Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (3): 274-280, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.274.280

Influence of Storage on the Volatile Oil Content of *Curcuma* Rhizome

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ABSTRACT

Curcuma xanthorrhiza is one the most important of Indonesian crude drug. Its pharmacological activities are usually associated with curcuminoid and the essential oil content. This study was aimed at studying the influence of the crude drug preparation on the content and the composition of volatile oil *Curcuma* rhizome. The bulky rhizomes were stored at room temperatures and their volatile oil contents measured. The composition of the oil was analyzed by GC-MS. The rhizomes were sliced, dried, comminuted and their oil content measured. Storage of bulky rhizome at room temperature continuously decreased the yield of volatile oil from bulky fresh rhizome. Storage did not change the number and the identity of the oil component: However, it altered their composition. Germacron, xanthorrhizol and α-curcumene increased while dipi-α-cedren decreased during storage. Upon slicing, drying under the sun, drying in an air oven, grinding and storage of the dried *Curcuma* rhizome, most of the volatile oil was still retained in the crude drug. Storage of bulky Curcuma rhizome continuously decreased its volatile oil content and the lost of oil reach 57% after 12 weeks. It is suggested that to assure the highest level of volatile oil content in the preparation of *Curcuma* crude drug, the rhizome should be immediately sliced and dried after harvest.

Key words: *Curcuma* rhizome, germacron, xanthorrhizol, α-curcumene and dipi-α-cedren

INTRODUCTION

The rhizome of *Curcuma xanthorrhiza* (Roxb), Zingiberaceae is intensively used in Indonesian traditional medicine. It is widely used for its hepatoprotective and anti inflammation activities (Li *et al*., 1995). In Holland, the extract combined with *Curcuma* powder has been prescribed by physician to stimulate the production of bile acid (Valema, 1995). Two major groups of compounds that have been known to be responsible for the pharmacological activities of *Curcuma* are curcuminoid and volatile oil (Syu *et al*., 1998). Recently, *Curcuma* extract have been studied for its use as cholesterol reducing agent (Wientarsih *et al*., 2002), antihyperlipidemic, antihyperglicemic agents (Nwozo *et al*., 2009; Sukandar *et al*., 2010), hepatoprotective agent (Li *et al*., 1995; Somchit *et al*., 2005; Prakash *et al*., 2008) and antimicrobial agent (Gul *et al*., 2004; Sunilson *et al*., 2009; Neogi *et al*., 2007; Bele *et al*., 2009; Butkhup and Samappito, 2011). Curcumin and the methanol extract of *Curcuma* have chemoprotective properties due to the capacity to arrest cell cycle and induce apoptosis (Reuter *et al*., 2008; Park *et al*., 2008). It was reported that curcumin inhibit the formation of proteosome in colon cancer cell line

(Milacic *et al*., 2008). The volatile oil and the hexane extract of *Curcuma* reduced triglyceride by lowering fatty acid syntheses (Yasni *et al*., 1994).

Storage of bulky crude drug is one of the important factors to be carefully considered. Fresh material that is not directly processed may undergo further metabolic process due to the enzymes activities which are still active in the tissue. Further, complication may arise from the contaminating microorganism that may rapidly grow in crude drug with high humidity (Prabha and Patwardhan, 1982; Wang *et al*., 2007). Therefore, some medicinal plants from rhizome have to be processed immediately to avoid deterioration. Fresh ginger for example, can only be stored for two weeks. Storage longer than two weeks will reduce the volatile oil content and also alter the composition of the volatile oil components (Sukrasno *et al*., 2000). Bulky *Curcuma* rhizome, however, can be stored for 12 weeks without significant reduction of the content and the composition of curcuminoid (Sukrasno *et al*., 2003). In daily practice, *Curcuma* rhizome is commonly processed through slicing followed by drying under sun light. This treatment may affect the essential oil content that constitutes as one of the components responsible for the pharmacological activities. The objective of this research was to study the effect of storage and related processes on essential content.

MATERIALS AND METHODS

Change of fresh weight upon storage: *Curcuma xanthorrhiza* Roxb was collected from Sumedang, West Java in December 2009 and identified in Herbarium Bandungense, The School of Life Science and Technology, Bandung Institute of Technology. Freshly harvested rhizomes were washed, dried and then divided into 9 parts with the weight of 300 g each. The rhizomes were then kept in a closed basket made from pleated bamboo that allows air circulation to take place for the period of 1, 2, 3, 4, 6, 8, 10 and 12 weeks. The weight of the rhizome was measured every week.

Determination of volatile oil: Rhizomes were crushed in the presence of water and the mixture was immediately filtered through muslin cloth. Starch in the filtrate was allowed to settle and the supernatant added to the distillation flask containing the residue after filtration. Volatile oil content was determined using Stahl distillation method (WHO, 1998).

The analysis of volatile oil quality: Volatile oil content was analyzed by TLC on Silica Gel GF-254 (Merck) with toluene-ethyl acetate (93:7) as the developing solvent and anisaldehyde as the spray reagent. The composition of oil obtained from fresh and 12 week stored rhizomes were further evaluated and compared by GC-MS. Gas chromatography was performed on Shimadzu GC-MS QP500 with column DB-17 30 m length and 0.25 mm inner diameter, helium as the carrier gas, pressure at 68 kpa, gradient elution from 40 to 250°C, injection temperature 250°C and injection volume 1 μL. All solvents (analytical and HPLC grade) were purchased from Merck.

Data analysis: The measurement of volatile oil content in each treatment was performed in three replicates. T-test with the level of significance $p<0.05$ was employed to analyze the data obtained.

RESULT AND DISCUSSION

Fresh *Curcuma* rhizome lost the water content slowly upon storage at Room Temperature (RT) with the average rate of lost was approximately 10% per-week. By the end of the storage, the rhizome was almost dry (Fig. 1). Fresh *Curcuma* rhizome contained 1.1% of volatile oil. To evaluate

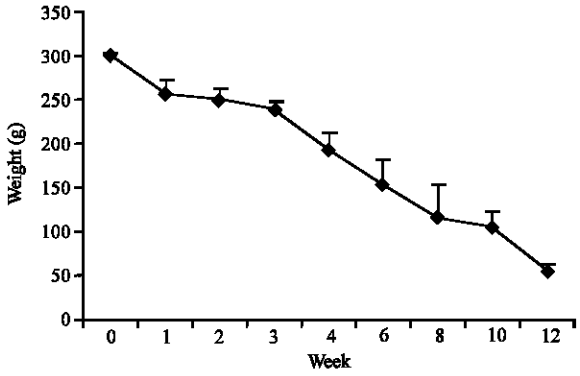

Fig. 1: Change in fresh weight of *Curcuma* rhizome upon storage at room temperature. Individual values expressed as Mean±SD in % are averages of three independent experiments

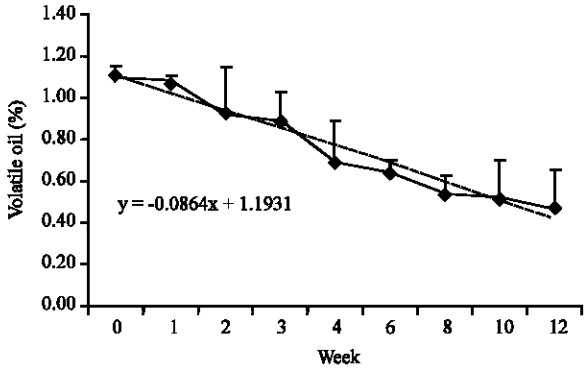

Fig. 2: Decrease of *Curcuma* oil during storage of fresh bulky rhizome. The percentage was calculated by dividing the oil obtained (mL) with the weight of fresh rhizome (g). Individual values expressed as Mean±SD in % are averages of three independent experiments

the volatile oil content in stored rhizome, the oil obtained during determination was presented as percentage toward the initial fresh weight, i.e., 300 g. The total yield of volatile oil remained constant in one week of storage and continuously decreased beginning from one week of storage up to the end of the experimental period. By week 12, the remaining volatile oil in the rhizome was only 0.47% and significantly different compared to the fresh rhizome (Fig. 2). In term of percentage toward the actual weight of the rhizome, the volatile oil content increased gradually on storage due to the evaporation of water. Substantial increase was observed between 11 to 12th weeks of storage. The percentage of volatile oil reached 3.71% toward actual weight of stored bulky rhizome by week 12 (Fig. 3). Although the percentage of volatile oil toward actual weight of rhizome increased, the total yield of volatile oil decreased from 1.1 to 0.47% as shown in Fig. 2. Similar observation was made with the volatile oil of the intact Zingiber officinal rhizome (Sukrasno *et al.*, 2000). Curcuminoid in an intact *Curcuma* rhizome, on the other hand, remains constant after storage for three months at room temperature (Sukrasno *et al.*, 2003). Choi *et al.* (2011) reported the increase of phenolic compounds in dried Citrus peal upon storage for three years.

The decrease on the yield of volatile oil might be due to the metabolism of volatile oil component that was still taking place during storage. The possibility of the evaporation of low boiling point

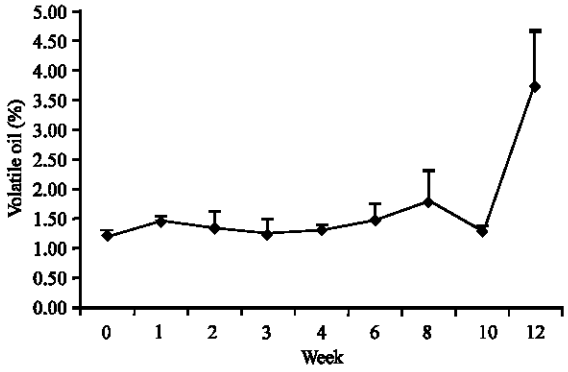

Fig. 3: Percentage of *curcuma* oil toward actual weight during storage of bulky rhizome. The percentage was calculated by dividing the oil obtained with the weight of rhizome after storage (g). Individual values expressed as Mean±SD in % are averages of three independent experiments

volatile oil components might also be considered. Therefore, from the volatile oil content point of view, fresh bulky rhizome should not be kept for longer than one week and it is better to be directly processed. Indeed, the crude drug of *Curcuma* rhizome is commonly prepared through slicing of freshly harvested rhizome followed by drying.

Attempt to evaluate the component of volatile oil from fresh and stored rhizome has been made by comparing the TLC and GC-MS chromatograms. It was observed that TLC chromatography can not distinguish the quality of volatile oil obtained from fresh, 6 week and 12 week stored bulky *Curcuma* rhizome. At least 12 components were separated and the chromatographic pattern of the oil obtained from three different periods of storage was similar. Further analysis of the volatile oil was performed by using GC-MS. Gas chromatographic analysis showed that there were at least 17 components separated (Table 1). Qualitatively the components of volatile oil from fresh and 12 week stored bulky rhizome were similar. Identification of the peaks was based on the mass spectra of the peaks and compared with the library data base and also by referring to Yasni *et al.* (1994). The identities of the peaks were as follows: (1) α-pinene (2) Camphene, (3) Isoborneol, (4) Camphor, (5) α-bergamotene, (6) Trans-caryophyllene, (7) γ-elemene, (8) β-farnesene, (9) α-longipinene, (10) Germacrened, (11) α-curcumene, (12) Dipi-α-cedrene, (13) Furanodiene, (14) Germacrene b, (15) β-elemene, (16) Germacron and (17) Xanthorrhizol. Substantial changed was observed with α-curcumene, dipi-α-cedrene, germacron and xanthorrhizol (peak 11, 12, 16 and 17). Oil obtained from fresh rhizome contained larger proportion of α-curcumene and dipi-α-cedrene (19.43 and 29.95%) and at lower level germacron and xanthorrhizol (3.50 and 7.10%). In oil from stored bulky rhizome, α-curcumene present at higher level (16.70%) than dipi-α-cedrene that decreased from 29.95 to 8.43%. Both germacron and xanthorrhizol increased compared to the oil from fresh bulb and present at 7.04 and 15.12%, respectively. These results suggest that storage of bulky rhizome will not only reduce the volatile oil content but also alter the % relative of its composition to the total volatile oil.

Curcuma crude drugs are commonly prepared by slicing followed by drying. Some times also prepared as powder. The volatile oil content during storage of dry sliced and powdered rhizomes was evaluated by determining the oil content at 0, 6 and 12 weeks of storage. Each data was obtained in triplicates. Data presented in Table 2 showed that the *Curcuma* crude drug maintained its volatile oil content at above 10% during 12 weeks of storage. Although, the average of 0 week

Table 1: Components of volatile oil from fresh and 12 weeks stored *Curcuma* rhizome

				Content (%)	
Peak No	Rt (min)	MW	Component	Fresh	Stored
1	7.342	136	α-Pinen	0.67	0.89
2	8.208	136	Camphene	1.45	2.08
3	17.325	154	Isoborneol	0.67	1.06
4	17.650	152	Camphor	5.61	8.99
5	22.350	204	α-Bergamotene	3.61	1.77
6	23.433	204	Trans-caryopyllene	1.10	0.57
7	23.817	204	γ-elemene	1.48	0.71
8	24.183	204	β-farnesene	3.70	1.58
9	25.108	204	α-longipinene	2.03	0.53
10	25.717	204	Germacrene d	1.51	0.52
11	26.442	202	α-Curcumene	19.43	16.70
12	26.817	204	Diepi-α-cedrene	29.95	8.43
13	27.792	216	Furanodiene	4.03	4.34
14	29.625	204	Germacrene b	4.42	2.74
15	32.858	218	β-elemene	1.06	1.66
16	35.892	218	Germacrone	3.51	7.04
17	36.875	218	Xanthorrhizol	7.10	15.12

Rt: Retention time, MW: Molecul weight

Table 2: Volatile content in dry sliced rhizome during storage

	Volatile oil content (%)	
Storage (week)	Sliced dry	Powder
0	10.33±1.32	11.60±0.54
6	11.62±0.70	10.33±0.42
12	12.14±0.85	10.34±0.78

Individual values expressed as Means±SD in % are averages of three independent experiments.

is lower than 6, 12 weeks of storage, statistically they are not significantly different. Similar results were observed with powdered crude drugs. The volatile oil content in powdered *Curcuma* crude drugs remained the same after storage for the period of 12 weeks.

Attempt to evaluate influence of crude drug preparation and the volatile oil content has also been made. The preparation methods included slicing followed by drying under the sun, drying in air oven at 85°C and powdered *Curcuma* from sun dried rhizome. The percentage of volatile oil content was calculated toward the original fresh weight of the rhizome. It was observed that slicing followed by drying under the sun did not reduce the volatile oil content of *Curcuma* rhizome. However, drying of sliced *Curcuma* in an air oven for four days slightly reduced the volatile oil content. Similarly with grinding of the crude drug, it slightly reduced the volatile oil content. Although, statistically significant, the decrease of volatile oil content on drying with an air oven and grinding was approximately only 20% i.e., from 1.19, 0.98 and 0.94% in sliced oven dried and powder from sliced sun dried, respectively (Table 3). These results were surprising, since most volatile oil in crude drug decreased upon drying and grinding (Sukrasno *et al*., 2000; Hassan *et al*., 2007a, b).

It is interesting to observe that exposure to relatively high temperature on drying and grinding did not substantially reduced the volatile oil content, while keeping the bulky rhizome at room

Table 3: Volatile oil content of fresh and processed *Curcuma* rhizome

Rhizome	Volatile oil content (%)
Fresh	1.19±0.05
Sliced sun dried	1.08±0.09
Sliced oven dried	0.98±0.05*
Powder from slice sun dried	0.94±0.08*

Individual values expressed as Means±SD in % are averages of three independent experiments.

temperature decreased the oil yield. These results suggests that the decrease of volatile oil content during storage of bulky rhizome is very likely due to the active metabolism that is still actively taking place in bulky rhizome. A drastic decrease of volatile oil content was also observed in ginger rhizome together with the formation of shoots after storage of bulky ginger rhizomes (Sukrasno *et al*., 2000).

CONCLUSION

Storage of bulky *Curcuma* rhizome decreased the volatile content and altered the % relative of its component to the total oil. Most of the volatile oil of *Curcuma* rhizome was still retained after slicing, drying and grinding. Since, volatile oil constitute as one of the active component of *Curcuma rhizome*, it is recommended that fresh rhizome should be immediately sliced and dried to assure its best quality as crude drug.

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