracts, Thin Layer Chromatography

INTRODUCTION

Indonesia is a country that is rich in sources of medicinal plants which have been used for generations as traditional medicinal ingredients. The efficacy of each plant varies depending on the presence of bioactive compounds and nutritive elements in the plant. Bioactive compounds in plants are usually contained in leaves, stems, roots, flowers, fruits, or seeds.

The avocado (*Persea americana* Mill.) is a plant that comes from Central America and can grow in tropical areas such as East Nusa Tenggara. Traditionally in Kabir-Alor Village, avocado leaves are used for asthma medication, by boiling two avocado leaves with two cups of water and adding one tablespoon of salt. The use of avocado leaves as traditional medicine can be attributed to its pharmacological activity. It has been reported that avocado leaf extract can function as anti-inflammatory, anticonvulsant, antioxidant, antibacterial and antidiabetic (Ranade and Thiagarajan 2015).

Carica papaya is a plant that can grow and spread from the lowlands to the highlands. Papaya has been used by the local people as traditional medicine. *C. papaya* leaf extract has been reported to treat malaria, fever, asthma, beriberi, abortion, jaundice, vermifuge, dressing wounds, gonorrhea, urinary complaints and antibacterial activity (Vij and Prashar 2015). People in Kabir-Alor village usually use papaya leaves to treat malaria, by boiling papaya leaves and drinking boiled water.

The use of combinations of medicinal plants to treat certain diseases is often carried out by the community with the aim of increasing the efficacy/effect of these medicinal plants. This is also done by the people in the village of Kabir-Alor. People usually use a combination of avocado and papaya leaves for the treatment of malaria by boiling and drinking boiled water.

Previous research has shown that a combination of papaya leaf extract and propolis helps reduce (Widya 2020). Another study showed that the ethanol extract of the combination of avocado and

johar leaves had an antibacterial effect against Salmonella typhi bacteria by 9.9 mm at a concentration of 100% (Pontoan 2016). However, research on the herbal combination of avocado and papaya leaves has not been reported, so it is necessary to conduct preliminary research to determine the secondary metabolite components contained in the combined extract.

METHODOLOGY

Materials and Instrumentals

The materials used in this study were avocado and papaya leaves collected on the island of Timor. Other materials used are Methanol for analysis (Merck), magnesium tape, HCl for analysis (Merck), acetone (Merck), Chloroform (Merck), diethyl ether (Merck), Propanol (Merck), Butanol (Merck), H_2SO_4 (Merck), Mayer's Reagent, Wagner's Reagent, $FeCl_3$, (Merck), Acetic anhydride, Whatman filter paper No 1 and distilled water.

Methods

Extraction

The sample was weighed (150 g) and macerated using methanol 96% (ratio 1:1:6) for 72 hours. After that, the mixture was filtered using Whatman No 1 filter paper and evaporated to dryness. The extract was weighed to determine the yield of extract. Hereinafter the extract is called EDAP.

Solubility Test of EDAP

To determine the solubility of the extract, EDAP was taken as much as 1 mL and added to a test tube containing 2 mL of distilled water, acetone, chloroform, 96% methanol and diethyl ether. After that shake the mix slowly until a few minutes (Hasti et. al., 2022).

Phytochemical Screening

Flavonoid test was carried out by 1 mL of EDAP was reacted with 2 mg of magnesium tape and 1 mL of concentrated HCl. The presence of flavonoid was expressed by the formation of a red, yellow or orange color (Ramadani, Karima, and Ningrum 2022). Test for alkaloids was done using Mayer's and Wagner's test. As much as 1 mL of the EDAP was put into tubes A and B then 0.5 mL of 2% HCl was added and mixed evenly. After that, 2-3 drops of Mayer's reagent were added to tube A and Wagner's reagent to tube B. The formation of a white precipitate in tube A and brown in tube B indicates the presence of alkaloids (Kopon, Baunsele and Boelan, 2020; Goa et al. 2021).

Tannins test is done by 1 mL of EDAP was reacted with 5 drops of 5% $FeCl_3$ (Ferric chloride). The formation of black or blue-green indicated the existence of tannins (Ramadani et al. 2022; Sariwati et al. 2021). Saponin test was carried out using the Forth method. The EDAP (1 mL) was put into a test tube and 2 mL of hot distilled water was added. The sample will form foam and then add 1 mL of 2 N HCl. If the foam does not disappear for 30 seconds, the extract is positive for saponins (Kopon et al. 2020). Test for steroids was carried out by the EDAP 1 mL were blended with 2 mL Acetic anhydride and a few drops of H_2SO_4. The Presence of steroids was expressed by the creation of the blue/green color (Sariwati et al. 2019).

Thin Layer Chromatography (TLC) Profiling

The TLC plates were prepared by using Silica gel G60F254 with a length of 8 cm and a width of 2 cm, washed with methanol, and then activated in an oven at 100 ^oC for 30 minutes. Samples were applied to a TLC plate with the help of a capillary tube (Yuda, Cahyaningsih, and Winariyanthi 2017). The mobile phase is prepared by making a solvent ratio according to the component to be tested as follows:

a. Alkaloids: Chloroform: Methanol: 5% HCl (5:5:3)
b. Flavonoids: Chloroform: Methanol: distilled water: Butanol (10:10:6:1).
c. Tannins: Butanol: Propanol: distilled water (2:1:3)
d. Saponins: Chloroform: Methanol: distilled water: Propanol (5:1:6:4).
e. Steroids: Heptane: Acetone: Methanol (5:1:4).

RESULTS AND DISCUSSION

Extraction Yield

Extraction of avocado and papaya leaves was carried out using the maceration method in a ratio of 1:1:6 (150 g of avocado: 150 g of papaya: 2400 mL of methanol). The solvent used during maceration will help the process of separating the active compound content from plant tissue (Saudale, Boelan, and Boelan 2018). The extraction process that takes place produces a yield of 91.4%. The yield resulting from the extraction process is based on the extraction method, extraction time, amount of extraction, and also the temperature used. The amount of yield obtained indicates the number of components that are extracted during the maceration process (Goa *et al.*, 2021). The diffusion process occurs during maceration, where the solvent methanol which has a high concentration diffuses into the cell nucleus of the avocado and papaya leaves through the cell wall causing the cell membrane

to rupture. It is at this time that the secondary metabolites in the center come out.

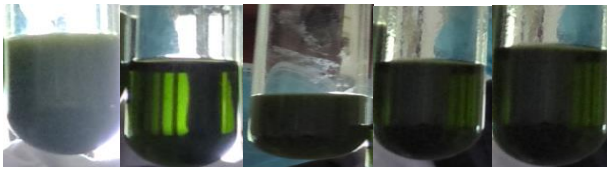
Solubility Test of EDAP

EDAP solubility test was carried out with several solvents, namely: distilled water, acetone, chloroform, 96% methanol, and diethyl ether and the results of observations can be seen in Table 1. The results of the solubility test EDAP was soluble in distilled water, acetone and methanol and insoluble in chloroform and diethyl ether solvents. Based on observations (Figure 1) when EDAP and distilled water are mixed, the solution becomes homogeneous and changes color to brown. The same result was also shown by the mixture of EDAP with methanol solvent.

The solubility of EDAP indicates the presence of hydrogen bonds between compounds in EDAP. The presence of compounds that have a lone pair of electrons such as in the group of alkaloid compounds and also O atoms in water, acetone, and methanol solvents cause hydrogen bonds to form with H atoms (Kido and Naja 2018; Mulyati and Panjaitan 2021). This causes the polar compounds in EDAP to dissolve in polar solvents such as water, methanol and acetone.

Table 1. Solubility Test Results of EDAP

Material Test	Observation	Result
EDAP + Distilled water	• homogeneously mixed • The color changes to brown	Dissolved
EDAP + Acetone	• homogeneously mixed • The color changes to light green	Dissolved
EDAP + Chloroform	• Heterogeneous • The solution does not change color but remains dark green	Not Dissolved
EDAP + Methanol+	• homogeneously mixed • Solution turns brown	Dissolved
EDAP + diethyl ether	• Heterogeneous • The solution does not change color but remains dark green	Not Dissolved



(a) (b) (c) (d) (e)

Figure 1. EDAP Solubility Test Results (a) EDAP + Distilled Water; (b) EDAP + Acetone; (c) EDAP + Methanol; (d) EDAP + Chloroform (e) EDAP + diethyl eter

Phytochemical Screening

Phytochemical screening was carried out to determine the secondary metabolites contained in the extract. The results of phytochemical screening were shown in Table 2. These results reveal the presence of important phytochemical components in the combined extract of avocado and papaya leaves. Flavonoids, alkaloids, tannins, saponins, and steroids were detected in EDAP. The results of the phytochemical test on the combined extract of avocado and papaya leaves gave different results from the single extract. Research conducted by Pontoan showed that methanol, ethanol and acetone extracts of avocado leaves contain groups of alkaloids, flavonoids and saponins (Pontoan 2016). In another study, papaya leaf methanol extract gave positive results in the flavonoid, alkaloid, tannins, and steroids test (Callixte, Baptiste, and Arwati 2020; Santi 2015).

Table 2. Phytochemical Screening of EDAP

Test	Reagent	Result
Flavonoid	Mg tape + HCl concentrated	+
Alkaloid	Mayer	+
	Wagner	+
Tannin	$FeCl_3$	+
Saponin	Hot Distilled Water + HCl 2 N	+
Steroid	Chloroform 98% + H_2SO_4	+

The flavonoid test showed positive results which were indicated by a yellow color change caused by a reduction reaction by Mg carried out in an acidic atmosphere when HCl was added. Reduction with magnesium and concentrated hydrochloric acid gives a brownish yellow color. The Estimated reaction on flavonoids test can be seen in Figure 2.

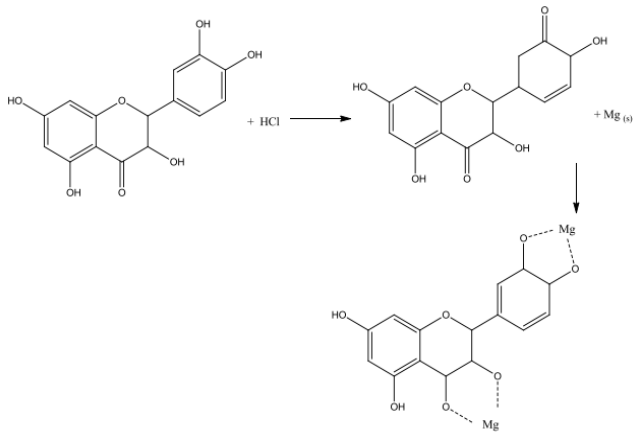


Figure 2. Flavonoid test reaction estimates (Nugrahani et al. 2016)

In the test, the alkaloid results were shown by the presence of a white precipitate when reacted with Mayer's reagent and a brown precipitate when reacted with Wagner's reagent. The formation of this precipitate is due to the change of the ligand. Alkaloids are semi-polar compounds that contain nitrogen atoms in the cyclic part and contain a variety of substituents such as amino groups, methoxy amides, and phenols (Puspitasari, Swastini, and Arisanti 2013). The approximate reaction that occurs in the alkaloid test using Mayer's reagent can be seen in Figure 3. During the manufacture of the reagent, a red precipitate of HgI_2 will be formed as a result of the reaction between a solution of $HgCl_2$ and KI. And if more KI is added it will produce $K_2[HgI_4]$, so that when reacted with the sample, the alkaloid compound which has a nitrogen atom will react with the metal ion K^+ from $K_2[HgI_4]$ and form a potassium-alkaloid complex.

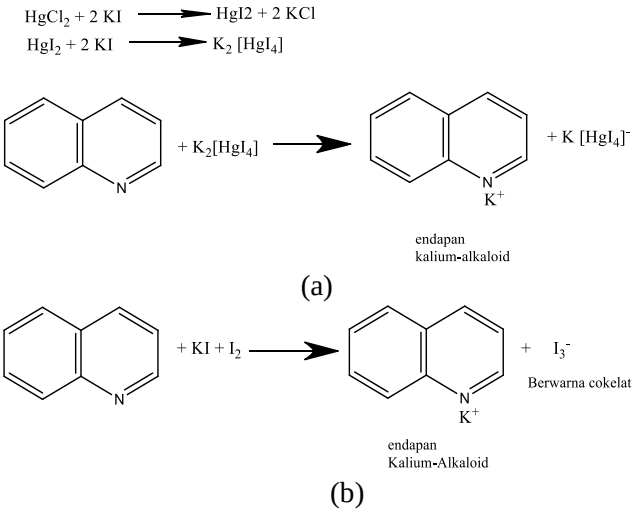


Figure 3 Alkaloid Test Estimation (a) Mayer's reaction estimates; (b) Wagner's reaction (Goa et al. 2021)

The class of tannin compounds tends to be polar because it has a hydroxyl group. Identification of the tannin compound was carried out by adding $FeCl_3$ and the sample showed a color change to green-black which indicates that the tannin compound underwent hydrolysis. The reaction can be expressed as follows:

$$FeCl_{3(aq)} + 6\ ArOH_{(s)} \rightarrow 6H^+ + 3Cl^- + [Fe\ (OAr)]^{3-}{}_{(aq)}$$

The results of the saponin test were positive because the sample formed foam. Saponins are a form of glycoside that has the ability to form foam in the air which shows positive results. The group of saponin compounds contains a polar glycosyl group and non-polar steroid and triterpenoid groups. Compounds that have non-polar and polar groups will be active on the surface. The foam formation reaction in the saponin test is shown in Figure 4.

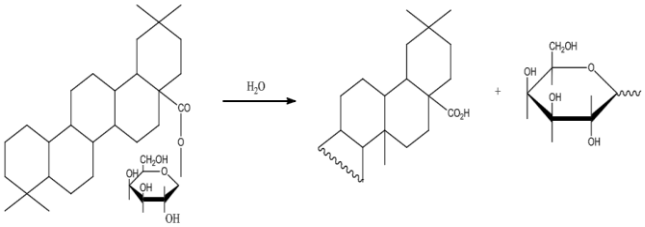


Figure 4. Saponin test reaction estimates (Marliana and Suryanti 2005)

The steroid compound group was identified by the Liebermann-Burchard test method, the results of this test showed a positive test with the formation of a dark green color. The principle in this test is the ability of the compound to form a color with concentrated sulfuric acid in an acetic anhydride solvent. During the reaction, there will be a release of H_2O and a combination with a carbocation.

Thin Layer Chromatography (TLC) Profiling

TLC is a method of separating a compound based on the distribution of two phases, namely the mobile phase in the form of an eluent and a stationary phase in the form of an adsorbent that has different polarities. TLC test was then carried out to confirm the positive results obtained from the phytochemical screening. The results TLC of EDAP can be seen in Table 3.

The Alkaloid TLC test was carried out with the mobile phase Chloroform: Methanol: HCl (5:5:3). This eluent produces one spot stain with an Rf value 0.86. Identification of flavonoid compounds by TLC test using mobile phase Chloroform: Methanol: Distilled Water: Butanol (10:10:6:1) and one spot is obtained from the separation with an R_f value 0.82. Furthermore, the TLC test was carried out to confirm the results of phytochemical screening of tannin compounds with a mobile phase Butanol : Propanol : Distilled Water

(2:3:3). From the elution results obtained two spots of separation with the value of R_f 0.79 and 0.62. Identification of saponin compounds by TLC test using the mobile phase Chloroform: Methanol: Distilled Water: Propanol (5:1:6:4) and obtained one spot of separation with an R_f value 0.76. Furthermore, a TLC test was carried out to confirm the results of phytochemical screening of steroid compounds with Heptane: Acetone: Methanol as mobile phase (5:1:4). From the elution results, one spot was obtained from the separation with an R_f value of 0.34.

These results are in line with the EDAP phytochemical test which showed that the extract contained a class of alkaloid compounds, flavonoids, tannins, saponins and steroids which was strengthened by the presence of a specific stain color when the TLC test was performed.

Table 3. R_f Values for Various Phytochemical

Phytochemical	Solvent system	Number of Strain	R_f Value
Alkaloids	Chloroform: Methanol: HCl	1	0.86
Flavonoids	Chloroform: Methanol: Distilled Water: Butanol	1	0.82
Tannins	Butanol: Propanol: Distilled Water	2	0.79; 0.62
Saponins	Chloroform: Methanol: Distilled Water: Propanol	1	0.76
Steroids	Heptane: Acetone: Methanol	1	0.34

CONCLUSION

Based on the results of a phytochemical test it can be concluded that EDAP produces many secondary metabolites of medicinal value including flavonoids, alkaloids, tannins, saponins and steroids. These results are confirmed by the TLC results.

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