Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 5 (2): 189-195, 2011
ISSN 1819-3455 / DOI: 10.3923/rjmp.2011.189.195

Influence of Drying Method on Flavonoid Content of *Cosmos caudatus* (Kunth) Leaves

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ABSTRACT

The aim of this study was to search for the optimal method to prepare crude drug with the highest flavonoid content from fresh *Cosmos caudatus* leaves. Flavonoid contents of *Cosmos caudatus* leaves were studied in relation to the processes involved in food and herbal medicine preparation. Quercitrin and rutin were identified as the major flavonoid component of the leaves. The flavonoid content substantially increased during boiling in water. This increase was not caused by the enhancement of extractability but more likely to be due to the endogenous biotransformation of precursor or intermediates into flavonoids. However, heating of fresh leaves in an air oven at elevated temperature from 30 to 100°C decreased the total flavonoid content except at 40°C by which the total flavonoid content increased approximately by 30%. Pre-heating of leaves at 60°C substantially reduced flavonoid content to only 30% compared to control and further drying at 60°C left the flavonoid to only 12% compared to control. The experiments suggested that drying temperature affects flavonoid content of *Cosmos* leaves and the highest content might be obtained when the leaves is prepared by drying at 40°C.

Key words: Quercitrin, rutin, HPLC, *Cosmos caudatus*, phenolic, Asteraceae

INTRODUCTION

Cosmos caudatus (Kunth), Asteraceae is one of Indonesian vegetable plants that is usually consumed freshly as salad or cooked by boiling with other spices. In East Java, it is also used in traditional medicine to reduce blood pressure. It is also used to improve blood circulation, as carminative, apetizer and insect repellent (Shui *et al*., 2005). Previous experiment showed that *Cosmos* leaves contained highest flavonoid levels compared to other 20 Indonesian vegetable plants (unpublished data). Flavonoid has been reported to have strong antioxidant and radical scavenging activities, which is expected to have beneficial effects in the prevention of degenerative diseases (Ebrahimzadeh *et al*., 2010; Makris and Rossiter, 2002; Weisburger *et al*., 2001; Tabak *et al*., 2001). The chemical components of *Cosmos* leaves previously reported include quersetin-3-O-**β**-arabinofuranoside, quercetin-3-O-**α**-rhamnosida, quersetin-3-O-**β**-glukosida and quercetin, proantocyanidin, chlorogenic acid, neo-chlorogenic and cryptochlorogenic acid (Shui *et al*., 2005).

We have previously found that the flavonoid content of *Cosmos* leaves increased substantially upon boiling (unpublished data). Similar effect of boiling was reported by Moriyama and Nagai

(2001) demonstrating the increase of kaempferol-3-O-gentibiose in *Cassia alata* leaves after heating at 85°C for 40 min. Although the flavonoid content decreased upon prolonged heating, compared to non-heated leaves the content was relatively higher in leaves exposed to 180 min heating. Rutin, a pure flavonoid glycoside, was stable upon boiling for 300 min (Buchner *et al*., 2006) or heated at 100°C for 3 h, but degraded when heated at 180°C (Murakami *et al*., 2004). It was surprising that roasting was also reported to substantially increase total phenol and procyanidin level in peel almond (Garrido *et al*., 2008). On the other hand, flavonoid of mulberry, Zingiberaceae leaves decreased when heated at high temperature under the sun, air oven or microwave (Katsube *et al*., 2009; Chan *et al*., 2009). These findings demonstrated that different type of flavonoid showed different stability upon heating. Thus, it was reported that luteolin of willow (*Salix purpurea*) was stable upon heating but not apigenin (Julkunen-Tiitto and Sorsa, 2001). Both compounds are flavone type and they are differed by hydroxylation pattern at ring B.

The aim of this study was to search for the optimal method to prepare crude drug with the highest flavonoid content from fresh *Cosmos caudatus* leaves. In particular, we investigated the effects of pre-treatment through heating prior to drying and drying with various conditions.

MATERIALS AND METHODS

Plant materials: *Cosmos caudatus* was harvested (in January 2009) and the leaves were separated for the experiments. The plant was authenticated at the Herbarium Bandungense, The School of Life Science and Technology, Bandung Institute of Technology.

Pre-drying and drying: Heating of *Cosmos* leaves was conducted in an air oven by keeping freshly harvested leaves in a closed Petri dish to minimize evaporation. Drying of pre-heated leaves was also conducted in the air oven.

Flavonoid extraction: One gram of dried leaves were powdered and macerated with 10 mL MeOH-H_2O (7:3) for 24 h, followed by percolation and re-percolation with 4×3 mL of solvent. The combined extracts were then adjusted to 25 mL with the same solvent. Further dilutions were made as necessary to obtain better separation of flavonoid components on High Pressure Liquid Chromatography (HPLC). Prior to injection, the extracts were filtered through 0.50 μm nylon membrane.

Determination of flavonoid: Flavonoid content in *Cosmos* extracts was determined by HPLC (HP 1100 series) and calculated as rutin content, which was used as reference in quantification of flavonoid. Chromatography was performed by injecting 20 μL extract into the HPLC apparatus, which was supported with ODS (Hypersil) column having 5 μm particle size, 100 mm length, 4 mm id., set at temperature of 35°C, flow rate 0.8 mL min^{-1} and detection at 350 nm (DAD). Gradient elution was performed with HOAC 5% (A) and MeOH (B) for 15 min in the following order: 0-5 min (30% B), 6-10 min (50% B), 11-14 min (100% B) and 15 min (30% B). Flavonoid peaks were identified based on their UV spectra and the amount was calculated by summing the area of flavonoid peaks and compared with the area of rutin peak that was used as reference.

RESULTS AND DISCUSSION

Identification of flavonoid content: Separation of *Cosmos* extract on HPLC (Fig. 1) gave two major flavonoid peaks shown by their UV spectra with two peaks at 350 and 270 nm, respectively. This spectrum indicated that the separated flavonoids were flavon or conjugated flavonol. Further

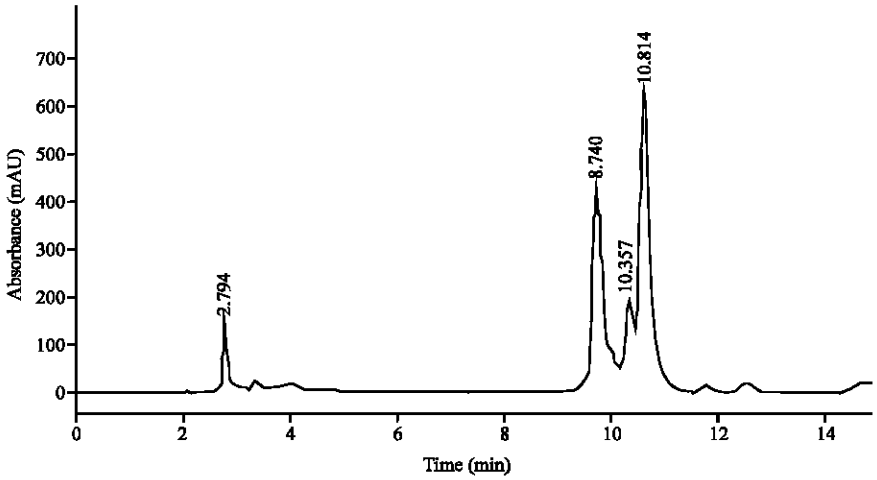

Fig. 1: Chromatogram of flavonoid extract from *Cosmos caudatus* leaves

Table 1: Flavonoid content in fresh, boiled in water and boiled in 70% MeOH

	Flavonoid (mg g^{-1})		
Extraction method	Aliquot	Marc	Total
Direct extraction with 70% MeOH	-	-	14.61±0.37
Boiling in water	22.75±1.19	3.48±0.15	26.23±1.20*
Boiling in 70% MeOH	10.64±0.36	1.27±0.02	11.91±0.38*

*$p<0.05$, compared to direct extraction from fresh tissue

identification was conducted by co-chromatography using rutin and quercitrin as markers. The major peak eluted at 10.814 min was then identified as quercitrin while peak at 9.740 min as rutin. These results were consistent with those reported by Shui *et al.* (2005) that identified flavonoid of *Cosmos* leaves using LC-MS. Peak eluted at 2.794 based on its UV spectrum was not considered as flavonoid and might be another type of phenolic compound.

Boiling and flavonoid contents of *Cosmos* leaves: Our previous experiment (unpublished data) showed that boiling substantially increased flavonoid content of *Cosmos* leaves. Such increase may be due to the increase of flavonoid extractability or *de novo* formation during boiling process. Experiment by boiling of *Cosmos* leaves in water and in 70% methanol was performed to test the above possibility. In this experiment, direct extraction was performed by grinding fresh tissue with 70% MeOH followed by maceration for 24 h and rinsing the marc three times with the same solvent in order to exhaustively extract the flavonoids present in the leaves. This extraction procedure was carried out as recommended by Xu *et al.* (2005). Extraction through boiling was conducted by boiling 5 g unground leaves in 50 mL of solvent for 15 min followed by analysis of flavonoids from the aliquot and the marc. Flavonoid from the marc was analyzed with the same procedure used to analyze that extracted from fresh tissue.

Results presented in Table 1 showed that boiling in MeOH 70% decreased flavonoid content of the leaves, while boiling in water substantially increased flavonoid content at almost twice as high. It is very likely that such increase was not due to the enhancement of flavonoid extractability but *de novo* formation of flavonoid during the heating of the tissue in the aqueous environment. In

Table 2: Flavonoid content in *Cosmos caudatus* leaves after pre-heating at various temperature followed by drying at 60°C

	Flavonoid content (mg g^{-1})	
Pre-heating temperature (°C)	Before drying	After drying at 60°C
Control	14.61±0.37	10.68±0.57
40	16.13±0.58	15.11±0.58
60	4.43±0.61	1.76±0.11
80	5.54±0.18	2.81±0.12
100	8.73±0.11	7.38±0.42

All drying treatment significantly decreased flavonoid content ($p<0.05$)

young tissue, common precursor for the synthesis of flavonoid together with other phenylpropanoid are available and upon heating, the activity of enzymes involved in flavonoid synthesis may increase resulting in rapid conversion of precursor to flavonoid. In addition, boiling temperature will inactivate polyphenoloxidase (Yamaguchi *et al*., 2003) that lead to the accumulation of flavonoid in the tissue. Further experiment is needed to support the proposed mechanism. Heating has been shown to influences phenolic content in plant materials. It has been demonstrated that roasting increased total phenol and procyanidin in peel almond (Garrido *et al*., 2008). Other authors demonstrated the decrease in total phenolic content of *Alpinia zerumbet*, *Etlingera elatior*, *Curcuma longa* and *Kaempferia galanga* leaves upon drying on air, microwave oven and air oven at 50°C, but the phenolic content was preserved if they freeze dried (Chan *et al*., 2009).

Pre-heating and flavonoid content of *Cosmos* leaves: In this experiment, fresh leaves were heated at various temperatures ranging from 40 to 100°C for the period of 30 min. During pre-treatment, leaves were kept in Petri dishes to avoid evaporation and drying. After pre-heating, leaves were dried at 60°C. Fresh leaves directly dried at 60°C was used as control. Flavonoid contents were measured before and after drying process. The objective of this experiment was to investigate the possibility to increase flavonoid content of the crude drug through pre-heating treatment before drying process. The experiment results showed that pre-heating decreased flavonoid content except at 40°C (Table 2). Pre-heating at 40°C increased flavonoid content by 10.4% compared to control. Increasing pre-heating temperature from 40 to 60°C decreased the flavonoid content. However, pre-heating at temperatures higher than 60°C increased the flavonoid content but not as high as the result after 40°C. The lowest level of flavonoid content was observed in dried leaves that was pre-heated at 60°C. This might be due to the increase of activity of flavonoid degrading enzyme and further increase in pre-heating temperature decreased the enzyme activity, resulted in increased flavonoid content. Our results were in line with the results of Prabha and Patwardhan (1982) showing that polyphenoloxidase had optimum activity at pH 5.4 and temperature of 50°C and the activity was still preserved at 60°C. However, at 70°C or above significant lost of activity was observed. Results presented in Table 2 showed that flavonoid level was lowest at 60°C and increase at 80 and 100°C. These results are consistent with the characteristic of polyphenoloxidase related to temperature as reported by Prabha and Patwardhan (1982).

After drying process, flavonoid content resulted form most of the treatments decreased compared to that before drying. The smallest decrease was observed after pre-heated leaves at 40°C. Although, the decrease was significant at $p<0.05$, the figure was only 6.32%. Substantial

Table 3: Flavonoid content of *Cosmos* leaves after drying at 40 and 60°C

Drying temperature (°C)	Flavonoid content (mg g^{-1})
Fresh leaves	14.61±0.37
40	19.12±0.80*
60	11.39±1.42*

*p<0.05 compared to fresh leaves

decrease was observed in control, after pre-heated at 60 and 80°C, reaching 26.90, 60.27 and 49.28%, respectively. While pre-heated leaves at 100°C only resulted in 15.46% decrease. Drying apricots at 55 and 75°C also resulted in the decrease in catechin, epicatechin, rutin and quercetine-3-glucoside (Madrau *et al*., 2009). Total phenolic content of a number plant materials also declined when they were dried at 55°C (Chan *et al*., 2009). The decreased in flavonoid and polyphenol when dried at temperature around 50°C might be due to the activity of polyphenoloxidase (Prabha and Patwardhan, 1982) and this explanation has also been proposed by Mrkic *et al*. (2006), Buchner *et al*. (2006), Rohn *et al*. (2007) and Madrau *et al*. (2009). The possibility of flavonoid decrease due to Mailard reaction (Piga *et al*., 2003; Durmaz and Alpaslan, 2007) is unlikely to take place at 60°C, since pre-heating at 80 and 100°C increased the flavonoid level.

Our present results suggested that pre-heating and drying at elevated temperatures will not increase flavonoid content of *Cosmos caudatus* leaves. However, the data did not explain the increase in flavonoid content of *Cosmos caudatus* leaves after boiling in water as shown in Table 1. It was expected that pre-treatment at elevated temperature will increase flavonoid content similar to that of boiling in water that enhanced flavonoid content to 79.53%.

It is interesting to observe the phenomenon after heating at 60°C, which demonstrated the lowest level of flavonoid content after pre-heating. Before the treatment, flavonoid was shown to be present, but after the treatment the level decreased. These results suggest that flavonoid of *Cosmos* leaves undergoes transformation into another metabolites that might be due to the increase in the flavonoid degrading enzyme activity such as polyphenoloxidase (Prabha and Patwardhan, 1982). Although, the level of flavonoid significantly decreased when fresh leaves was dried at 60°C, the flavonoid content was still retained to a level of 73.11%. During drying, water in the leaves evaporated might lead to a decrease in the activity of flavonoid degrading enzyme that needs aqueous media to exert its catalytic activity. These observations suggest that heat treatment at 60°C such as pre-heating or drying is not recommended for the preparation of crude drug from *Cosmos* leaves if the level of flavonoid is to be maintained.

Drying of *Cosmos caudatus* leaves: Most crude drugs are kept dry to facilitate long term storage and more efficient processing, such as the need for smaller amount of solvent used for extraction. Since the pre-heating of *Cosmos* leaves did not produce substantial increase in flavonoid content and a slight increase was found in pre-heating at moderate temperature (40°C), pre-heating process is not worthwhile to be included in crude drug preparation from *Cosmos* leaves. As shown in Table 3, it is interesting to note that drying at 60°C decreased flavonoid content by 22%, while at 40°C increased the content by 30.87%. The findings further necessitate considering these two temperatures for the efficiency of flavonoid isolation from *Cosmos caudatus* leaves.

CONCLUSION

Pre-heating of *Cosmos* leaves at elevated temperature did not increase flavonoid content except at 40°C and pre-heating at 60°C leads to highest decrease in flavonoid content. Based on the experimental results, crude drug of *Cosmos* leaves was best prepared by drying at 40°C.

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