



Research Article

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Effect of drying methods and extraction solvents on anti-amylase activity of selected medicinal plants

Peries CM¹, Navarathne SB², Wijesinghe WAJP³, Henagamage AP¹, Cooray R⁴

¹ Department of Science & Technology, Faculty of Applied Sciences, Uva Wellassa University, Sri Lanka

² Department of Food Science & Technology, Faculty of Applied Sciences, University of Sri Jayawardenepura, Sri Lanka

³ Department of Export Agriculture, Faculty of Animal Science & Export Agriculture, Uva Wellassa University, Sri Lanka

⁴ School of Molecular and Life Sciences, Curtin University of Technology, Perth, Western Australia

ABSTRACT

Presently, there is a blooming recognition in using the herbal medicine to treat the Diabetic mellitus due to the negative side effects of usage in synthetic drugs. The present study was aimed to evaluate the potential of anti-amylase activity of three different leaf extracts of Thebu (*Costus speciosus*), Kowakka (*Coccinia grandis*) and Masbadda (*Gymnema sylvestre*) following different drying techniques i.e; shade drying at room temperature, oven drying at 45 °C and freeze drying. Dried plant leaves were macerated with n-hexane, ethyl acetate and ethanol for 24 hours at room temperature separately. Obtained extracts were used to determine the anti- amylase activity. Results revealed that freeze dried ethanolic leaf extracts of all selected species showed the highest anti- amylase activity compared to the other drying methods. Further, there was a significant effect (p <0.05) on the drying techniques and solvents used to extract in inhibition of alpha amylase activity. Freeze dried *C. speciosus* and *C. grandis* ethanolic extracts displayed an effective inhibition against alpha amylase with an IC₅₀ value of 4 mg/ml and 4.16 mg/ml respectively. Therefore, this study revealed that the drying methods significantly affected on the inhibition of alpha amylase enzyme. Freeze-drying was the most promising drying method and ethanol was the best solvent in extracting anti-amylase bioactive compounds from leaf extract of *C. speciosus*, *C. grandis* and *G. sylvestre*.

Keywords: Diabetic mellitus, Anti- amylase activity, Drying techniques, Medicinal plants.

INTRODUCTION

Diabetic mellitus is a major health issue that is widely spreading in the world in alarming rate. The World Health Organization predicted that more than 300 million people will be suffered from diabetes by year 2025 [1]. Currently, the blood glucose level of diabetic patients is regulated by insulin or the chemically synthesized drugs such as acarbose, metformin. However, these drugs have serious detrimental effects [2]. Therefore, herbal medicines have been gaining significant importance recently in worldwide for the treatment of diabetes due to their effectiveness, fewer side effects in clinical experience and relatively low costs [3]. Further, the herbal products are rich in secondary metabolites which can help to reduce blood glucose levels and these secondary metabolites can be derived from different parts of the medicinal plants [4].

Even though, medicinal plants are rich in wide range of secondary metabolites, moisture content can critically influence on their chemical and physical properties. Hence, drying process is playing a major role to reduce the moisture content of plant parts while inhibiting of undesirable enzymes and growth of microorganisms. Previous literature revealed that different drying methods have shown significant influences on stability and the availability of bioactive compounds [5]. Though several studies showed the anti-amylase potential of Thebu (*Costus speciosus*), Kowakka (*Coccinia grandis*) and Masbadda (*Gymnema sylvestre*), there are limited reports on the effect of different drying methods on anti-amylase activity of those herbs. Therefore, this study focused in evaluating the effects of different drying techniques on anti-amylase activity of aforementioned medicinal plants.

MATERIALS AND METHODS

Chemicals and reagents

Soluble starch, D-glucose, 3,5- di nitro salicylic acid, dimethyl sulfoxide (DMSO), ethanol, n-hexane, ethyl acetate, α - amylase (porcine pancreatic) and acarbose were purchased from Sigma Aldrich, Germany. All other chemicals used for the preparation of buffers and solvents were in analytical grade.

***Corresponding author:**

Maheshika Peries

Department of Science & Technology, Faculty of Applied Sciences, Uva Wellassa University, Sri Lanka

Email:

maheshika.peries85[at]gmail.com

Collection and preparation of plant materials

Three medicinal plants namely *Thebu* (*Costus speciosus*), *Kowakka* (*Coccinia grandis*) and *Masbadda* (*Gymnema sylvestre*) were selected based on their ethnopharmacological use in traditional medicine to treat Diabetes. Fresh matured leaves of *C. speciosus* and *C. grandis* were collected from Badulla and *G. sylvestre* was collected from Colombo, Sri Lanka and authenticated from the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. The collected leaves were washed with cleaned water and left to dry on a cheese cloth for 15 min at room temperature separately.

Application of different drying techniques

Different drying techniques were applied namely shade drying at room temperature (25±5 °C), oven drying at 45°C and freeze-drying at -80 °C for the fresh leaves. All the drying methods were triplicated and performed until the leaves were reached to a constant weight. Fresh leaf samples used as control without drying. Finally, dried leaf samples were ground and stored in a freezer at -20°C for the subsequent use of the study.

Extraction of the plant materials

The well dried, ground leaf samples of three plants were subjected to extract the bio active compounds by maceration method with n-hexane, ethyl acetate and 95% ethanol separately. Therein, ten grams (10g) of each plant material was soaked with 100 mL of different solvents separately for 24 hours at room temperature in a shaker with 120 rpm. The well shaken samples were filtered through a Whatmann 01 filter paper. Obtained residues were extracted again following the same procedure. The filtrates were evaporated using a rotary evaporator at 40°C. The obtained residues were stored separately in sterile tubes at -20 °C [6].

Determination of anti- amylase activity

DNSA (3, 5-Dinitrosalicylic acid) method was used to analyze the inhibition of amylase enzyme activity. Different plant extracts (5 mg/mL) were dissolved in deionized water with 10 % DMSO. A 100 µL of α-amylase (8U /mL) was mixed with 100 µL plant extract and incubated at 25 °C for about 30 min. A 100 µL of this mixture was mixed with 100 µL starch (1 % w/v) solution and incubated at 37 °C for 10 minutes. Thereafter, DNSA reagent (100 µL) was added, incubated at 85 °C for 15 min in a water bath, allowed to cool and diluted with distilled water (900µL). Negative controls were conducted in the same manner with 10 % DMSO (100µL) in distilled water. Blanks were prepared by adding DNSA reagent prior to the addition of starch solution and kept for 37°C for 10 min. After that, starch was added to the mixture and kept in the water bath at 85 °C for 15 min and finally

diluted with distilled water (900 µL) as done before. Absorbance was measured at 540 nm with using 96- well microplate reader (Thermo Scientific Multiskan Go). The % of alpha amylase inhibition was calculated using the following equation [7].

$$\text{Percentage of alpha amylase inhibition} = \frac{(Ac - As)}{Ac} \times 100$$

Ac - Absorbance negative control, As- Absorbance of sample

The acarbose served as the positive control and the α-amylase inhibition percentage of freeze-dried leaf samples were plotted against the different extract concentration and the IC₅₀ values were obtained from the graph.

Statistical analysis

All the trials were conducted in triplicates. Minitab -16 was used to analyze the data and the results were presented as mean deviation. Factorial analysis and Tukey paired comparisons tests were performed to compare the different drying conditions, solvents and plant varieties for the inhibition of alpha amylase activity. The differences were considered at the significance level of p<0.05.

RESULTS & DISCUSSION

Effects of different drying techniques on the drying yield

Out of all the drying techniques, freeze-drying method was the most efficient drying method for all three selected leaf samples (figure 1) since, it was taken the least time to get the constant weight (table 1). Out of all three plant species, the significantly (P< 0.05) highest drying yield was obtained from *G. sylvestre* for all drying techniques. Drying is utmost prevalent technique that can be used to preserve the food quality. Sun drying and air drying at room temperature are the most popular low-cost drying methods used in many parts of the world. In addition to that, freeze-drying and oven drying methods are used for drying. However, the composition of bioactive compounds and their stability can be altered due to many drying techniques [8]. Thus, it is essential to investigate the effect of different drying methods in order to enhance the product quality. Among the drying methods adapted for this study, freeze-drying is mostly preferable method to obtain high drying yield. Previous literature also stated that the freeze-drying process has been given the quality of a dried product with more yield, high nutritional and marketing value [9]. Furthermore, it can preserve the heat labile compounds in the medicinal plants and minimize the colour changes during the drying process [10]. In spite of those, this is a complex and expensive method of drying due to its high energy consumption and operational costs [11].

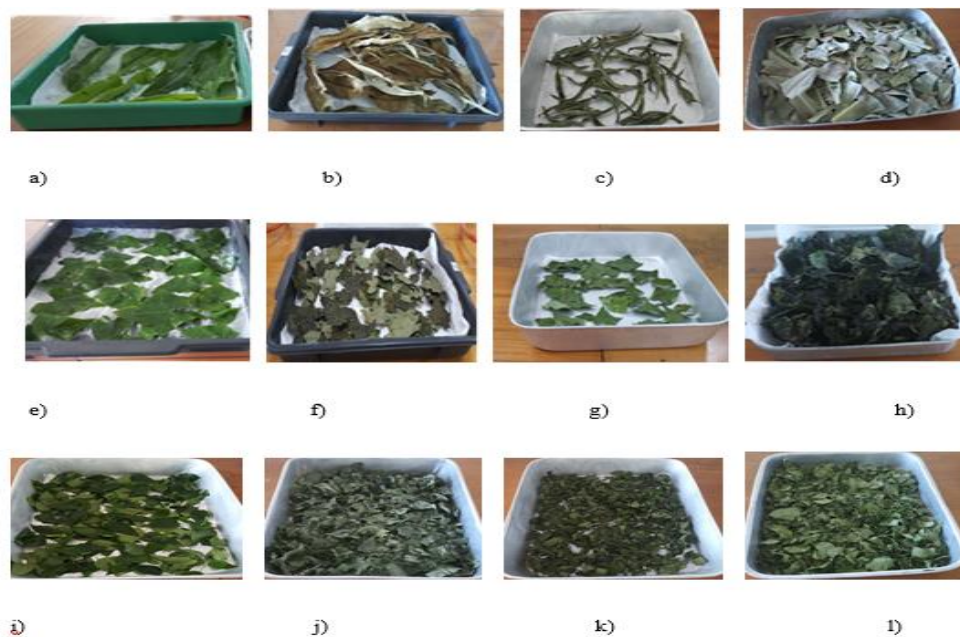


Figure 1: Applying different drying techniques for different leaves 1a. Fresh, 1b. Shade dried leaves, 1c. Oven dried (45 °C) leaves, 1d. Freeze dried leaves of *C. speciosus*, 1e. Fresh, 1f. Shade dried leaves, 1g. Oven dried (45 °C) leaves, 1h. Freeze dried leaves of *C. grandis*, 1i. Fresh, 1j. Shade dried leaves, 1k. Oven dried (45 °C) leaves, 1l. Freeze dried leaves of *G. sylvestre*

Table 1: Effects of different drying techniques on the drying yield of *C. speciosus*, *C. grandis* and *G. sylvestre*

Plant leaves type	Drying Techniques					
	Shade drying		Oven drying (45°C)		Freeze drying	
	Drying time	Drying yield (w/w%)	Drying time	Drying yield (w/w%)	Drying time	Drying yield (w/w%)
<i>C. speciosus</i>	30 days	11.13+ 0.815b	48 hours	11.42+0.440b	12 hours	12.03+0.455b
<i>C. grandis</i>	14 days	11.10+ 0.020b	24 hours	12.43+0.194b	15 hours	13.69+0.130b
<i>G. sylvestre</i>	12 days	27.7+4.010a	12 hours	25.98+4.350a	09 hours	27.93+ 3.738a

All values are means of triplicate + SD

Effect of different drying methods on extracted yield

Three different solvents i.e; ethanol (high polar), hexane (non polar) and ethyl acetate (medium polar) were used in the current study based on their polarity and low toxicity to extract secondary metabolites. It was observed that different extraction yields were obtained from different solvents for the selected plant species dried using different drying techniques (table 2). This is due to the polarity differences of the extraction solvents and it causes wide variations in the level of bioactive compounds in the extract. A significant difference was observed between extraction yield and plant type, and drying technique and solvent type ($p < 0.05$). Extraction yields were high in all selected medicinal plants after drying techniques compared to fresh leaves. Among the three solvents, ethanol (95%) was given the highest yield compared to hexane and ethyl acetate. Ethanolic extracts of *C. speciosus* and *G. sylvestre* obtained the maximum extracted yields of 8.27% and 6.95% respectively for freeze dried samples. This indicates that the extraction efficiency is highly entangled with polar solvents

disclosing that polar compounds are easier to be extracted compared to non-polar compounds. Although ethanol contains hydroxyl group that can form hydrogen bonding with the solute and this nature can enhance the extracting process [12]. These findings are in consistent with the extraction yield of *Severinia buxifolia*. According to that literature, yields obtained from ethanol, acetone, chloroform and dichloromethane for *Severinia buxifolia*. were 12.2%, 8.6%, 7.2%, 4.9% respectively [13].

Yield of extract is important for ethnobotanical usage since plants with low extracted yield are not commonly preferred by the pharmaceutical industry even though they are rich in their activity [10]. Furthermore, there are several factors that can be influenced by the extractions i.e; method of extraction, temperature, extraction time, the configuration of phytochemicals, and the solvent [13]. In addition to that, the extraction solvents have an effect on the extraction yield and the content of bioactive compounds; thus, those bioactive compounds significantly effect on the biological activity of the extract [14].

Table 2: Effect of different drying methods on extracted yield (w/w%) of *C. speciosus*, *C. grandis* and *G. sylvestre* with different solvents

Drying Application	Solvent	<i>C. speciosus</i>	<i>C. grandis</i>	<i>G. sylvestre</i>
Fresh	Hexane	0.685 ± 0.685 ^b	1.98 ± 0.346 ^{bcd}	1.31 ± 0.474 ^d
	Ethyl acetate	1.21 ± 1.21 ^b	1.39 ± 0.247 ^d	2.61 ± 0.728 ^{cd}
	Ethanol (95%)	1.375 ± 1.375 ^b	2.25 ± 0.247 ^{abcd}	3.60 ± 0.467 ^{bc}
Shade drying	Hexane	1.64 ± 0.552 ^b	1.85 ± 0.877 ^{cd}	5.68 ± 0.636 ^{ab}
	Ethyl acetate	1.06 ± 1.153 ^b	1.92 ± 0.735 ^{cd}	3.57 ± 0.445 ^{bc}
	Ethanol (95%)	3.97 ± 0.742 ^b	3.47 ± 0.410 ^{abcd}	6.77 ± 0.615 ^a
Oven drying (45°C)	Hexane	0.90 ± 0.396 ^b	4.18 ± 0.516 ^{ab}	4.76 ± 0.424 ^{abc}
	Ethyl acetate	2.55 ± 1.237 ^b	3.13 ± 0.410 ^{abcd}	2.87 ± 0.467 ^{cd}
	Ethanol (95%)	3.39 ± 0.870 ^b	4.31 ± 0.354 ^a	5.53 ± 0.580 ^{ab}
Freeze drying	Hexane	3.18 ± 1.393 ^b	3.77 ± 0.552 ^{abc}	2.66 ± 0.735 ^{cd}
	Ethyl acetate	2.66 ± 0.962 ^b	3.50 ± 0.382 ^{abcd}	3.48 ± 0.516 ^{bcd}
	Ethanol (95%)	8.27 ± 0.431 ^a	3.91 ± 0.997 ^{abc}	6.95 ± 0.445 ^a

Three plants were statistically analyzed separately for extracted yield with different drying techniques and solvents. All values are means of triplicate + SD

Alpha amylase inhibitory effect of selected medicinal plant extracts by DNSA method

The drying techniques and solvents were significantly affected ($p < 0.05$) on all three selected plant species in inhibiting alpha amylase activity at the concentration of 5 mg/mL. Out of all drying techniques, freeze drying technique showed the highest alpha amylase inhibition percentage for all the solvents in all selected plant species (figure 2). Out of all the extracts, ethanolic extracts of the freeze-dried leaf samples of all selected plant species showed the significantly ($p < 0.05$) highest alpha amylase inhibition percentage. Literature revealed that the anti-amylase activity has been identified due to the presence of phytochemicals such as phenolic, flavonoids, alkaloids, and terpenoids compounds in medicinal plants [15]. They can act as natural inhibitors of alpha amylase enzyme and delay the absorption of starch into the body by interrupting in the hydrolysis of 1,4-glycosidic linkages of starch [16]. Fresh samples of selected species have showed lower anti amylase activity compared to the drying techniques as a result of the limited separation of secondary metabolites corresponding to the solvent. This might be ensued due to the biochemical reactions which are activated by the high moisture content in fresh leaves than the dried samples [17].

According to figure 2a, 2b and 2c, ethanolic extracts from freeze dried *C. speciosus*, *C. grandis* and *G. sylvestre* samples imparted the maximum anti-amylase activity of 56.55%, 58.04% and 35.38% respectively compared to oven drying and shade drying techniques. The results are obtained from this research study was consistent with the earlier findings of [18] which, a freeze dried *Moringa oleifera* leaves were

shown the highest alpha- amylase inhibition compared to other drying methods namely air drying, sun drying and oven drying. Similarly, [19] found that the freeze-dried extracts have a positive effect in increasing the percentages of inhibition of α -glucosidase, and α -amylase in selected traditional Mexican herbs that contribute to the treatment of Diabetes. The lower anti amylase activity of extracts of oven dried and shade dried might be attributed to the influence of high temperature and longer drying-time duration. Literature also revealed that the volatile and heat labile active compounds which are responsible for bio activity can be degraded more on oven drying and shade drying compared to the freeze drying [20]. The variations observed in the alpha amylase inhibition of the selected medicinal plant leaves might be due to the different heat-induced chemical modifications that took place during the drying process.

The graph revealed that the α -amylase inhibition is dose-dependent (Figure 3). Freeze dried ethanolic leaf extracts of *C. speciosus*, *C. grandis* were exhibited the most effective inhibition against alpha amylase with an IC_{50} value of 4.00 mg/mL and 4.16 mg/mL respectively. However, alpha amylase inhibition of standard acarbose (IC_{50} = 2.24 mg/ml) was significantly higher than that of the selected plant extracts. According to the literature, the use of acarbose is reported to be associated with gastrointestinal side effects caused by the excessive inhibition of pancreatic α -amylase [21]. Therefore, previous literature stated [22] that any bioactive compounds having lower inhibitory activity against α -amylase may be an effective therapeutic agent for the control of diabetes than acarbose.

CONCLUSION

In conclusion, this study revealed that the drying methods and solvents used in extracting bio active compounds were significantly influenced in inhibiting alpha amylase enzyme. Freeze-drying was the most effective drying method and ethanol was the best solvent in extracting bioactive compounds from leaf extract of *C. speciosus*, *C. grandis* and *G. sylvestre*. However, further phytochemical identification, structural elucidation and characterization methodologies are required to explore and isolate the bio active compounds in the selected medicinal plants.

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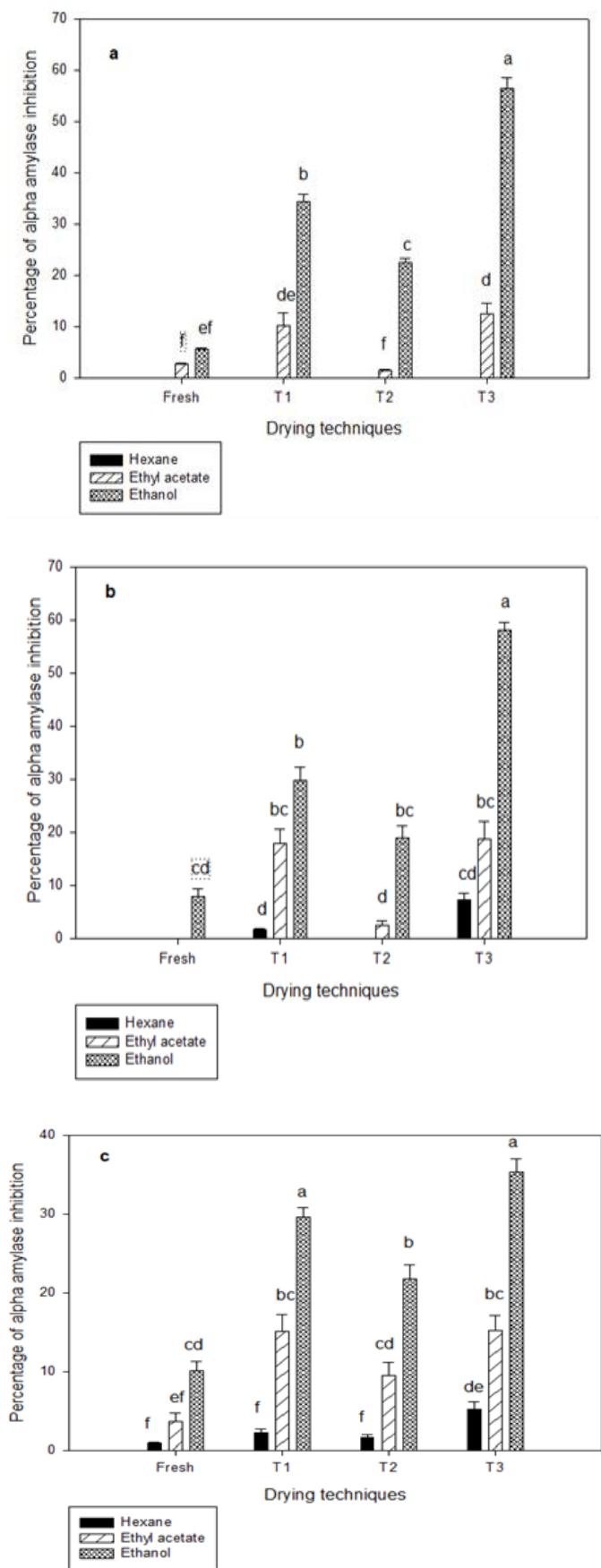


Figure 2: (a). Effect of drying methods on anti- amylase activity of *C. speciosus* with different extracts, **(b).** Effect of drying methods on anti- amylase activity of *C. grandis* with different extracts, **(c).** Effect of drying methods on anti- amylase activity of *G. sylvestre* with different extracts Treatments: T1- Shade drying, T2- Oven drying at 45 °C, T3- Freeze drying

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