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RESEARCH

Enhancement of Antioxidant Properties of the Medicinal Plant, *Costus Pictus* by Optimization of its Drying and Extraction Criteria

Saranya Selvakumarasamy[1] · Balakrishnaraja Rengaraju[1]

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Abstract

Antioxidants act as major protective factors against different infections and diseases. The search for natural antioxidants has gained significant momentum due to its associated health benefits. It prompted the investigation of the antioxidant properties of widely recognized medicinal plants, considering their prominent role in conventional medicine. The incorporation of natural antioxidants derived from medicinal plants into food products has the potential to enhance their health benefits. The present investigation is the first study on the optimization of drying and extraction techniques in *Costus pictus* leaves. *C. pictus* leaves were dried under varying conditions (40, 50 and 60 °C) and dried powders were subjected to various solvents, namely water, ethanol, methanol and ethyl acetate. The leaves dried at 60 °C and treated with ethanol showed improved activities and were subsequently selected for further extraction. Among the various extraction methods, ultrasound-assisted extraction demonstrated superior antioxidant properties and increased phytochemical contents, making it the optimal technique for our study. Fourier transform infrared spectroscopy (FTIR) reports also substantiated these quantitative results. The extraction process played a significant role in enhancing the desirable attributes and properties of the leaf extracts, surpassing the results obtained from both dried and fresh leaves. The application of liquid chromatography-mass spectrometry (LC-MS) analysis to the leaf extracts facilitated the identification of phenolic compounds and flavonoids, presenting a comprehensive insight into the composition of the extract. Exploration of antioxidant properties, phenolic compounds and flavonoids would validate the benefits and expand the applications of *C. pictus* in functional foods and nutraceuticals.

Keywords *Costus pictus* · Drying · Extraction · Antioxidant activity · Plant foods

Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
FC	Flavonoid content
FRAP	Ferric reducing antioxidant potential
FTIR	Fourier transform infrared spectroscopy
GAE	Gallic acid equivalents
LC-MS	Liquid chromatography-mass spectrometry
MAE	Microwave-assisted extraction
PM	Phosphomolybdenum
QE	Quercetin equivalents
SE	Solvent extraction
SOX	Soxhlet extraction
TE	Trolox equivalents
TPC	Total phenolic content
TPTZ	2,4,6-Tripyridyl-S-triazine
US	Ultrasound-assisted extraction

Introduction

Oxidative stress, which could be attributed to lifestyle changes and environmental factors, is imperative for the cause of diabetes, cancer and neurological disorders. Lack of an efficient antioxidant defense was a vital factor in increased diabetes rates [1]. However, no appropriate curative therapies were available against these diseases. Moreover, synthetic antioxidants also have detrimental health effects owing to their toxicity [2]. So, plants have been

✉ Saranya Selvakumarasamy
saranselvakumar@gmail.com

[1] Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode, India

perceived as a natural source, due to their profuse bioactive compounds, to have a positive militating impact in preventing these disorders.

Medicinal plants have been inextricably intertwined in the therapy for many medical ailments since their primeval days. The atavistic nature of utilization for healthcare applications has paved for more tapping on medicinal plants. Diverse bioactive compounds, namely alkaloids, phenolic compounds and flavonoids, contribute to a diverse array of biological properties like antioxidant and anti-inflammatory activities [3]. Furthermore, the diverse nature of phytochemicals has also intrigued the derivatization of dietary supplements from these plants [4]. It was indispensable to investigate its biological properties, to have an efficacious functional food based on plant fortification [5]. The antioxidant efficacy of the plant was influenced by the parameters involved in diverse processing techniques, which affected the availability of antioxidants and other phytochemicals. Processing techniques positively impacted carotenoids and flavonoids; while also attenuating the oxidation of polyphenols through thermal processing, which led to its elevated levels in processed forms like dried powders [6]. The stability of the flavonoids also depends on the methods employed for extraction [7].

The utilization of plants as food additives can be done by adding either dry plant powders or plant extracts obtained through a contemporary extraction approach. For the isolation of bio-actives from botanical sources, extraction acts as a vital step, and solvents play an indispensable part in the extraction. Solvent extraction (SE) is commonly utilized for plant-based extractions. Soxhlet extraction (SOX) involves repeated interaction between the sample and the solvent, and the bioactive molecules get leached away into the solvent, thus circumventing the requirement for filtration [8]. Advanced technologies like microwave-assisted extraction (MAE) and ultrasound-assisted extraction (US) are gaining more attention due to their reduced extraction time and requirement of minimal solvents [9–11]. The application of plant extracts in diversified areas, such as the food and pharmaceutical sectors, has been observed. Yesil-Celiktas et al. [12] have documented the potential utilization of rosemary extracts as powerful anti-cancer supplements, attributing their cytotoxic properties to their effectiveness against cancer cells.

Costus pictus originated from the sub-Himalayan parts and South and Central America and is a renowned medicinal plant. It can thrive in a tropical climate which aids its cultivation. It is highly acclaimed as the 'Insulin plant' and is attributable to its anti-diabetic traits. Earlier investigations on *C. pictus* leaves demonstrated their anti-diabetic properties by evaluating their impact on rats with induced diabetes [13]. Quantitative analysis conducted by Jayasri et al. [14] revealed the antioxidant attributes of the leaves [14]. No previous studies have explored the optimization of various processing stages for *C. pictus* leaves.

The objective of the study is to (i) investigate the influence of various solvents on antioxidant properties of *C. pictus* leaves processed at different drying temperatures, (ii) analyze various extraction methods, and validate by Fourier-transform infrared spectroscopy. The study also analyzed the efficacy of processing conditions on the antioxidant characteristics and flavonoid content. The objective is to enhance the extract to achieve its maximum potential in terms of antioxidant properties and flavonoid composition through optimization, thus enabling its utilization in various industrial applications.

Materials and Methods

The Materials and Methods has been provided as supplemental information.

Results and Discussion

All figures and tables were presented in the supplementary material section.

Optimization of the Solvent and Drying Temperature

Fresh *C. pictus* leaves and leaf powders dried at different conditions were dispersed in various solvents. It could give a brief understanding of the role of these solvents on the antioxidant properties and phytochemicals. The effect of temperature and solvents on various quantitative assays of the dried powders was illustrated in Fig. (S1).

For the 2,2–diphenyl–1–picrylhydrazyl (DPPH) free radical scavenging assay, IC_{50} values were far lesser at 60 °C, followed by 50 and 40 °C, respectively. There was a 4-fold reduction in the IC_{50} values at 60 °C compared to the fresh samples for ethanol and ethyl acetate dilutes, whereas a 3-fold reduction was noticed for water and methanol samples. Among the solvents, methanolic dilute had fewer IC_{50} values and ethanol samples (267.958 ± 3.08 μg/mL) only had very minimal differences from the methanol samples (272.570 ± 2.42 μg/mL). IC_{50} values increased in water and a vast increase was seen in the ethyl acetate samples (Fig. S1 1a). Methanolic samples at 60 °C (1.641 ± 0.019 μg/g Trolox equivalents (TE)) revealed better results for 2,2'-azinobis(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) assay; nevertheless, only minimal variations were seen among the different drying temperatures (40 °C – 1.593 ± 0.013 μg/g

TE and 50 °C – 1.618 ± 0.006 μg/g TE) and the polar solvents. Ethyl acetate dilutes had very minimal values for the dried and fresh samples (Fig. S1 1b). Ferric reducing antioxidant potential (FRAP) analysis results signified that higher temperature (60 °C) had more appreciable antioxidant properties than the other temperatures and was significantly higher than the fresh leaves (approximately 4-fold increase). Methanol (11.034 ± 0.02 mM/g TE at 60 °C) gave the best results and ethyl acetate samples (4.590 ± 0.09 mM/g TE at 60 °C) revealed the lowest antioxidant properties (Fig. S1 1c). Phosphomolybdenum (PM) assay also revealed methanol and ethanol showed better antioxidant properties; however, the latter had minor variations than the former solvent. Minimal changes were noticed among the different temperatures and antioxidant activity was considerably better than in the fresh leaves (Fig. S1 1d). Total phenol content (TPC) was higher in the ethanol samples and was consecutively lesser in methanol, water and ethyl acetate samples. Ethanol revealed greater TPC due to its high affinity for phenolic constituents. Leaves dried at 60 °C (water – 12.979 ± 0.03 mg/g gallic acid equivalents (GAE), ethanol – 22.338 ± 0.06 mg/g GAE, methanol – 22.068 ± 0.04 mg/g GAE and ethyl acetate – 6.619 ± 0.04 mg/g GAE) displayed similar TPC to the fresh leaves (water – 12.108 ± 0.02 mg/g GAE, ethanol – 20.463 ± 0.02 mg/g GAE, methanol – 16.321 ± 0.04 mg/g GAE and ethyl acetate – 5.053 ± 0.01 mg/g GAE), thus confirming the slightest variation caused to the phenolic compounds owing to the less drying time of the samples (Fig. S1 1e). Flavonoid content (FC) was more evident in methanol and ethanol samples than in water and ethyl acetate and was more at 60 °C compared to other drying temperatures and the fresh leaves (Fig. S1 1f).

The results were analogous to the other studies in *C. pictus*, wherein methanol was reported as the best solvent [13, 15]. As methanol was a class 2 GRAS (Generally Recognized As Safe) solvent, its utilization was prone to the concentration guidelines due to its toxic effects. Therefore, using it would hinder the application of dried powders as food additives, as it might exceed the utilization limit of 3000 ppm. Moreover, the results of ethanolic samples were on par with methanol; therefore, ethanol was chosen for further extraction. Similar results were observed for another medicinal plant, *Limnophila aromatica*, wherein ethanol gave superior results compared to other solvents [16].

The antioxidant activity of dried powders was more than the fresh leaves and leaves dried at 60 °C had the best antioxidant properties. The increment in the activity of the dried leaves could be due to the unbinding of bound antioxidants caused by cell structural disruption on drying. Another contributing factor could be the potential thermal inactivation of enzymes, including polyphenol oxidase, which may play a role in suppressing oxidation and thereby maintaining the integrity of the antioxidant property [17]. The observed outcomes matched the results of Pinela et al. [18], wherein dried samples had better antioxidant properties than wild plants.

Drying expedited the release of phenolic compounds, leading to higher total phenolic content in dried samples compared to fresh leaves. Moreover, some plants might remain metabolically active on drying and they recognize moisture loss as a stress inducer and produce more phenolics during drying. It might also contribute to the increased TPC levels in dried leaves. Enzymes, polyphenol oxidase and peroxidase, found in fresh leaves can facilitate the oxidation of phenolic compounds, resulting in lower TPC levels. However, during the drying process, these enzymes become thermally inactive, leading to an increase in TPC in the dried leaf powders [19]. Therefore, the dried leaf powders displayed higher total phenolic content, with a notable increase observed at a drying temperature of 60 °C. These results followed a similar pattern to the dried leaves of *Camellia sinensis* [19] and *Urtica dioica* [17]. FC was higher in the leaves dried at higher drying temperatures owing to the shorter drying time, thereby minimizing the prolonged exposure of flavonoids to heat. Thus, 60 °C had more pronounced FC. The findings were consistent with the hot air drying of lemon myrtle leaves, which also demonstrated increased phytochemical content at higher drying temperatures. Sustained exposure to lower temperatures could potentially induce the degradation of phytochemicals, while higher temperatures reduced drying time and minimized phytochemical loss [20].

Hence, leaves dried at 60 °C revealed unrivaled results and were taken for extraction. These observations suggested that dried powder could be ideal for incorporation in various nutraceutical and food formulations owing to its better antioxidant properties and improved phytochemical contents.

Optimisation of the Extraction Method

Ethanol was used as the solvent and the dried powders were subjected to various extraction techniques. Different extraction criteria were set for diverse methods and the optimal conditions were found depending on its phytochemical contents and antioxidant properties. Fig. (S2) illustrates the optimum extraction conditions for all the extraction methods.

The higher yield was noticed in ultrasonicated extracts and was nearly 2-fold higher than solvent extraction (SE) and Soxhlet extracts. Microwave-assisted extracts showed a minimal extraction yield. Ultrasonicated extracts demonstrated enhanced antioxidant properties than other extracts and displayed the highest values in all four quantitative assays (Fig. S3 a). The pulsed and continuous modes in the

ultrasound-assisted extraction revealed minor variations. The results of the antioxidant assays varied among themselves due to the variations in the radical neutralizing mechanism. Similar antioxidant properties were reported for the leaf extracts of *Psidium cattleianum* [21]. TPC was highest in US extracts (61.000 ± 0.011 mg/g GAE), followed by SE (57.540 ± 0.002 mg/g GAE), MAE (49.278 ± 0.007 mg/g GAE) and SOX (48.595 ± 0.004 mg/g GAE). A similar trend was also seen in the flavonoids, where the US had the maximum FC (159.19 ± 0.002 mg/g quercetin equivalents (QE)) and SOX had the minimum FC (34.83 ± 0.003 mg/g QE) (Fig. S3 b).

Thus, ultrasonicated extracts exhibited improved antioxidant activities with the greatest TPC and significantly higher FC. As the ultrasound-assisted extraction was a non-thermal method, it aided in sustainment of the functionality of plant bioactive, thus imposing a great advantage to it. Moreover, shorter extraction time and minimal solvent utilization also favored it [22]. Acoustic cavitation was the chief principal behind this extraction and these cavitations accelerated the solubilization of the bioactive compounds, thereby yielding superior results. Also, the ultrasonication performed with an ultrasonic probe imparted an additional benefit over an ultrasonic bath due to the uniform energy distribution and high-intensity ultrasonic waves, thereby causing better cavitation [23]. Higher extraction time and increased utilization of solvents were the major setbacks for solvent extracts. Microwave extracts also showed lower results, as microwaves could cause degradation of the heat sensitive phytochemicals. Soxhlet extracts had minimal antioxidant property and phytochemicals due to thermal decomposition of the bioactive compounds. However, the requirement of an additional concentration step also makes Soxhlet extraction less favourable [24]. Hence, the *C. pictus* leaf extracts had better antioxidant properties than their dried powders.

FTIR Analysis

Optimal extracts from all the extraction methods were subjected to FTIR analysis to analyze the impact of different extraction methods on the dried *C. pictus* leaf samples (Fig. S4). All the leaf extracts revealed characteristic ethanol bands in the FTIR spectra at ~ 3700 cm^{-1}, as ethanol was the solvent in all the extraction processes. Peaks at 3366 cm-1 and 3273 cm^{-1} indicated O-H stretching and C-H stretching was seen at 2924 cm^{-1} and 2883 cm^{-1} [25]. A broad peak attributed to O-H stretching, along with a peak for C = O at 1270 cm^{-1}, signified the occurrence of aliphatic carboxylic acid; it was uniformly present in all the extracts. C-H stretching vibrations in the aromatic aldehyde resulted in a peak around 2300 cm^{-1} and were reported only in the US samples.

Peaks associated with C-O stretching in the saturated primary alcohol and asymmetric stretching of S = O in sulfonamides were noticed at 1057.7 and 1327 cm^{-1}, respectively, for all the methods [26]. All *C. pictus* leaf extracts exhibited peaks around 3320 and 1045 cm^{-1} and implied phenolic O-H stretching and phenolic C-OC bending, respectively. The presence of phenolic compounds was indicated by peaks at 1237 cm-1 and 1245 cm-1, corresponding to the stretching of phenolic C-OH and C-O bonds in the aryl ether ring, respectively [27]; it was seen in the US and SE samples, and it was comparatively lesser in MAE extracts. These peaks were absent in SOX extracts. These substantiated the variations in the polyphenol content among these extraction methods.

A sharp peak at 1045 cm^{-1} signified the symmetric stretching observed in the C-O groups of unmodified polyflavonoids and was seen in all the extracts. Peak around 1445 cm^{-1} denoted the bending vibrations in the C-H groups of the flavonoids [28]. In-plane deformations in the C-H group of the flavonoids were observed in the wavenumbers from 600 to 980 cm^{-1} [29]. This range was observed as a broad peak in ultrasound-assisted extracts and solvent-extracted extracts; nevertheless, the former had a more intense peak than the latter, which could have contributed to the differences in the flavonoid content. The intensities of the peaks in this range were lesser in microwave-assisted extracts followed by the Soxhlet extracts and might be related to their lower flavonoid concentrations. Thus, these FTIR results confirmed that ultrasound-assisted extracts had better phytochemical contents and were concordant with the quantitative assays.

Identification of Compounds

Following quantitative analysis, the optimized ultrasound-assisted extract underwent compound identification through liquid chromatography-mass spectrometry. The resulting chromatogram (Fig. S5) and relevant studies on plant extracts [30–34] aided in the tentative identification of compounds, as presented in Table S1. Phenolic acids, including caffeic acid, 4-O-feruloylquinic acid, and dihydroxybenzoic acid pentoside, were noticed in the leaf extract. Additionally, there were observed ionizations with mass-to-charge ratios of 173 and 453, suggesting the occurrence of phenol derivatives. In the optimized leaf extracts, flavonoids such as quercetin and its derivatives, as well as kaempferol 3-O-glucoside, were detected. Ashwini et al. [35] reported major flavonoids such as kaempferol 3-O-glucoside, isoquercetin, and quercetin in *C. pictus* leaf extract and our results align with those findings.

Conclusion

The present investigation explored the efficacy of different solvents on fresh and dried samples of *C. pictus* leaves. Antioxidant properties and phytoconstituents acted as crucial elements for the optimization. Leaf samples dried at 60 °C and ethanol were chosen for further extraction. Dried powders were extracted using different methods and the US emerged as an unequivocal technique based on the critical optimization parameters. FTIR analysis also substantiated these experimental outcomes. Thus, the leaf extracts were more desirable than dried powders and fresh leaves. As *C. pictus* was widely acknowledged for its anti-diabetic properties, these leaf extracts would be a propitious choice for functional foods and pharmaceutical industries, as they would nutritionally enrich it. This study also suggested further phytochemical characterization and clinical trials with these leaf extracts as it would give a comprehensive overview of this medicinal plant and aid its implementation in varied industrial applications.

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Data Availability The published article incorporates all the generated and analyzed data.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors affirm that there are no conflicts of interest.

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