Extraction and Screening of Phytochemicals in Broccoli Florets (*Brassica oleracea var. italica*) with Different Polarity Solvent

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Broccoli, scientifically known as *Brassica Oleracea var. italica* is a vegetable with dense and green flower heads. It is grown for its edible stalk and flowering head, which is called florets. Additionally, it possesses a significant amount of nutrients and antioxidants. The objective of this research was to extract bioactive components from *Brassica oleracea var. italica* florets using a maceration method. The extraction method involves the use of solvents that have different polarities: ethanol (polar), acetone (semi-polar), and toluene (non-polar)—the percentage yield of *Brassica oleracea var. italica* was highest when ethanol was used (7.54%), compared to toluene (6.88%) and acetone (5.94%). A phytochemical test was conducted to identify the presence of bioactive compounds such as tannins, saponins, alkaloids, terpenoids, flavonoids, and phenolics. *Brassica Oleracea var. italica* has been shown to possess all the active compounds that were tested.

Keywords: *Brassica Oleracea var. italica;* different polarity solvent; maceration; active compound; phytochemical test

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Green in color, Brassica oleracea var. italica is a famous vegetable belonging to the Brassica family [1]. Brassica oleracea var. italica is a healthy vegetable that is part of the *Brassica oleracea* species, which also includes cabbage, cauliflower, and Brussels sprouts. It is also widely recognized for its exceptional nutritional value. This vegetable is a rich source of vitamins C and K, as well as folate (vitamin B9), dietary fiber, antioxidants like beta carotene, and several minerals like potassium and manganese [2]. The consumption of Brassica oleracea var. italica is linked to a wide range of health advantages. It enhances immune function, promotes heart health, aids digestion, and may possess anti-cancer qualities due to its abundance of antioxidants [3]. Brassica oleracea var. italica possesses several functional attributes related to secondary metabolites, such as phenolic compounds, carotenoids, chlorophyll, alkaloids, and glycosylates, among others, during the extraction process [4].

Maceration is a widely used extraction method that is cost-effective and straightforward. This extraction process can be completed in less than seven days. Furthermore, this technique can be used to extract active compounds and essential oils from plant sources. An extraction procedure is described wherein a container is filled with a coarse stem bark, leaf, or root bark [5]. A suitable solvent such as methanol, ethanol, acetone, or toluene is then poured over the substance until it completely envelops it [6]. The efficiency for the removal of active compounds from plant material depends on the type of solvent and the type of plant material. The polarity of solvent is a crucial determinant of the efficiency of solvent extraction [7]. In this work, different polarity of solvent was used to determine the highest percentage of extraction method. Besides, the phytochemical test was also employed to detect the presence of active compounds in *Brassica oleracea var. italica*.

EXPERIMENTAL

Chemicals and Materials

The fresh broccoli (*Brassica oleracea var. italica*) was purchased from a local market in Muar, Johor, Malaysia. Ethanol (99.9%), acetone (99.5%), and toluene (99.8%) were bought from Sigma Aldrich. The instruments used are analytical balance (Mettler Toledo, Malaysia) and rotary evaporator (RE 100-Pro, China). 247 Alya Athirah Mohd Idris, Nurul Fatihah Hisamuddin, Riana Rayner, Siti Balqis Mohamad Shah, Kavirajaa Pandian Sambesavan and Saliza Asman

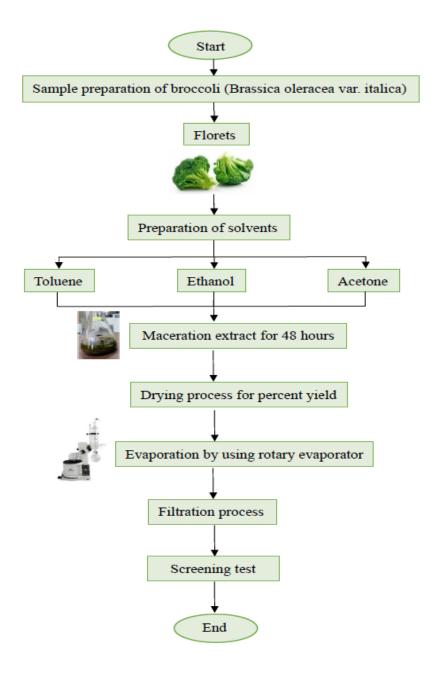


Figure 1. Flow chart of extraction of Brassica Oleracea Var. Italica.

Methodology

Extraction of Brassica Oleracea Var. Italica

The *Brassica oleracea var. italica* florets were cleaned and cut into small pieces separated from their stems— 150 grams of *Brassica oleracea var. italica* florets were weighted and submerged in three conical flasks containing 450 mL of ethanol (polar), acetone (semipolar), and toluene (non-polar) solvent respectively. The maceration was carried out at room temperature for 48 hours, with continual agitation at 150 rpm in a water bath. After that, the solvent was concentrated in a rotary evaporator for crude extract. The extract obtained with the solvent was weighted, and the percentage of yield was calculated [8].

Phytochemicals Test

Several tests were employed to conduct phytochemical screening:

Test for Tannins: Several drops of FeCl₃ solution were added to 2 mL of aqueous extract. The presence of tannins was revealed by the formation of a yellow or red precipitate [9].

Test for Saponins: A 5 mL aliquot of distilled water was introduced into the solution containing 2 mL of extract in a test tube, and the mixture was vigorously agitated for approximately 15 minutes. If foam remains present for 10 minutes, it signifies the existence of saponins [9].

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Test for Alkaloid: 5 mL of aqueous extract was added to 2 mL of potassium bismuth iodide solution, and the formation of an orange-red precipitate indicates the presence of alkaloids [9].

Test for Terpenoids: Following the Salkowski test, 2 mL of concentrated H_2SO_4 and chloroform were added to 2 mL of extract and shaken. The layer of chloroform was observed to be red, while the layer of acid exhibited greenish-yellow fluorescence [10].

Test for Flavonoid: A total of 2-3 drops of NaOH were introduced into a 2 mL sample of the extract. At first, a vivid yellow hue was observed, but it progressively turned transparent upon the addition of a small amount of diluted HCl, showing the presence of flavonoids [10].

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Test for Phenolic

1 mL of aqueous extract was added into 2 mL of distilled water, followed by 10% of ferric chloride. The formation of a bluish-black color indicated the presence of phenols [10].

Preparation of Percentage Yield Extraction of Broccoli

Prior to maceration, the initial weight of the *Brassica* oleracea var. italica was measured. The remaining broccoli residue from the maceration process was air-dried for 5 days at room temperature. The dried residue was then weighed to determine the percentage yield. The percentage yield of each extraction from

 $Percentage \ yield \ (\%) = \frac{Actual \ yield}{Theoretical \ yield} x \ 100$

Phytochemicals	Ethanol Extract	Acetone Extract	Toluene Extract
Tannins			
Saponins			
Alkaloids			
Terpenoids			
Flavonoids			
Phenolics			

Table 1. Colour change of each phytochemical test.

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Phytochemicals	Ethanol Extract	Acetone Extract	Toluene Extract
Tannins	+	+	-
Saponins	-	-	+
Alkaloids	+	+	-
Terpenoids	+	-	+
Flavonoids	+	+	+
Phenolics	+	+	-

Table 2. The phytochemicals found in each solvent.

(+) =presence; (-) =absence

Table 3. Percentage yield from extraction of *Brassica oleracea var. italica*.

Phytochemicals	Ethanol Extract	Acetone Extract	Toluene Extract
Mass before maceration (g)	150	150	150
Mass after maceration (g)	138.69	141.09	139.68
Mass after drying (g)	22.24	26.91	25.62
Percentage yield (%)	7.54%	5.94%	6.88%

RESULTS AND DISCUSSION

Phytochemical Test

The extracted crude from Brassica oleracea var. italica undergoes identification by several phytochemical tests of active compounds. Table 1 shows the color change of each phytochemical present (+) and absence (-) of three chosen solvents (ethanol, acetone, and toluene). Tannins, saponins, and alkaloids show positive results in the presence of yellow or red precipitate, foam, and orange-red precipitate, respectively. In addition, terpenoids, flavonoids, and phenolic compounds showed positive results with the formation of a radish-brown color and an intense green color. The extraction using ethanol showed that Brassica oleracea var. italica has positive results for tannins, alkaloids, terpenoids, flavonoids, and phenolics. Extraction by acetone, tannins, alkaloids, flavonoids, and phenolic compounds gives positive results.

At the same time, extraction by toluene gives positive results for three active compounds: saponins, terpenoids, and flavonoids. The result showed that *Brassica oleracea var. italica* contains tannins, saponins, alkaloids, terpenoids, flavonoids, and phenolic compounds based on the type of solvents used. The extraction using ethanol gives the highest number of positive results of phytochemical compared to acetone and toluene. This result agreed with Le et al. (2020), which proved that the extract of *Brassica* oleracea var. italica contains flavonoids, alkaloids, terpenoids, and phenolic compounds [4]. Compared with Kusmiati and Agustini, similar findings are the presence of terpenoids and flavonoids only [12]. Due to different results, multiple complementary tests and analytical techniques may be needed to characterize the phytochemical profile of the plant studied comprehensively.

Percentage Yield Extraction of *Brassica oleracea* Var. Italica

The crude extract was observed under the parameter of percentage yield to indicate the efficiency of three chosen solvents on the extraction of Brassica oleracea var. italica. Table 3 and Figure 2 show the percentage yield of Brassica oleracea var. italica crude extract. The percentage yield from ethanol extraction is the highest (7.54%) compared to toluene (6.88%) and acetone (5.94%). This is because ethanol, a polar solvent with both positive and negative polarities, enables it to dissolve a broad spectrum of compounds and thus has the broadest extracting range. Both polar (water-soluble) and non-polar (lipid-soluble) substances can be extracted efficiently [11]. Additionally, ethanol is non-hazardous and straightforward to handle. It is conveniently extracted through techniques like distillation or evaporation, yielding a concentrated extract containing the specific compounds of interest [13].

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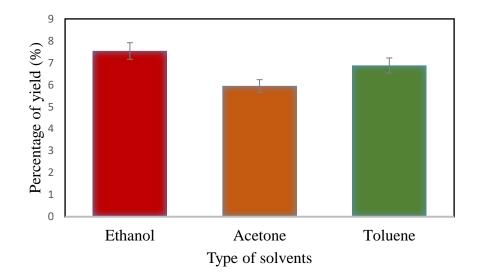


Figure 2. Percentage of Yield.

CONCLUSION

Broccoli, *Brassica oleracea var. italica* a nutrientdense brassica vegetable, is rich in high antioxidant and bioactivity compounds. The extraction of crude extract using ethanol as a polar solvent indicated the highest percentage of yield compared to toluene and acetone. The screening phytochemical test proved that *Brassica oleracea var. italica* contains alkaloids, terpenoids, saponins, tannins, phenolics, and flavonoids.

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