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Effect of different drying treatments on the biochemical components of java tea (*Orthosiphon stamineus* Benth.)

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Abstract
Orthosiphon stamineus is a Lamiaceae-family medicinal plant that is native to Tropical Asia. It is used as a diuretic, and can also be used to treat urinary dysfunctions and a variety of other ailments such as rheumatism. Pertaining to java tea's various health benefits and the commercial importance the herb possesses; it is necessary to standardize the postharvest aspects of *Orthosiphon stamineus*. With this main objective, to identify the best suited drying method for Java tea leaves (*Orthosiphon stamineus*) an experiment was conducted at Department of Medicinal and Aromatic crops, Horticultural College and Research institute, Tamil Nadu Agricultural University, Coimbatore during the year 2020-2021. The experiment was laid out in Completely Randomized Design with 5 treatments: solar drying, shade drying, cabinet drying (40 ^{0}C), cabinet drying (50 ^{0}C) and cabinet drying (60 ^{0}C) and replicated four times. The results revealed that shade dried leaf samples were better in quality aspects such as retention of biochemical parameters like total phenols, total flavonoids and antioxidant activity. From this study, it was concluded that among the various drying methods adopted, shade drying of java tea leaves for 5 days is an effective method in terms of quality preservance.

Keywords: Antioxidants, drying, flavonoids, java tea, phenols, quality

Introduction
Orthosiphon stamineus is a Lamiaceae-family medicinal plant that is native to Tropical Asia. A perennial herb that grows wild and along roadsides in Java, *O. stamineus* has been used in folk medicine for ages (Arifullah *et al*., 2014) [2]. The leaves of this plant are known as "Java tea" and are mainly used for the purpose of making herbal tea (Indubala *et al*., 2000) [3]. This crop is mostly used as a diuretic, but it can also be used to treat urinary dysfunctions and a variety of other ailments. In Malaysia, a decoction of the aerial portions of java tea, namely the leaves, is used to manage blood pressure and clear bladder and kidney stones (Tezuka *et al*., 2000) [10]. In India, java tea is used to treat diabetes in conjunction with medicinal plants such as *Andrographis paniculata*.
Pertaining to java tea's various health benefits and the commercial importance the herb possesses; it is necessary to standardize the postharvest aspects of *Orthosiphon stamineus*. The quality of raw herbal materials used in herbal-based nutraceutical and food supplement products is strongly related. The quality of herbs is influenced by a number of factors, one of which is drying. The objective of this study was to determine the relationship between drying methods and the composition of bioactive metabolites in *O. stamineus* leaves.

Materials and Methods
Drying Experiment
O. stamineus plants were grown at the Department of Medicinal and Aromatic plants, Horticulture College and Research Institute, Coimbatore. The plants were produced from cuttings and maintained according to standards. Fresh leaves of java tea were harvested in the morning hours between 7:00 – 9:00 AM. The leaves were then washed and rinsed to remove extraneous matter if any. The twigs were removed off prior subjection to further drying treatments. Leaf samples of 100 g were weighed and were dried using 5 different drying treatments; solar drying, shade drying, cabinet drying at 40ºC, cabinet drying at 50 ºC and cabinet drying at 60 ºC. The samples were dried for 9 hours, 4 hours and 3 hours by cabinet drier at the temperature of 40 ºC, 50 ºC and 60 ºC respectively. The samples in the shade and solar drying treatments were dried for 5 days and 5 hours respectively. The experiments were replicated four times for each of the treatment and the mean data was recorded.

Extraction Process
Metabolites from dried *O. stamineus* leaves were extracted by using a centrifuge (M/s Remi-R8C) and methanol was used as the solvent. 1 g of dried and pulverized leaf samples of *O. stamineus* was weighed accurately and extracted in 10ml of methanol for 10 minutes in hot water bath. The samples were then centrifuged at 5000 rpm for 5 minutes. The leaf extracts were stored at -20 ^{0}C until further analysis.

Total phenolic content (TPC) determination
The total phenolic content was estimated by the Folin-ciocalteau method. One ml methanolic extracts of the java tea leaf extract was taken in a test tube and one ml of Folin-ciocalteau reagent was added to it. After 10 minutes, 2 ml of 20% sodium carbonate was added and mixed thoroughly using a cyclomixer. The absorbance was read at 765 nm in a UV-VIS Spectrophotometer. Total phenol content of the sample analyzed was expressed in mg GAE/ g.

Total antioxidant activity (AOA) (%) determination
The antioxidant activity of the java tea samples was estimated by the DPPH radical scavenging assay. 2, 2, -diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that is purple in colour and is light sensitive. The antioxidant activity of the samples is based on its ability to decolourize the DPPH solution. The absorbance was read at 517 nm.
The percentage of radical scavenging activity (RSA percentage) or percentage of DPPH inhibitions of the sample extract was determined using the following formula:

$$\% \text{ RSA} = \{A(C) - (A(S)\} A(C) \text{ X } 100$$

Where
A(C)- absorbance of negative control,
A(S)- absorbance of sample

The total antioxidant activity analysed was expressed in %.

Total flavonoids content (TFC) (mg QE / g) determination
The total flavonoid content was calculated using the aluminium chloride method. A test tube was filled with 1ml of leaf extract and 4ml of water (10 ml volume). After 5 minutes, 0.3 ml of 5% sodium nitrite was added, followed by 0.3 ml of 10% aluminium chloride. After 6 minutes of room temperature incubation, 1ml of 1 M Sodium hydroxide was added to the reaction mixture. With distilled water, the final volume was quickly increased to 10 ml. A spectrophotometer was used to measure the absorbance of the sample against the blank at 510 nm.

Statistical Analysis
The results were statistical analyzed using Analysis of Variance (ANOVA) in Statistical Software for Science Social (SPSS). The data were expressed as means of four replications measurements. The probability of $P< 0.05$ was considered to show considerable differences for all comparisons made.

Result and Discussion
The effect of five different drying treatments on the phytochemical contents of *O. stamineus* leaves are presented in Table 1.

Table 1: Effect of drying treatments on total phenols, flavonoids and antioxidant activity of *Orthosiphon stamineus* Benth.

Treatment	Drying method	Duration of drying	Phenol (mg GAE/g)	Flavonoids (mg QE/g)	AOA (%)
T_1	Solar drying	5 hours	7.70	107.98	72.18
T_2	Shade drying	5 days	10.55	125.63	57.45
T_3	Cabinet drying at 40 ^{0}C	9 hours	10.43	116.57	63.78
T_4	Cabinet drying at 50 ^{0}C	4 hours	10.20	103.84	75.94
T_5	Cabinet drying at 60 ^{0}C	3 hours	3.75	96.29	83.63
	Mean	5.2	895.898	110.062	70.596
	S. E(d)	0.16	4426.48	5.55	2.09
	CD (P=0.05)	0.33	9383.72	11.76	4.44

Duration of drying
The duration of drying exhibited significant variation among the different drying methods (Table 1). Cabinet at 60 ^{0}C recorded the least time (3 hours) for drying of the herb, followed by cabinet 50 ^{0}C- 4 hours. The maximum drying duration of 5 days was registered by shade drying. Higher drying temperatures reduced the duration of drying in comparison to shade drying (Pirbalouti, *et al*., 2013) [8]. But most of the nutrients can be retained with significant difference in proximate and mineral contents by shade drying. These results are on par with that of Abdullah *et al*. (2012) [1], wherein, shade drying took 4 days to dry java tea leaves and found that shade dried leaves seemed to retain certain bioactive constituents unlike other drying methods such as sun and oven drying used in their study.

Estimation of total phenol
The total phenolics content of the dried java tea leaves in the different drying methods ranged between 3.75 to 10.55 mg GAE/g d.b. The experimental results revealed that the shade drying retained the highest total phenols (0.05), whereas the Cabinet drying at 60 ^{0}C had the lowest TPC. Exposure to higher temperature could have caused degradation of certain phytochemical components. The results of the study were in line with that of Abdullah *et al.* (2012) [1] wherein, drying of *O. stamineus* leaves in oven had significantly reduced the content of phenolic compounds to 1.572 mg/g as compared to 4.350 mg/g when leaves were dried under the shade. The losses of TPC due to thermal degradation may be due to the bindings of polyphenols with other components or the alteration in the chemical structure of polyphenols which cannot be extracted and determined by available methods (Miranda *et al*., 2010, Zakhama *et al*., 2019) [5]. Considering the results from this investigation, it is worth mentioning that shade drying is suitable for enhancing the extractability of phenolic compounds to a great extent, but their efficacy is dependent on the nature of the vegetal matrix being used and the type of compounds to be extracted (Multari *et al*., 2018) [9].

Estimation of total flavonoids
Total flavonoid content (TFC) of the dried java tea leaves

ranged between 96.29 mg to 125.63 mg QE/ g d.b. Shade drying retained the highest total phenols (0.05), whereas the Cabinet drying at 60^0C had the lowest TFC. The results are similar to that of Rita Mansour (2016) [16] in *Thymus vulgaris* wherein, the air shade drying contained more total phenolics, and flavonoids than air –sun drying of thyme. Temperature could have been the reason for the loss of flavonoids. Heating might cause breakdown of certain phytochemicals which disrupt the integrity of cell walls and there by cause movement of some flavonoid components. In addition, the loss in flavonoids may also be due to the breakdown or leakage by chemical reactions including oxygen, enzymes and light.

Total antioxidant activity

The antioxidant activity of dried java tea leaves using different drying methods were in the range of 57.45% to 83.63% as illustrated in Table 1. Higher DPPH radical scavenging activity was indicated by the higher % inhibition. The leaves subjected to shade drying exhibited higher % inhibition and thereby higher AOA ($p< 0.05$) just like high TPC. The higher total phenolics correlated the high antioxidant activity, this was probably due to the combined effect of the phenolic compounds and their high hydrogen atom-donating abilities. Similarly, a correlation between DPPH radical scavenging activity and TPC was reported for some vegetables and fruits (Jimenez *et al*., 2001; Park *et al*., 2006) [7].

Conclusion

The results of the study revealed that the technique for drying of fresh leaves is an important factor in determining the quality and the bioactive constituents of the herb. Shade drying of java tea seems to retain higher content of total phenolics, total flavonoids and antioxidant activity. Cabinet drying at 40^0C - 50^0C can also be considered in order to retain important bioactive compounds of java tea wherein, drying duration or time is a limiting factor.

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