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Short Communication

PHYTOCHEMICAL SCREENING AND COMPARISON OF ANTIOXIDANT ACTIVITY OF WATER AND ETHANOL EXTRACT PROPOLIS FROM MALAYSIA

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ABSTRACT

Objective: In an attempt to compare extraction yield, phytochemical screening, radical scavenging activity, and total phenolic and flavonoid contents of water extract propolis (WEP) and ethanol extract propolis (EEP) from Malaysia.

Methods: Raw propolis was extracted using distilled water and ethanol at various concentrations (10-100%) and the extraction yields were presented in percentage. The phytochemical screening, radical scavenging activity, total phenols using Folin-Ciocalteu method and flavonoid using aluminum chloride method of WEP and EEP were determined.

Results: Malaysian propolis extracted using 70% ethanol produced the highest extraction yield and had significantly higher radical scavenging activity as well as total phenolic and flavonoid contents compared to WEP.

Conclusion: Malaysian propolis extract using 70% ethanol is potential to be developed as a good antioxidant agent.

Keywords: Antioxidant, Extractions, Malaysian, Propolis, Phytochemical screening

Propolis is a dark to yellow or brown lipophilic resinous substance collected by worker bee from different exudates of the plants [1]. It is a natural compound from plants buds and known to contain resins, wax, and balsams. It also contains flavonoids, phenolic acids, terpenoids, prenylated *p*-coumaric acids, lignans, caffeoylquinic acids and mineral elements [2, 3]. Propolis has been reported to have medicinal values such as antioxidant and hypoglycemic effects in rats [4]. Brazilian and Chinese propolis has been shown to have antioxidant activity when assessed using *1,1-diphenyl-2-picrylhydrazyl (DPPH)* radical scavenging activity [5]. Furthermore, gas chromatography-mass spectrometry analysis shows that ethanol extract of Malaysian propolis contains more volatile compounds compared to water extract propolis [6]. Flavonoids and phenolic acids are seen to be useful in preventing free radical-induced organ damage [7]. Phenolic compounds have a good antioxidant potential due to the presence of hydroxyl substituents and their chemical structure, which give them an ability to scavenge free radicals [8].

The chemical composition of propolis varies greatly depending on geographical location, plant vegetation, season, and time of collections, type of bees, and the concentration and nature of the solvents used for the extraction [9]. Total phenols and flavonoids are found to be higher in Brazilian propolis extracted with higher ethanol content compared with the lower ethanol content [10]. Meanwhile, the ethanol extract of Beijing propolis possesses more antioxidant activity as compared to water extract propolis [11]. However, to date, no study has been reported on antioxidant activity of propolis from Malaysia. Therefore, the aim of this study was to compare extraction yield, phytochemical screening, radical scavenging activity, and total phenols and flavonoid contents of water extract propolis (WEP) and ethanol extract propolis (EEP) from Malaysia.

All chemicals and reagents used were of analytical grade, purchased from Sigma-Aldrich, Merck KGaA (Darmstadt, Germany). Raw Malaysian propolis was purchased from local bee farm (Min House Camp, Kota Bharu, Kelantan, Malaysia), collected during the period of the dry season (March to June). WEP and EEP at various concentrations (10, 30, 50, 70, 90 or 100%) were prepared as described previously [6, 12, 13]. The extraction yield was calculated as a percentage of the initial amount of raw propolis used. The EEP with the highest extraction yield was used for further analysis. Phytochemical screening was performed using standard methods and evaluated for the presence or absence of alkaloids, phenols [14], flavonoids [15], tannins, cardiac glycosides, xanthoproteins, terpenoids and resins [16]. The radical scavenging activity (RSA) was done using DPPH assay [17]. Total phenolic and flavonoid contents were carried out using Folin-Ciocalteu method with gallic acid as a standard [18] and aluminum chloride colorimetric method with quercetin as a standard [19], respectively. Data were presented as mean±standard deviation (SD) and analyzed using Graphpad Instat Exe version 3.1 (GraphPad software, Inc. San Diego California, USA). Independent *t*-test was used to compare the differences between WEP and EEP results. The P value of<0.05 was considered as statistically significant.

This is the first study on extraction yield, phytochemical screening and antioxidant properties of Malaysian propolis extracts. The seventy percent ethanol produced the highest percentage of extraction yield (12.12%), followed by 100% and 90% ethanol (11.94% and 11.38%, respectively) when compared to distilled water (4.59%). Meanwhile, 10% ethanol produced the lowest percentage of extraction yield (1.69%), followed by 50% and 30% ethanol (2.16% and 2.25%, respectively). This result is consistent with Brazilian green propolis in which 70% ethanol produces more extraction yield compared to methanol [20]. This finding is also in line with other studies whereby Brazilian propolis extracted with 70% ethanol produces more extraction yield compared with 80% ethanol and water [21] while Indian propolis extracted with 100% ethanol produces more extraction yield compared with methanol and water [22]. The present finding is supported by a study using gas chromatography-mass spectrometry analysis which shows that Malaysian EEP produces more volatile phytochemical compounds as compared to WEP [6]. These differences may probably depend on the compounds solubility at different concentration of the solvents. It has also been suggested that the difference may be because of the hydroxyl group that turns water to a poor solvent for many organic compounds. Hence, the polarity may be the main reason for the differences in the extraction yield [23].

A phytochemical screening test is useful in the detection of the bioactive compounds and subsequently, may lead to the drug discovery and development. In this study, all the nine compounds were present in both WEP and EEP (table 1). The color intensity was

more in EEP compared to WEP for cardiac glycosides, saponins, flavonoids and phenols which might suggest the higher concentration of these compounds in EEP. This is in accordance with a study in which Indian propolis contains compounds such as alkaloids, tannins, saponins, terpenoids, flavonoids, phlobatanins, reducing sugars and anthraquinones which are more in ethanol extract compared with methanol and water extracts [22]. European propolis has been reported to contain phenolic acids, flavonoids, terpenoids, cinnamic acids and prenylated derivatives of *p*-coumaric acid [24]. However, it is reported that chemical composition of propolis may depend on factors such as plant vegetation, season, and time of harvesting [9].

Table 1: Phytochemical screening results of Malaysian propolis extracts

Compounds	WEP	EEP
Alkaloids	+	+
Cardiac glycosides	+	++
Tannins	+	+
Saponins	+	++
Flavonoids	+	++
Phenols	+	++
Xanthoproteins	+	+
Terpenoids	+	+
Resins	+	+

WEP: water extract propolis, EEP: ethanol extracts propolis. A positive sign (+) indicates the presence of compound while double positive sign (++) indicates the presence of a compound with higher intensity of color change (n=3).

In this study, both WEP and EEP had antioxidant property, and EEP had significantly higher RSA compared to WEP (Table. 2). A similar study showed a higher percentage of RSA (92%) in ethanol extract of Brazilian propolis [13]. These findings are in accordance with the previous study on South Portugal propolis showing that propolis is an important source of antioxidant compounds [25]. The higher RSA of EEP in the present study might be due to the presence of higher concentration of phytochemical compounds that possess antioxidant property such as cardiac glycosides, flavonoids, and phenols as shown by our findings (table 1).

Table 2: Antioxidants property of Malaysian propolis extracts

Antioxidant property	WEP	EEP
Radical scavenging activity (%)	70.69±0.49	82.44±0.05*
Total phenolic content (mg gallic acid Eq per g)	119.00±7.00	646.67±30.44*
Total flavonoid content (mg quercetin Eq per g)	87.58±5.20	209.83±1.42*

Data are mean±standard deviation (n=3). WEP: water extract propolis, EEP: ethanol extracts propolis. *P<0.05 compared to WEP (Independent *t*-test).

Total phenolic content in EEP was significantly higher compared to WEP (table. 2), which is similar with our finding (table 1). This is also in line with other studies showing that Beijing and Algarve propolis have more phenolic compounds when extracted with ethanol as compared to water [11, 25]. This difference may be due to the nature of the solvents used and the solubility of the phenols in the solvent [26]. Furthermore, it has been reported that total phenolic content in Beijing and Chinese propolis are ranging from 6.68 to 164.2 [11] and from 42.9 to 304 mg gallic acid Eq per g [5], respectively, which are lower than the total phenolic content of Malaysian EEP in the present study.

Total flavonoids content of EEP was significantly higher than WEP (Table. 2). The higher phenolic and flavonoid contents in Malaysian EEP may be responsible for the higher RAS of EEP in the present study. Our finding on total flavonoid content in Malaysian EEP was slightly lower than Chinese and Beijing EEP, which has total flavonoid content of 232.1±3.2 mg quercetin Eq per g [27] and 282.83 mg rutin Eq per g [11], respectively. On the other hand, Cameroon propolis has been reported to have total flavonoid content of 152±6 mg quercetin Eq per g [28], which is lower than Malaysian EEP of the present study. These differences may further support the suggestion that, the chemical composition of propolis may depend on geographical location apart from plant vegetation and season of harvesting [9].

The present study conducted on Malaysian propolis had revealed the extraction yield, phytochemical contents and *in vitro* antioxidant properties which appeared more with 70% ethanol extraction, suggesting its potential use as a good antioxidant agent. However, further studies are suggested to evaluate antioxidant and biological activities of this extract in an experimental animal model.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests

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