

Spectrophotometric Determination of Total Phenol and Flavanoid Content in Manjakani (*Quercus Infectoria*) Extracts

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ABSTRACT: Previous research showed that manjakani (*Quercus infectoria*) has the antioxidant property. However, the entity involved in antioxidant properties remained unknown. Manjakani might have some amount of phenolic and flavonoid compounds, which are often correlated with the antioxidant property and therefore this study aimed to investigate the total phenolic and flavonoid content in manjakani extracts (MKE). Manjakani samples used in this study were chosen by their colour types (i.e. mixed, gray and black coloured) and were extracted with water and absolute ethanol respectively using soxhlet extraction. The total phenolic compounds content was determined using Folin-Ciocalteu assay while the total flavonoid compounds was determined by aluminum chloride colorimetric assay. Both assays were analysed by UV-Vis Spectrophotometer. By comparing with the phenol and catechin standards, the concentration of phenolic and flavonoid content in manjakani extracts were determined using regression analysis. The results show that manjakani extract contains substantial amount of phenolic content (144.5 mg – 177.5 mg PEPC/ g MKE) but negligible or absent of flavonoid content.

Keywords: flavonoid compounds, phenolic compounds, spectrophotometer, *Quercus infectoria*

Introduction

Quercus infectoria Olivier (Fagaceae) is a small tree native to Greece, Asia Minor and Iran (Basri and Fan, 2005) commonly known as oak tree. The tree produces galls that emerge on its shoots or leaves as a consequence of invasion of gall wasp, *Cynnis gallae tinctoria* (Samuelsson, 1992) or *Adleria gallae-tinctoria*. The metamorphosis process taking place on the hatched insect's eggs produces enzymes that stimulate food supply on the leaf cells, and a hard round "ball" is formed there. This ball-like structure is known as Gall (Samuelsson, 1999). In Malaysia, the galls of *Q. infectoria* are called manjakani (Muhammad and Mustafa, 1994).

It was reported that manjakani contains large amount of tannins, gallic acid, syringic acid, ellagic acid, β -sitosterol, amentoflavone, hexamethyl ether, isocryptomerin, methyl betulate, methyl oleanate, hexagalloylglucose and others (Dar et al., 1976; Ikram & Nowshad, 1977; Hwang et al., 2000). The main constituents found in the galls of *Q. infectoria* are tannin (50 - 70%) and small amount of free gallic acid and ellagic acid (Ikram and Nowshad, 1977; Evans, 1996; Wiart and Kumar, 2001).

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In Asian countries, manjakani has been used for centuries as traditional medicine for treating inflammatory diseases (Galla, 1911; Anonymous, 1995; Kaur et al., 2003). Using the hot water extract of manjakani as a mouth antiseptic can control the inflammation of tonsils, while the direct application of it onto the skin cures swelling or inflammation (Chopra et al., 1956). Hemorrhoids caused by inflammation of the skin can also be treated by applying the powdered manjakani in the form of ointment to the skin (Anonymous, 1995). In Malaysia, manjakani is very popular among women for restoring the elasticity of uterine wall after childbirth when used in combination with other herbs as drinking remedy (Muhammad and Mustafa, 1994).

Phenols are found naturally in plant kingdom such as in western poison oak to discourage herbivory. It is also found in decaying dead organic matter such as rotting vegetables and in coal (Harrison, 1998). It has antiseptic properties and was first used by Sir Joseph Lister (1827-1912) in the technique of antiseptic surgery. Phenolic compound from various solid and liquid foods also possesses antioxidant activity (Gunduc and El, 2003). The phenolic compound concentration in certain food and drinks vary, depending on the variety, degree of maturity, method of processing and climatic factors (Shahidi and Naczk, 1995).

Flavonoids are widely found in plants and possess many functions including giving yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects. They are also found in fruits, vegetables, and certain beverages that have diverse

dietary antioxidants including vitamins C and E (Buhler and Miranda, 2000). Flavonoids are polyphenolic compounds and have many potential in health application including antiviral, anti-allergic, antiplatelet, anti-inflammatory, anti-tumor and antioxidant activities (Buhler and Miranda, 2000).

In this study, we aimed to determine the total phenolic and flavonoid content in various manjakani extracts (MKE).

Materials and Methods

Samples

Manjakani or gall of *Quercus infectoria* was purchased from three different local shops in Kota Bharu, Kelantan, Malaysia. The samples were extracted using soxhlet extractor (Ross, Germany) and analysed for flavanoid and phenolic content using the UV-Vis Spectrophotometer (Cary 100 Bio, Varian).

Procedure

For the determination of total phenolic content in manjakani extracts, aliquots of extract or standard solution of phenol was added to Folin-Ciocalteu reagent (FCR), and sodium carbonate. Spectrum (200 nm – 800 nm) of the solution and the absorbance at 765 nm (Marinova *et al.*, 2005) against a reagent

blank was then recorded using a UV-Vis Spectrophotometer (Cary 100 Bio, Varian).

To determine the total flavonoid content in manjakani extracts, sodium nitrite was added to the aliquots of extract or standard solution of catechin. Aluminum trichloride was added to the solution 5 mins later and at the sixth min, sodium hydroxide was added. Spectrum (200 nm – 800 nm) of the solution and the absorbance at 510 nm (Marinova *et al.*, 2005) against a reagent blank was then taken.

Result and Discussions

Spectroscopic Study with the Phenol Standard and Evaluation of Total Phenol Content in the Prepared Manjakani Extracts

Five replicates of the spectral measurements were done with each of the extracts of manjakani in the presence of folin-ciocalteu and Na_2CO_3 . (FIG. 1 & 2). Total content of phenol corresponding to the absorbance at 765 nm of the respective manjakani extract was calculated using the regression equation ($y = 0.523x - 0.475$, $R^2 = 0.971$) obtained earlier from the standard phenol calibration curve (figure not shown). TABLE 1 shows the absorbance at 765 nm for the manjakani extracts and the calculated amount of total phenol content in the respective manjakani extract.

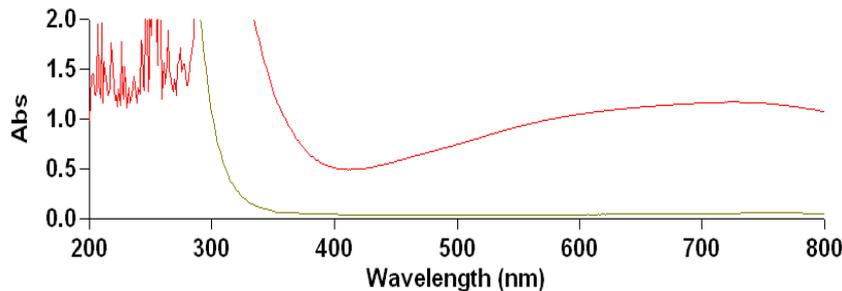


FIG. 1- Spectrum of manjakani (water extract) in the presence of FCR and Na_2CO_3 in water

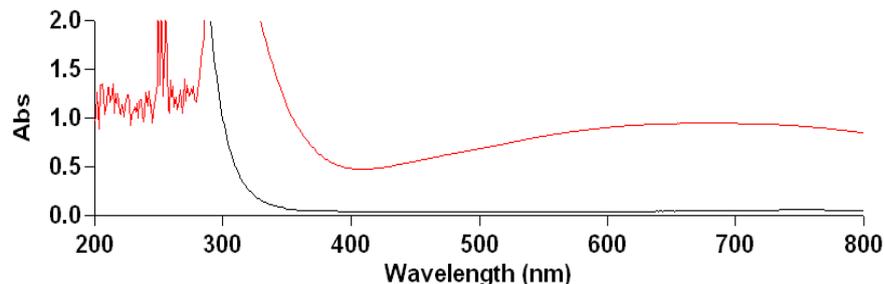


FIG. 2- Spectrum of black manjakani (ethanol extract) in the presence of FCR and Na_2CO_3 in water

TABLE 1- Absorbance at 765 nm for the manjakani extracts and the corresponding total phenol content in the respective manjakani extracts

Extracts	Type of manjakani used								
	Mixed			Black			Gray		
	A _{765 nm}	Conc (ppm)	*Amount (mg PEPC/g MKE)	A _{765 nm}	Conc (ppm)	*Amount (mg PEPC/g MKE)	A _{765 nm}	Conc (ppm)	*Amount (mg PEPC/g MKE)
Water Extract	1.26	3.32	166	1.07	2.95	147.5	1.21	3.22	161
Ethanol Extract	1.23	3.26	163	1.38	3.55	177.5	1.16	3.13	156.5

* MKE = Manjakani Extract and PEPC = Phenol Equivalent Phenolic Compound

It is important to note here that each of the manjakani samples contained substantial amount of phenolic compound (**TABLE 1**). Moreover, regardless of the type of manjakani as well as the extracting solvent used, there was not much difference in the content of total phenol in the manjakani extracts (**TABLE 1**). On the other hand, as the polarity of ethanol and water is comparable, no big difference was observed in the total phenol content in the manjakani extracts extracted by the two solvents.

Spectroscopic Study with the Flavonoid and Evaluation of Total Flavonoid Content in the Prepared Manjakani Extracts

Standard catechin in the presence of NaNO₂, AlCl₃ and NaOH showed an absorbance at about 510 nm (**FIG. 3**), indicating that catechin reacted with the reagents (NaNO₂, AlCl₃ and NaOH) and was then changed into a product that absorbs at 510 nm

(Marinova et al., 2005). Thus it was evident that if a manjakani extract containing flavonoid compound which react with the reagents mixture (NaNO₂, AlCl₃ and NaOH), this will appear at 510 nm.

Five replicates of spectral measurements were taken with each of the extracts of manjakani in the presence of NaNO₂, AlCl₃ and NaOH. Representative spectra of manjakani extracts are shown in the **FIG. 4** and **FIG. 5**.

The total content of flavonoid corresponding to the absorbance at 510 nm of the manjakani extract was calculated utilizing the regression equation ($y = 0.655x - 0.745, R^2 = 0.971$) obtained earlier from the standard catechin calibration curve (figure not shown). **TABLE 2** shows the absorbance at 510 nm for the manjakani extracts and the calculated amount of the total flavonoid content in the respective manjakani extracts.

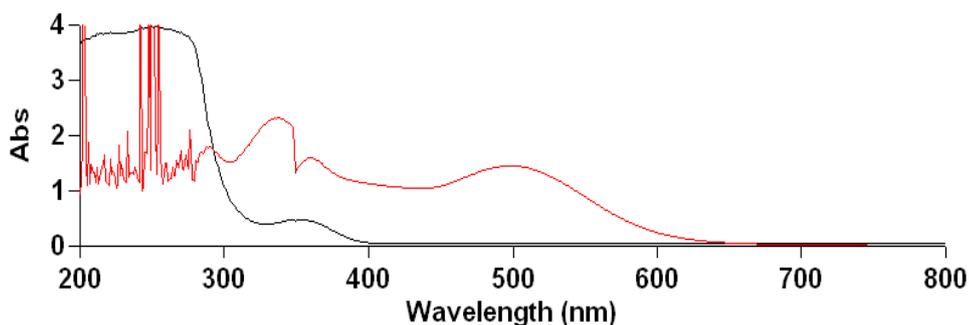


FIG. 3- Spectrum of catechin in the presence of NaNO₂, AlCl₃ and NaOH (Red spectrum: 50 ppm Catechin (1 mL), NaNO₂ (0.3 mL), AlCl₃ (0.3 mL), NaOH (2.0 mL), dH₂O (6.4 mL)) and the blank (Black spectrum: NaNO₂ (0.3 mL), AlCl₃ (0.3 mL), NaOH (2.0 mL), dH₂O (7.4 mL))

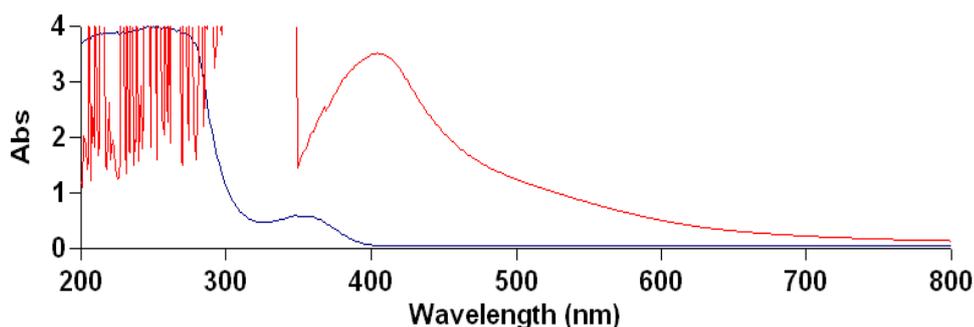


FIG. 4- Spectrum of ethanolic extract of manjakani (gray) in the presence of NaNO₂, AlCl₃ and NaOH in water

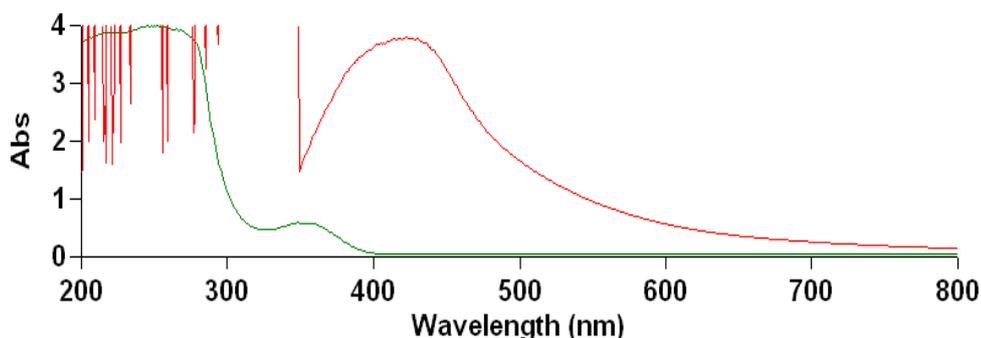


FIG. 5- Spectrum of aqueous extract of manjakani (black) in the presence of NaNO₂, AlCl₃ and NaOH in water

TABLE 2- Absorbance at 510 nm and the corresponding concentration and amount of flavonoid in the manjakani extracts.

Extracts	Type of Manjakani used								
	Mixed			Black			Gray		
	Abs ₅₁₀ nm	Conc (ppm)	*Amount (mg CEF/g MKE)	Abs ₅₁₀ nm	Conc (ppm)	*Amount (mg CEF/g MKE)	Abs ₅₁₀ nm	Conc (ppm)	*Amount (mg CEF/g MKE)
Water Extract	1.37	3.23	3.23	1.53	3.47	3.47	1.74	3.79	3.79
Ethanol Extract	1.01	2.68	2.68	1.63	3.63	3.63	1.50	3.43	3.43

*MKE = Manjakani Extract and CEF = Catechin Equivalent Flavonoid

It is important to note here that the absorption spectrum of the manjakani extract (FIG. 4 and FIG. 5) in the presence of NaNO₂, AlCl₃ and NaOH is quite different from that of the standard flavonoid (FIG. 4) in the presence of the same reagents. Thus, it is difficult to conclude that manjakani extracts contain flavonoid compounds. However, the total flavonoid content (TABLE 2) can be based on the observed absorbance at 510 nm (TABLE 2). The calculated amount, based on the absorbance at 510 nm without observing the spectrum and its features, could be mistakenly considered as total the flavonoid content in the respective manjakani extract. The low amount of flavonoid content (3.63 – 2.68 mg CEF/g MKE) compared to that of high content of phenol (177.5 – 147.5 mg PEPC/g MKE) indirectly support

our claim earlier that the manjakani extracts hardly contains flavonoid compounds. Further experiments are necessary to conclude the calculated amount as the total flavonoid content in the respective manjakani extract.

Conclusion

Our results indicated that manjakani extracts tested contained considerable amount of phenol equivalent total phenolic compounds while no catechin equivalent to the total flavonoid content was quantitated. The amount of phenol equivalent to the phenolic compounds in the prepared manjakani extracts show no observable difference using either

water or methanol as solvent. Decreasing order of phenol equivalent to the total phenolic compound content (mg/g of dried extract) in the water extracts of manjakani was: mixed group (166 mg PEPC/g MKE) > gray group (161 mg PEPC/g MKE) > black group (147.5 mg PEPC/g MKE), and in the ethanolic extracts of manjakani was: black group (177.5 mg PEPC/g MKE) > mixed group (163 mg PEPC/g MKE) > gray group (156.5 mg PEPC/g MKE). Reported antioxidant activity of manjakani extracts could be due to the presence of phenolic compounds in them.

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