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EFFECT OF DRYING TREATMENT ON THE CONTENTS OF ANTIOXIDANTS IN *Cardiospermum halicacabum* Linn.

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ABSTRACT

Fresh and dried materials of *C.halicacabum* was evaluated for their total phenolic Content (TPC) and antioxidant activity using DPPH (1, 1-diphenyl-2-picrylhydrazyl), TPC (Total phenol content) and FRAP (Ferric Reducing Antioxidant Power) methods showed a significant reduction in antioxidant property for microwave treated plant material when compared to other drying treatments. Highest DPPH radical scavenging effect was observed in methanol extraction of microwave treated sample. The highly significant total phenol content (3.08±0.002) was recorded with freshly used plant material extracted with distilled boiled water. Proposionate to the phenolic content, extract from boiling water showed significant ferric reducing activity (5.080±0.006),due to greater solubility of compounds, breakdown of cellular constituents as well as hydrolysis of tannins. A strong free radical scavenging activity in the chosen plant material suggests that it has great potential in the food industry as a functional food ingredient.

KEY WORDS

Antioxidant activity, DPPH free radical scavenging activity, *Cardiospermum halicacabum*, Ferric reducing activity, Functional food ingredient.

INTRODUCTION

Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism.ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2**.)** and hydroxyl radicals (OH**.),** as well as non-free radical species (H_2O_2) and the singled oxygen ($1O_2$) and are excessively generated by induction of various stimuli[1] .Oxidative damages caused by free radicals mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases including, malaria, acquired immunodeficiency syndrome diabetes, anemia and cardiovascular diseases[2]. Since endogenous antioxidant defenses are not completely efficient it requires dietary antioxidants to moderate the collective effects of oxidative damage caused by ROS because antioxidants are not only needed to fight ROS but are also important as food additives; they can be either synthetic or naturally occurring. Some synthetic antioxidants possess carcinogenic activity and required to be replaced with naturally occurring ones [3].

Cardiospermum halicacabum Linn. (Sapindaceae) is an herbaceous climber found throughout the plains of India[4].This plant, commonly known as Mudakathan in Tamil is also called as Kanphuti, Kapalaphoti in Hindi, Uzinja in Malayalam and Balloon wine in English which annually spread with tendril hooks. It is used in ayurveda and folk medicine for the treatment of rheumatism, lumbago, earache and fever[5]. Experimental pharmacological studies have shown analgesic, anti-inflammatory and vasodepressant activities of this plant [6, 7].

Some herbs or crops are consumable in their fresh state and may deteriorate within a few days after harvest. One way to preserve these plant products is to dry them in either by traditional sun/shade drying or microwave drying/oven drying although it functions to inactivate the enzymes polyphenol oxidases may lead to significant changes in the composition of phytochemicals[8]. Generally, these processes may cause negative attribute to the final food product, however studies proved that the overall antioxidant properties of certain foods may instead be enhanced due to the formation of Milliard Reaction Products (MRPs),which results from a condensation reaction between amino acids (or proteins) and reducing sugars or lipid oxidation products[9]. These MRPs exhibited antioxidant activity as measured by 1, 1-diphenyl-2-picrylhydrazyl assays, however, the reducing power and iron-chelating abilities of MRPs were also reported to increase upon irradiation to scavenge hydroxyl and superoxide anion radicals under *in vitro* conditions in glucose-amino acid model system[10].

Phenolic compounds possess a wide spectrum of biological effects including antioxidant and free radical scavenging properties[11]. Phenolics are classified into two groups such as polyphenols and simple phenols[12]. Polyphenols function by trapping and scavenging free radicals and also regulate nitric oxide, decrease leukocyte immobilization, inhibit cell proliferation and angiogenesis and exhibit phytoestrogenic activity. Polyphenol Oxidases (PPOs) are copper-containing enzymes

comprised of catecholoxidases and laccase that catalyzes the aerobic oxidation of variety of phenolic substrates in the plant material into o-quinones with a concomitant O_2 reduction thus causing browning of damaged fruits or vegetables [13].

2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical and is used to measure the radical scavenging activity [14]. Moreover, the total antioxidant activity could also be measured by Ferric Reducing Antioxidant Power (FRAP), which depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH[15]. Therefore, the objective of this study is to evaluate the effects of various drying methods on the total phenol contents and antioxidant properties such as DPPH and FRAP activities of the herb *Cardiospermum halicacabum* under different solvent.

MATERIALS AND METHODS

Chemicals and Reagents

Gallic acid, Folin-Ciocalteu's reagent, linoleic acid, iron (III) chloride and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma, Potassium dihydrogen phosphate, di-potassium hydrogen phosphate, potassium ferricyanide and ascorbic acid were obtained from Merck (Germany). Solvents used were from Fisher Chemicals (Springfield, NJ)

Collection of Plant Materials

The wild plant of *Cardiospermum halicacabum* was collected in from the campus of Karunya University. The species identification was examined by comparing its morphological features and microscopic examination of the anatomy as per the standard methodologies at Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India (Ref No. BSI/SC/5/23/09-10/tech.-1).

Sample Preparation

The plants were collected on the day of extraction itself in order to maintain their freshness. Plant materials obtained were separated into two groups; one fresh and another to be subjected to various drying conditions. The whole plant was subjected to three different drying conditions namely, shade, sun and microwave oven dry, however for all the three strategies, approximately five hundred grams of the fresh plant material was washed, drained and used. For shade dry, the pre washed and drained plant material was placed on a filter paper (90x60 cm) at room temperature (27±1°C) for 3 days. For sun dry, the fresh material was placed in to the greenhouse for 3 days. For microwave oven drying, the plant material was placed in the middle of the turntable of a commercial microwave oven (SAMSUNG Model CE1031LFB; 900W) for 4 min. Once, the drying process was over, the dry weights were measured to calculate the percentage of water loss and were powdered using a laboratory blender and stored for further work.

Sample Extraction

Fifty-milligrams of each treated powder were crushed with 1 mL each of methanol (100%), distilled water and distilled boiling water (100°C) separately. It was allowed to stand for 30 min without any disturbance at room temperature and then swirled with a vortex for 5 min after which was centrifuged at 10,000 rpm for 10 min to collect the supernatant. This extract was stored at -20°C until further use [16].

Determination of Total Phenolic Content

The Total Phenolic Content (TPC) of the plant extract was determined spectrophotometrically using Folin-Ciocalteau's reagent according to the modified method[17]. Fifty microliter of the sample in triplicate was added into the test tubes followed by 1.5 mL of

2 N Folin-Ciocalteu reagent (diluted 10 times) and 1.2 mL of 20% sodium carbonate. The contents of the tubes were mixed thoroughly and stored at dark for 30 min. Phenols react with phosphomolibdic acid of Folin-Ciocalteau's reagent in alkaline medium and produce blue colored complex, that could be measured at 765 nm and expressed as mg Gallic acid per gm of plant material with Gallic acid as the standard.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay

Free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured according to the modified method[18].Different dilutions of the extracts were prepared in triplicate. Then 1 ml of each dilution was added to 2 ml of 0.15mM DPPH and vortexed for 15 to 30 sec and allowed to stand without any disturbance for 30 min at room temperature. Indication in the activity of DPPH was observed with a change in the color from purple to yellow and was measured by reading the absorbance at 517 nm. Ascorbic acid was used as the standard, while the inhibition ratio for DPPH scavenging activity was calculated from the equation:

$$AA(\%) = \frac{A_c - A_s}{A_c} \times 100$$

Where:

AA = Ascorbic acid

A_c = Absorbance of control

A_s = Absorbance of test sample

Determination of Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing property of the extracts was determined using assay described by Yen and Chen [19]. To about 50 µL of the extract, 1.5 mL of 0.1 mM FRAP solution (0.2 M Potassium phosphate buffer, pH 6.6 and 10 mM 2, 4, 6- Tris (2-pyridyl)-S-Triazine) was added and vortexed for 15 to 30 sec and incubated at 50°C for 20 min. In order to terminate the reaction, 2.5 mL of 10% trichloroacetic acid and equal volume of ultra pure water was added to the mixture after which was added 0.5 mL of $FeCl_3$ (1 g L^{-1}). The procedure was carried out in triplicate and allowed to stand for 30 min before measuring the absorbance at 593 nm. The absorbance obtained was converted to Gallic acid equivalents in milligrams per gram fresh material (mg g^{-1}) using a Gallic acid standard.

Statistical Analysis

The entire assay such as FRAP, Total phenol content and DPPH were done in triplicate for all the treated plants extracted under different solvents. The data obtained was subjected to statistical One Way Analysis of Variance (ANOVA) and the significant difference among the means were compared with Duncan's Multiple Range Test (DMRT) at ($p \leq 0.05$) level using the SPSS/PC+Student Ware software(version17.1.2).

RESULTS

The results seemed to be higher in fresh samples; this may be due to the reason that they had been a loss of moisture content approximately 35, 21.70 and 20.09% during the process of shade, sun and microwave dry, respectively. However, drying resulted in considerable shrinkage thereby making the plant material crispier and ease to powder coarsely for further analysis.

The results of antioxidants such as Total Phenol Content, DPPH and FRAP gave an interesting observation. In general, three different drying methods were involved. Although, the biochemical analysis performed with different solvents such as 100% methanol, distilled water (at room temperature) and boiled water (100°C), invariably, the samples extracted fresh showed higher activity for all the analyzed antioxidants. Different drying treatments and various solvent extracts affected the TPC and subsequent antioxidant activities of *C.halicacabum* plant extracts; in fact using methanol for microwave dried sample caused the highest TPC loss compared to fresh samples, which consequently resulted in significant decrease in the antioxidant activity as exhibited by the reduction in DPPH free radical scavenging activity too.

Highly significant (3.15±0.013 mg g^{-1}) phenol content was recorded with freshly used plant material extracted with distilled boiled water (100° C), which revealed to be higher compared to methanol extract of the fresh material (3.08.±0.002), however the same in distilled water yielded only 2.05±0.002 mg g^{-1}. Among the drying conditions, both sun and shade dried extracts recovered good percentage of TPC when compared to microwave dried extract.

Effect of drying treatments on Total phenol contents of C.halicacabum

Total phenol content

Plant part (leaves)	Fresh		Sun dry	
	(mg g^{-1}) phenol	Mean±SEM	(mg g^{-1}) phenol	Mean±SEM
Methanol	3.08	1.491±0.0026	2.32	0.941±0.0025
Distilled water	2.05	1.525±0.0020	1.54	1.552±0.0012
Distilled boiled water	3.15	1.451±0.0136*	1.88	1.046±0.0020

Plant part	Shade dry		Microwave	
	(mg g^{-1}) phenol	Mean±SEM	(mg g^{-1}) phenol	Mean±SEM
Methanol	2.18	0.878±0.0042	1.24	0.990±0.0025
Distilled water	2.05	1.135±0.0081	1.11	1.644±0.0006
Distilled boiled water	2.15	1.404±0.0237	1.99	1.980±0.0125

*Data expressed as Mean±SEM of triplicates. * Indicates the significant difference (p≤0.05)*

Microwave dried sample yielded very poor content of TPC and was revealed to be lesser approximately 20.93, 16.27 and 25.58% in extracts of methanol, distilled water and distilled boiled water, respectively, when compared to the high significant values of fresh sample.

The activity of DPPH under different conditions was observed and compared in *C.halicacabum*. In contrast to the availability of TPC, the activity of DPPH declined in the freshly extracted sample. Methanolic extraction of Microwave treated sample showed significantly increasing value of DPPH activity (82.039±0.221 mg AA g^{-1}). Extraction of shade dried plant material ranked second in the order of DPPH activity. It showed an activity of 48.679±0.2623 mg AA g^{-1} which was 63.2 % in methanolic extract that revealed to be lesser compared to the microwave dried methanolic extract.

Effects of drying treatments on DPPH activity of C. halicacabum

DPPH radical Scavenging activity

Plant part (leaves)	Fresh		Sun dry	
	(mg AA g^{-1}) DPPH activity	Mean±SEM	(mg AA g^{-1}) DPPH activity	Mean±SEM
Methanol	22.075	22.075±0.3359	24.045	24.045±0.1477
Distilled water	33.670	33.670±0.2392	35.793	35.793±0.1081
Distilled boiled Water	15.439	15.439±0.2141	9.625	9.625± 0.2153

Plant part	Shade dry		Microwave	
	(mg AA g^{-1}) DPPH activity	Mean±SEM	(mg AA g^{-1}) DPPH activity	Mean±SEM
Methanol	23.270	23.270±0.1454	82.039	82.039±0.2216*
Distilled water	48 .679	48.679±0.2623	72.216	72.216±0.2793
Distilled boiled water	16.045	16.045±0.2730	61.907	61.907±0.3278

Data expressed as Mean±SEM of triplicates. * Indicates the significant difference (p≤0.05)

However, the least activity of 9.625±0.2153 mg AA g^{-1} was recorded in the extract of sun dried plant material, which was revealed to differ about 90.59% especially in extracts obtained from distilled boiled water.

The ferric reducing activity of *C.halicacabum was* increased in the extracts obtained from fresh materials. However, extract from boiling water showed significant Ferric reducing activity of 5.080±0.006 mg g^{-1}. Among the drying process, great variation was observed, where shade dried plant material yielded in FRAP compound than sundry and microwave treated material

Effect of drying treatments on ferric reducing activity of *C. halicacabum*

Ferric reducing antioxidant power

Plant part (leaves)	Fresh		Sun dry	
	(mg AA g^{-1}) FRAP activity	Mean±SEM	(mg AA g^{-1}) FRAP activity	Mean±SEM
Methanol	4.245	1.020±0.0353	2.920	0.963±0.0090
Distilled water	3.945	0.830±0.0075	3.590	0.764±0.0069
Distilled boiled Water	5.080	0.916±0.0059*	3.600	0.612± 0.0069

Plant part	Shade dry		Microwave	
	(mg AA g^{-1}) FRAP activity	Mean±SEM	(mg AA g^{-1}) FRAP activity	Mean±SEM
Methanol	3.240	0.735±0.0156	0.36	0.193±0.0075
Distilled water	3.170	0.522±0.0097	0.45	0.268±0.0072
Distilled boiled water	3.190	0.598±0.0066	0.61	0.171±0.0032

*Data expressed as Mean±SEM of triplicates. * Indicates the significant difference (p≤0.05)*

Among the shade dried material, boiled water and methanolic extract produced the higher activity of 0.598±0.006 and 0.735±0.015 mg g^{-1} respectively and showed a negligible difference between them. However, it was approximately higher than the extracts in distilled water. Nevertheless, microwave treatment totally declined the activity and proved to be fatal for the examination. Data obtained from this study indicate that drying of plant materials tend to lower the TPC values to a varying extent.

DISCUSSION

Present data showed that *C.halicacabum* extracts possessed high phenolic content, and exhibited strong free radical scavenging activity and ferric reducing property. Large quantity of phenolic compounds in *C.halicacabum* extract makes it a strong free radical scavenger, which indicates that the extract has good potential as a source for natural antioxidants to prevent free radical mediated oxidative damage. Of course there could be few explanations for the decrease in both TPC and antioxidant activity of the extracts during the process of drying that attributes the deactivation of the degradative enzymes.

Perhaps sun drying leads to an uneven loss of TPC, while oven heating at 100^0C itself rapidly inactivates polyphenol oxidases present in plant materials by absorbing the water molecule through microwave energy. Heating treatment not only deactivates enzymes, but also degrades phytochemicals, some phenolic compounds decompose rapidly in direct sunlight or if dried at elevated temperature[20].

Drying process would generally result in a depletion of naturally occurring antioxidants in raw materials from plants[21]. Intense and/or prolonged thermal treatment may be responsible for a significant loss of natural antioxidants, as most of these compounds are

relatively unstable. Yet in some cases, drying process causes little or no change to the content and activity of naturally occurring antioxidants, such as carotenoids and vitamin C (e.g. lycopene)[9].Another cause of depletion of antioxidants could be due to operations such as peeling, cutting and slicing as they induce a rapid enzymatic oxidation of natural antioxidants. In contrast to the result obtained in this investigation, numerous other studies reported the increase in both TPC and antioxidant activity of samples after processing. One of the researcher reported that sun drying of green leafy vegetables caused a significant increase in TPC, reducing property, and free radical scavenging activity, even though there was a significant decrease in vitamin C content[22]. Another investigation reported that air drying resulted in a considerable increase of TPC in oregano and peppermint leaves but no significant difference was observed for lemon balm. This investigation clearly explains that the drying process may result in high or low levels of TPC depending on the type of phenolic compounds present in the plant material and their location in the cell[8].

Solvent used for extraction is another factor to be considered to influence the quantification of antioxidants. Methanolic extracts of fresh samples possessed both higher TPC and antioxidant activity than cool water extracts as methanol was able to denature polyphenol oxidases. Because being an organic and volatile solvent, it is more efficient in plant cell wall degradation, therefore, able to extract a greater amount of endocellular materials than water[16]. However, processed plant materials extracted by methanol led to the decrease in both TPC and antioxidant activity but not in their aqueous extracts. Infact, boiling water extracted significantly higher TPC from both fresh and processed plant materials than cool water.

Higher TPC in boiling water extracts led to stronger radical scavenging activity in DPPH and FRAP assay. Boiling water and methanol were able to completely inactivate degradative enzymes present in fresh plant materials, thus yielding higher TPC than cool water. However, in dried plant materials where polyphenol oxidases have been inactivated, methanol was still found to yield lower TPC than both boiling and cool water. One explanation could be the presence of certain very polar compounds in dried plant materials, which can only be extracted with very polar solvent, such as water. Then again, boiling water extracted more polyphenols from dried plant materials than cool water, which means the heat from boiling water was the factor that led to higher total phenols being extracted from dried plant materials. It was reported that intense heat from boiling water was able to release cell wall phenolics or bound phenolics due to the breakdown of cellular constituents, thus causing more polyphenols to be extracted[23]. Moreover, the high temperature from boiling water also increases the solubility of phenols[24].

Researchers mentioned the possible breakdown of tannin to simple phenol when high temperature was used during extraction process, which increased the number of compounds with free hydroxyl groups[18]. Extraction of hydrolysable tannins from plants with hot water is accompanied by different degree of hydrolysis of tannins, resulting in variation of the antioxidant activities. Data in the effects of drying, antioxidant activity and TPC of herbs and vegetables are conflicting due to several factors. Such as different drying conditions, type of extraction solvents, and antioxidant assays used. In conclusion, this study indicated that methanolic extracts of fresh plant materials possessed lower antioxidant properties than dried samples. Plant phenolic compounds are present in different binding status depending on plant species.

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