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Identification of Phytochemical Extract of a Combination of Young Coconut Water, Ginger and Turmeric

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Abstract

Turmeric is a tropical plant that is widely found on the Asian continent which is extensively used as a food coloring agent and fragrance. Ginger is a spice plant that it often found in South Asia which is widely has spread throughout the world. Coconut is an annual plant, has a hard stem and is generally not branched (monopodial), has fibrous roots, and contain water that is clear and tastes sweet, young coconut water has benefits such as antibacterial, beauty treatment, as an isotonic agent, and carry out the urination. This research was conducted to obtain information about the physicochemical properties and secondary metabolites contents of the coconut water, ginger and turmeric combination extract. The research method was carried out by mixing the mashed turmeric and ginger with the young coconut water and then macerated. The resulting extract was then tested for physicochemical properties (solubility) and identified groups of secondary metabolites (flavonoids, tannins, saponins, and steroids/triterpenoids. The extract was soluble in 70% ethanol. In addition, the combination of young coconut water, ginger, and turmeric extract contain a group of secondary metabolites among others flavonoids, tannins, saponins, and triterpenoids.

Keywords:Phytochemical screening, physicochemical, extract, secondary metabolites, ethanol

INTRODUCTION

Indonesia is one of the third largest tropical forests in the world besides Brazil and Zaire that can produce secondary metabolite compounds with many potential to be antioxidants, dye substances, food aroma enhances, perfume, insecticides, and medicine (Nola, Putri, Malik, & Andriani, 2021). Biodiversity is the basis of various treatments and the discovery of the pharmaceutical industry in the future, For example, avocado plants as traditional medicine (Kopon, Baunsele, & Boelan, 2020).

The phenomenon of food consumption has a new paradigm for food science and technology development by the modification of food processed products towards functional properties. Functional food is food that is natural and has gone through the process, containing one or more bioactive molecules such as compounds in phytochemical components, phytochemical or saturated fatty acids, unsaturated fatty acids, fiber, and others (Lantah, Montolalu, & Reo, 2017). In addition to the content of phytochemicals, the content of bacteria that exist in natural ingredients is a finding that can be utilized in the field of food because the abundance of microorganisms in nature certainly has a major influence on food quality (Missa & Baunsele, 2020; Mere, Bintang, & Safithri, 2021).

Phytochemical screening is a method for determining the group of secondary metabolites that have the biological activity of a plant. Plant phytochemical screening was used as initial information in knowing the group of chemical compounds found in a plant (Savalas, Loka, & 'Ardhuha, 2021).

Many secondary metabolite-producing plants are found in Indonesia, one example is turmeric. Turmeric is also used as a dye, treatment, and flavoring since 600 BC. Turmeric has been trusted as one of the highly valuable herbs. The important chemical content of turmeric rhizomes is curcumin, essential oil, desmethoxy curcumin, bisdesmethoxy curcumin, fat, protein, calcium, phosphorus, and iron. The chemical content of essential oils of turmeric consists of artumeron, β-caryophyllene, linalool, and 1.8 cineol. Essential oils of turmeric rhizome, contain a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpenes (53%). Curcumin (diferuloylmethane) (3-4%) is an

active component of turmeric that plays a role in producing yellow dye and consists of curcumin I (94%), curcumin II (6%), and curcumin III (0.3%) (Yuan Shan & Iskandar, 2018).

The secondary metabolites of curcumin can be used as a medicine for some diseases (Suprihatin, Rahayu, Rifa'i, & Widyarti, 2020). According to Cobra, (2019) turmeric contains compounds that are affected by drugs, called curcuminoids that are self-conduct from curcumin, desmethoxy curcumin, and bisdesmethoxy curcumin. Curcuminoid is useful for preventing various disease infections. The main content of curcuminoids is yellow curcumin. The curcumin content in turmeric ranges from 3-4%.

One of the herbal plants that is often used besides turmeric is ginger. Ginger is a spice plant from South Asia and has now spread worldwide (Redi Aryanta, 2019). According to Wardani (2012) ginger can be utilized in various industries, including the beverage industry (ginger syrup, instant ginger), the cosmetics industry (perfume), food industry (ginger candy, ginger, enting-entering ginger), traditional medicine industry, or herbal medicine, kitchen spice industry.

The phenol component is the main component of ginger, which contains active substances or identity, including Gingerol. Gingerol has the effect of pharmacology and giver, it is proven to be able to inhibit the formation of tumors on the skin of the trial mouse, inhibit the proliferation of cancer in humans through induction of apoptosis, both in leukemia blood cancer cells, colon cancer, and others (Syukur, Yusron, & Trisilawati, 2016). While Sari (2011) explains that the chemical composition of ginger consists of, starch, resin, malic acid, oxalic acid, fat, carbohydrates, vitamins A, B, and C, flavonoid compound minerals, phenol, polyphenols, proteolytic enzymes called zingibain. The active ingredient of ginger consists of essential oil, zingiberin, zingerone, famesene, geraniol, paradol, shagol and gingerol. Ginger also contains flavonoids, alkaloids, steroids/terpenoids and saponins.

In addition to turmeric and ginger herbal plants, another example of a secondary metabolite-producing plant is coconut. Coconut Fruit is one of the unique tropical plants products because besides the fruit component of the fruit can be consumed directly, as well as the fruit water components can be drunk directly without going through processing (Barlina, 2004). Coconut fibers are capable as ingredients to adjective blue methylene dyes that have a negative effect on the environment (Baunsele & Missa, 2020, 2021). According to Kristina and Syahid (2020) coconut water contains ZPT kinetin (cytokinin), zeatin (auxin), tannins, vitamins (vitamin C, riboflavin, vitamin B5, inositol, biotin, pyridoxine, thiamin), minerals (N, P, K, Mg, Fe, Na, Zn and Ca) and sugar (glucose, sucrose, and fructose).

Coconut plants are used by almost all parts of humans so they are considered multi-purpose plants, especially for coastal communities. Coconut results traded since ancient times are coconut oil. Coconut plants utilized by Indonesian people can be in the form of fibers, shells, sticks, and coconut leaves as handicraft materials and household appliances (Sutara, 2013). According to Nayoan et al., (2018) young coconut water has benefits such as Antibacterial, beauty, isotonic, facilitating urination. The water of young coconut is also widely used by the community in Indonesia as a material for treating fever and bioethanol (Malle, Kapelle, & Lopulalan, 2014).

METHODOLOGY

Materials and Instrumentals

The research natural materials were used the Young coconut water (*Cocos nucifera)*, ginger (*Zingiber officinale*), and turmeric *(Curcuma Longa L)*. the other ingredients, namely: cotton (no brand), 70% ethanol for analysis (Merck), zinc (Flinn Scientific), HCl for analysis (Merck), distilled water, gelatin (Merck), acetic acid glacial (Merck), Sulfuric acid (Merck), kitchen knife, plastic bag, basin, sieve (no brand), aluminium foil, stirrer, scale, spoon, filter paper, test tube, dropper, scale glass, reagent bottle, stove and reagent bottle, mortar and pestle.

Methods

Preparation of Young Coconut Water, Ginger and Turmeric extract

The ginger and turmeric were firstly cleaned and then mashed using mortar and pestle. The mashed materials can accelerate the maceration process. The maceration process used the young coconut water as a solvent. The scaled glass was heated at a temperature of 50-60 ^{o}C for 20-30 minutes then dried at room temperature and got a constant weight of the glass. A total of 170 mL of young coconut water and wix with 50 g of ginger and 50 g of turmeric that has been mashed. The mixture is then stirred until evenly distributed, then transferred into a jar and covered with aluminum foil. The mixture was macerated for ± 24 hours (Bustanussalam, Apriasi, Suhardi, & Jaenudin, 2015). After the maceration is complete, it is filtered using cotton which is placed on filter paper. The combination extract of young coconut water, ginger, and turmeric was then used for physicochemical and phytochemical analysis. This extract is called ECGT.

Solubility Test of ECGT

The extract was taken as much as 5 mL and then entered into a test tube containing 5 mL of 70% ethanol. Shake the mix slowly until homogeneous. The homogeneous indicated the ECGT was a polar solution

Flavonoid Test of ECGT

About 0.5 mL ECTG was put on the test tube and then added 0,5 grams of zinc powder. Dripping 4 mL of 0.2N chloride acid slowly. Let it about 2 until 5 minutes, and observe the colour changes. If the mix became yellow that indicated that ECGT contained flavonoids (Guntarti, Annisa, Mughniy, & Rizqi, 2017).

Test of Saponin ECGT

A total of 3 mL of ECGT was put into the test tube then added 2 mL of hot water and KOH then shaken for 5 minutes. If a solid foam is formed and stable for 15 minutes, it indicates the presence of saponin compounds (Aprilia & Yanti, 2019).

Tannin test of ECGT

The extract of ECGT is dissolved with 10 mL of distilled water while stirred to a homogeneous mixture. Add gelatin solution, if a precipitate appears, it means that the ECGT contains tannins (Desinta, 2015)

Test of Triterpenoids and Steroids Of ECGT

A total of 0.5 mL of the ECGT was put into a test tube and then added 0.25 mL of acetic acid and 0.15 mL of 0.2N H_2SO_4. The presence of terpenoid compounds is indicated by the formation of a dark blue or green-black color of the mixture (Aprilia & Yanti, 2019).

RESULTS AND DISCUSSION

Preparation of Young Coconut Water, Ginger and Turmeric extract (ECGT)

The results of ECGT obtained a yellow filtrate with the total yields presented in Table 1. The data in Table 1 uses the average of triple repetition.

Tabel 1. The Data Extract of ECGT

Initial weight (grams)		Young coconut water		Sample Weight	Maceration Results (mL)	
Turmeric	Ginger	mL	Grams	Grams	mL	Grams
50	50	170	84.7	184.7	130	49.2
50	50	170	84.7	184.7	132	49.4
50	50	170	84.7	184.7	128	49.0
Average			84.7	184,7	130	49.2

Maceration is the simplest extraction method (Ansel, 1989). The maceration process is by immersing the combination extract in a solvent. The solvent will penetrate the cell of the sample and will interact with the active substance. This method is low cost and protects the damage of chemical substances of the natural ingredient (Kiswandono, 2011). At the maceration stage, the extract of the combination of young coconut water, ginger, and turmeric, macerated for ± 24 hours. Young coconut water will enter the cell walls of ginger and turmeric that have been mashed so that the active substances in the ginger and turmeric cells will be pushed out and will mix in the analyte. After going through the maceration stage, the sample was filtered using filter paper that had been added with cotton. From this process, 130 mL of yellow extract was obtained.

The maceration process causes the discolouration of yellow color in the extract. These changes were due to the occurrence of complex bonds between vitamin C, curcumin, and gingerol, with a percentage yield of 26.64%. The Maceration process depends on the polarity of the solvent and solute, and the surface reaction area (Susanty & Bachmid, 2016).

Phytochemical Screening

Table 2 presents data on the physicochemical test of the Solubility and phytochemical of ECGT. The data analysis includes testing of the solubility, the content of flavonoids, saponins, tannins, triterpenoids, and steroids. The analysis was carried out to determine the secondary metabolites of the ECGT, and then the chemical compounds can be used for various purposes.

Extract solubility test to determine the polarity of the extract. The more polar a material is, the better the tendency to use it for consumption or medicine. The results are shown in Table 2 and Figure 2 show that ECGT is soluble in 70% ethanol solvent. This data was obtained based on the observation that a homogeneous mixture was formed and the color changed from dark yellow to light yellow. The solubility of ECGT with 70% ethanol as a solvent indicates the hydrogen bonds between the compound molecules in the ECGT extract and the solvent. Molecularly the formation of hydrogen bonds between gingerols and ethanol solvents is detailed in Figure 1.

The formation of hydrogen bonds between gingerol compounds and ethanol solvents occurs due to the presence of atoms that have high electronegativity such as O and H atoms in ethanol and gingerols so that electrostatic interactions will form between the two functional groups.

Table 2. Solubility and phytochemical test results ECGT

Test Material	ECGT (mL)	Observation	Test results
a. Solubility Test ECGT			
2 mL 70% ethanol	5	The solution is homogeneously mixed and the color changes to light yellow	Late
b. Flavonoid Test ECGT			
Wilstarer Cyanidine reagent	1.5	A yellow solution is formed and there is foam	Positive **(+)**
c. Uji Saponin ECGT			
hot water + 0.1N HCl	10	There is a stable foam for 30 seconds	Positive (+)
d. Tannin Test ECGT			
Gelatin	3	Chocolate Deposit	Positive (+)
e. Triterpenoid and steroid test ECGT			
Liebermann-Burchard reagent.	1.5	A yellow solution and precipitate formed	Positive (+)

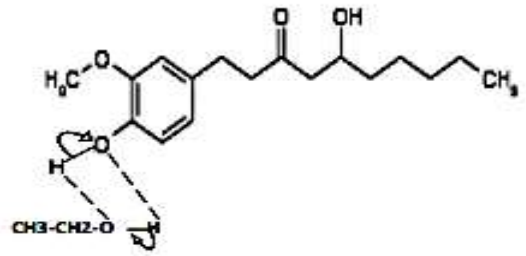

Figure 1. Interaction prediction between Gingerol Molecules and Ethanol

This causes the polar compounds formed in the extract of the combination of young coconut water, ginger, and turmeric to be soluble in polar solvents such as ethanol.

(a) (b)

Figure 2. The results of the solubility test (a), the flavonoid test (b) of the ECGT extract

The flavonoid content test was analyzed using a comparison of theoretical data with a positive result for the Wilstater cyanidin reagent. The results of the group test for flavonoid compounds obtained positive ECGT data containing groups of flavonoid compounds which are described in Table 2 dan Figure 2. Based on observations, it is known that a yellow solution and foam formed in the test tube where ECGT and test reagents were reacted.

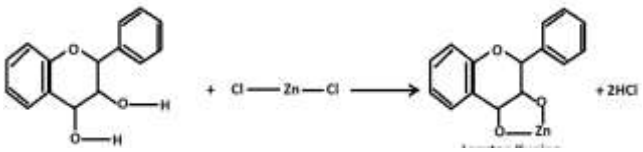

Figure 3. Reaction of Wilstater Cyanidine Reagent with flavonoids

Zn metal dissolved in acid will form Zn^{2+} and Cl^- ions. In the o-hydroxy propiophenone compound, there is a difference in electronegativity between O and H, where O is more electronegative than H causing the O and H bonds to break. O- interacts with Zn^{2+} ions to form zinc salt 2-(1-chloro-1-oxido propyl phenolate) and H^+ interacts with Cl^- to form HCl. Thus, a yellow complex is formed which indicates the presence of flavonoids in the extract of the combination of young coconut water, ginger, and turmeric.

Saponification is the ability of a material to have soap-like properties. The data from the test results for the group of saponin compounds are described in Table 2 shows that the combined extract of young coconut water, ginger, and turmeric contains a group of saponin compounds. The test results for the group of saponin compounds using the Forth method formed stable foam, indicating that the extract of the combination of young coconut water, ginger and turmeric contained positive saponin compounds. The appearance of foam in the Forth test indicates the presence of glycosides that have the ability to form foam in water which is hydrolyzed into glucose and other compounds (Robinson, 1983).

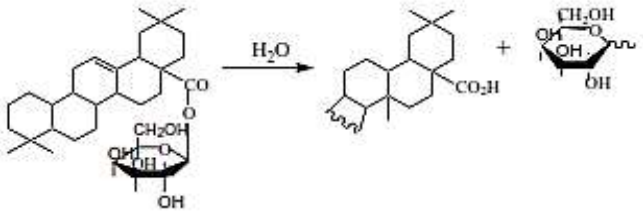

Figure 4. Froth formation reaction (Marliana, Suryanti, & Suyono, 2005)

The formation of CO_2 gas in ECGT is caused by the presence of high electronegativity atoms such as C-O. If the bond is broken between C atoms, the CO_2 molecule will be released because at the same time the negative C atom that attracts the C-C electrons will attack the positive H, then the negative C atom will attack the positive H atom. Positive C will be attacked by negative O atoms so that CO_2 gas is formed which is indicated by the presence of gas bubbles/foam on the surface of the extract of the combination of ECGT. The glycosyl group is the polar compound that is contained in the saponin compound. Glycosides can cause the presence of froth in the water (Marliana et al., 2005).

(a) (b) (c)

Figure 5. The results of the steroid and triterpenoid test (a), the tannin test (b) the saponin test (c) of the ECGT extract

One of the phytochemical properties of natural materials is the presence of tannins in raw materials. In this study, ECGT was also tested for tannin content using gelatin. The results of the analysis shown in Table 2 dan Figure 5 show that ECGT is positive for a group of tannin compounds. When gelatin is mixed with ECGT it will form a yellow solution with a brown precipitate. The color change that occurs in the reaction tube to brown is evidence of the presence of tannins. The resulting brown precipitate comes from hydrogen bonds between gelatin and tannins. There is a difference in the electronegativity of O and H atoms in the OH group in tannin compounds where O is more electronegative than H so that O becomes partially negative, H becomes partially positive. And the presence of high electronegativity of O and N atoms in gelatin, so that there is a partial positive H interaction with partial negative O and N in gelatin to form hydrogen bonds as a gelatin-tannin complex precipitate. The precipitate was formed because tannins can coagulate protein from gelatin to form stable nonpolar copolymers (Desinta, 2015).

The data from the identification of the triterpenoid content of the ECGT were analyzed using a comparison of the theoretical data of the Liebermann-Burchard reagent. The results of the group test of triterpenoid and steroid compounds are presented in Table 2 and Figure 5. By the reaction, H_2SO_4 releases H^+ ions because H_2SO_4 is more acidic than CH_3COOH. In triterpenoid compounds there is also a difference in electronegativity between O and H atoms, O is more electronegative than H so the bonding electrons will break to O, O produces a negative charge and H produces a positive charge. The CH_3COO^+ ion will interact with the O^- group and HSO_4^- will interact with the H^+. Based on this reaction, the resulting color is yellow. The yellow color indicates the presence of triterpenoid compounds. While the solution is green, the combination extract of young coconut water, ginger and turmeric contain steroids. The data described in Table 3 shows the comparison between each natural ingredient and the compounds of ECGT.

Table 3. Data Content Of each Ingredient

Ingredients	Secondary Metabolic Compound			
	Flavonoid	Tanin	Saponin	Steroid/ triterpenoid
Ginger	-	-	√	√
Turmeric	-	-	-	-
coconut water	-	-	√	-
ECGT	√	√	√	√

Based on the data in Table 3, it can be explained that although there are materials that do not contain certain metabolites, when combined with other materials, there is a possibility of a reaction to form other secondary metabolites. For example, turmeric, ginger, and pure coconut water do not contain the groups of flavonoid and tannin compounds. But after being combined and extracted, it will contain flavonoids and tannins. This idea provides an advantage where ECGT can be used as a natural ingredient in herbal medicines because in addition to being commonly consumed, ECGT also contains many secondary metabolite compounds.

CONCLUSION

The conclusion from this study is that the combined extract of young coconut water, ginger, and turmeric has good solubility in 70% ethanol solvent. If the natural ingredients used are tested partially, then not all groups of secondary metabolites are contained in each natural ingredient, but if the extract used is a mixture or combination of all the ingredients referred to, ECGT will contain all secondary metabolite compounds including flavonoids: tannins, saponins, steroids, and triterpenoids.

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