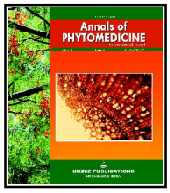
Annals of Phytomedicine 10(2): 399-408, 2021

DOI: http://dx.doi.org/10.21276/ap.2021.10.2.53

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Effect of drying methods on proximate analysis and antioxidant activities of ripe and unripe fruits of *Diplocyclos palmatus* (L.) C. Jeffery

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Article Info

Article history

Received 11 October 2021

Revised 27 November 2021

Accepted 29 November 2021

Published Online 30 December 2021

Keywords

Antioxidant assay

Freeze-drying

Hot-air/tray drying

Phenolic content

Proximate analysis

Abstract

Ethnopharmacology has been the mainstay of traditional medicines of the entire world and currently is being integrated into mainstream medicine. The objective of the present study was to examine the impact of two drying techniques on proximate and bioactivity assays of two stages, i.e., green and red fruits using different solvents of the selected under utilized plant, *Diplocyclos palmatus* (L.) C. Jeffery. The proximate, reducing power assay, hydrogen peroxide assay, nitroblue tetrazolium (NBT) assay has not been reported from the selected plant. All the experiments were carried out using standard methods with slight modifications. Proximate confirms with highest protein content from tray dried red fruit 17.55% high amount of fat 5.8% from freeze-dried red fruit and tray dried green fruit shows high moisture content 9.45%. The most remarkable findings of this experimental study were EtOHTR Soxhlet extract have shown maximum antioxidant activity of 95.21 ± 1.95 with DPPH, EATR Soxhlet extract showed highest total flavonoid content with 11.9 ± 0.5 and similar impact for NBT assay with the highest percentage of activity 38.9 ± 1.2 reveals temperature with ethyl acetate as favored for green fruits. The freeze-dried temperature as favored for both the fruits with ethanolic cold maceration showed the highest TPC, RPA, and on contrary to this freeze-dried ethyl acetate Soxhlet extract of both the fruits have shown highest activity with ABTS++ 60.8 ± 1.9 for red and 55.3 ± 0.3 for green fruits. The obtained experimental data can be utilized for further evaluation of enzyme activity and toxicity assays which will be useful for the bioprospecting of traditional Medicinal plants.

1. Introduction

Traditional dietary guidelines, health professionals, AYUSH, Pharmacognosy literature including food and agricultural organization , insist on the quality utilization of plant-based foods for the avoidance of chronic diseases and maintaining good health. There has been extensive importance in the bioactive constituents derived from plants, which include a mass of compounds with diverse identities and mechanisms of action that could be the source of nutraceuticals. Plants are natural reservoirs of constituents consumed by living organisms reveals that are almost free from side effects and have better compatibility with the human system when compared to synthetic chemicals. Thus, plant-based products have been an integral part of nutrition and primary human healthcare from time immemorial (Venketeshwer, 2012; Bhavani, 2013; Zengina *et al.*, 2016).

Analytical challenges exist right from the extraction, establishment of bioactivity, and characterization to the level of application as nutraceuticals. Conventionally extracts are subjected for bioactivity assays not giving much importance to the impact of extraction methods on bioactive constituents. However, information regarding qualitative changes that occur during extraction on phytoconstituents as well as bioactivities and suitable methods of sample preparation before extraction of herbal drugs are limited to date (Puranik *et al.*, 2012; Devi *et al.*, 2019).

Many reports are substantiating that phytoconstituents have been proved to be the best source of antioxidants (Monte *et al.*, 2014; Jiménez *et al.*, 2015). In the past years, considerable researches have demonstrated that oxidative stress is the root cause for a wide variety of human disorders (Halliwell and Gutteridge, 1984; Gelderman *et al.*, 2007; Williams *et al.*, 2014; Wang *et al.*, 2017).

The present investigation is focused mainly to ensure the precise content of nutrients during bioactivity estimation of the selected fruits of *D. palmatus* is a wild less widely recognized Cucurbitaceae species. During the experimental period, chances of variations in the quality of constituents may occur, therefore, the present study is to find out the effect of temperature as quality criteria and also on bioactive potency, by subjecting the sample for proximate analysis and antioxidant assays.

Cucurbitaceae comprises about 120 genera and 775 species (Mabberley, 1997), India 31 genera and 94 species are widely distributed (Renner and Pandey, 2013) which are commonly known as squashes, gourds, melons, *etc*., despite large and diverse species are well known as vegetables and fruits, there is cause to wonder why some of the wild cucurbits have been largely ignored by crop

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reporting services, planners, nutritionists and others responsible for the quality and quantity of our food supply (Bates *et al*., 1990). The wealth of ethnopharmacological applications of many rare species has not been recognized.

D. palmatus is a wild creeper commonly found distributed in the warmer forests of India, Australia, Africa, Malaysia, and Southern regions of China, and in most parts of the world, it is still a wild and underexplored creeper. But, in many regions, though the plant is wild, there are no reports due to a lack of awareness regarding its distribution and applications. Many reports are available regarding its ethnopharmacological relevance such as fruits are used by the Australian aboriginal for skin disorder and ringworm (Packer *et al*., 2011), fruits are edible/consumed in a few regions of India (Tripathy *et al*., 2013), leaves and fruits are common vegetables in Kenya, South East Asia (Njoroge and Newton, 1994) leaves and fruits are used as a laxative, expectorant and also for the treatment of impotency, female infertility and leucorrhea (Singh and Malviya, 2006). Ethnopharmacological relevance with comprehensive documentation of *D. palmatus* has been reported by many investigators (Mosaddik *et al*., 2003; Kokate *et al*., 1993; Gupta and Wagh, 2014; Kumar and Totawar, 2015).

Enzymatic degradation and microbial contamination can be prevented by drying techniques (Harbourne *et al*., 2009). An exhaustive review of the literature reveals that absolutely no reports are available concerning the impact of temperature on proximate analysis as well as the bioactive composition of the selected fruits.

2. Materials and Methods

2.1 Plant material

D. palmatus (DP) is a slender, highly branched seasonal creeper with a 6 m long stem. Leaves simple, alternate, palmately lobed, with obnoxious odor when mashed. Flowers are unisexual, white to yellowish, female flowers solitary and male flowers in small fascicles. It flowers from August to September. Fruit green with white longitudinal stripes, which turn out to be intensely red with ripeness (Sanbi.org/resource/Infobase). The samples of the unripe green and ripe red fruits of DP were collected, identified, and authenticated. The Voucher Specimen (UOMBOT19DP1) was deposited in the herbarium of the Department of Studies in Botany Manasagangotri, Mysore. The unripe (green) and ripened (red) fruits were separated, washed thoroughly with distilled water, and surface dried with the muslin cloth.

2.2 Physical measurement of fruits

The fruit length and size were taken using a vernier caliper MITUTOYO in/mm Lcd Digital Electronic Carbon Fiber Vernier Caliper Gauge Micrometer (Figures 1 and 2).

Figure 1: Ripe red fruits.

Figure 2: Unripe green fruits.

2.3 Estimation of pH and brix

As a prerequisite pH was determined, 10 ml of both the green and red fresh fruit extracts were taken in two beakers and the pH meter was calibrated with phosphate buffer of pH 4.0 and 7.0, then the probe was immersed into the extracts and the readings were recorded. Estimation of °Brix (Total soluble solids) using a HI96801 refractometer (0 to 85% °Brix/0 to 80 dC (32 to 176 dF) (Horwitz and Latimer 2006; Nonga, 2014). The refractometer was standardized with distilled water at 0, then the samples were placed and readings were recorded.

2.4 Preparation of samples

The cleaned fresh succulent fruits were subjected to two different drying processes to study the impact on proximate as well as bioactivity.

2.5 Hot air/tray drying method

The cleaned fruits were sliced to make uniform-sized slices (Figures 3 and 4), then spread onto a clean tray for proper drying using a dryer (Drying model 0 24 E, Kilburn, and a company limited, Bombay-1) (Sun *et al*., 2015). To decide the best suitable temperature for drying, samples were first subjected to different temperatures, and then it was subjected at 50 °C 2m/s air flow rate and 45% relative humidity for 10 h. After drying, samples were ground with a blade mixture, and the size of powder varied from 0 to 0.63 mm, the powders were stored in zip lock covers and stored in desiccators until further use. These samples were named as TR for tray dried red fruits, TG for tray dried green fruits.

Figure 3: Tray dried red fruits.

Figure 4: Tray dried green fruits.

2.6 Freeze-drying method

Sliced fruits (Figure 5) were subjected to freeze-dry at -85°C and for 12 h using a Laboratory freeze dryer (GAMMA 2-16 LSC plus Germany), (Hossain *et al*., 2010). After drying, samples were ground with a blade mixture and stored at desiccators, and the size of powder varied from 0 to 0.63 mm, the powders were stored in zip lock covers until further use. These samples were named FR freeze-dried red fruits, FG for freeze-dried green fruits.

Figure 5: Freeze dried red and green fruits.

2.7 Proximate analysis

The parameters include moisture, crude protein, ash, crude fat; and carbohydrate, color all of these were carried out by the methods (Horwitz and Latimer, AOAC 2006).

2.8 Moisture

Moisture content in the sample was determined by using a hot air oven (Drying model 0 24 E, Kilburn and company limited, Bombay-1). To a previously weighed clean empty Petri dish add approximately 5 g of the sample and spread on the Perti dish homogeneously. The sample was heated at the temperature of 110°C for 5-6 h until a stable weight was achieved. Moisture percentage was obtained from the weight loss due to heating.

% of Moisture = (Initial weight of sample – weight after drying) ×100/Initial weight of the sample.

2.9 Total crude protein

The crude protein of the fruit extracts was represented by measuring the nitrogen (N) content. This determination was done based on the standard. Briefly, 0.5 g of sample was placed in a Kjeldahl flask. Subsequently, 2 g of catalyst mixture (10 parts K_2SO_4 to one part of $CuSO_4$) and 25 ml of concentrated H_2SO_4 were added into the Kjeldahl flask. All the components were mixed thoroughly and heated until the mixture became clear. Wash the flask with distilled water and swirl to mix. Transfer the digest to a 250 ml volumetric flask using a funnel. Measure approximately 15 ml of 2% of boric acid solution into the conical flask with a mixed indicator of 2-3 drops (10 ml of 0.1 ml bromocresol green and 2 ml of methyl red) in a 150 ml conical flask to the distillation assembly using a pipette. Add a small amount of distilled water and then add 20 ml of NaOH connect steam to distillation assembly. Distillation was done within 10 min and then the produced NH_3 was collected (in the form of NH_4OH) in the conical flask was determined by titrating with N/70 HCL. The amount of total nitrogen and crude protein was calculated by using the formula:

Crude protein content (%) = % of Nitrogen × 6.25

2.10 Total ash

An empty crucible was accurately weighed, and approximately 0.5-1.0 g of samples were weighed in it using a weighing balance. The sample in the crucible was charred with Bunsen burner, then placed in a muffle furnace at 550°C for more than 5 h until the ash completely turn to white ash, then crucible was carefully removed from the furnace to a dissector to cool, and then weighed.

2.11 Crude fat

The crude fat content of samples was determined by the Soxhlet unit. About 1 g of moisture-free sample was tied with a whatmann filter paper, placed in a soxhlet extraction unit. Extraction was continued for 8 h using petroleum ether as a solvent, after the extraction the pre-weighed round bottom flask containing the extracted fat was dried on a water bath at 98°C ± 5°C. After complete drying, the round bottom flask was cooled in desiccators and the weight was recorded. The percentage of crude fat was calculated by the differences in the initial and final weight of the round bottom flask on a wet weight basis.

2.12 Carbohydrates

The value of total carbohydrate was obtained by = 100 – (Percentage of protein + ash + fat + fiber).

2.13 Color measurement

Color measurement was made by using a lab scan XE spectro-photometer (Hunter Lab scans XE, USA) and it was calibrated to white and black standard sites of samples. The tristimulus L*a*b* measurement model was used as it relates to the human eye response to color. The L* variable lightness (L*=0 for black, L*=100 for white), a* scale represents the red/green. (+a* intensity in red and –a* intensity in green) and the b* scale represents the yellow/blue (+b* intensity in yellow and –a* intensity in blue). The fruit samples were filled into a clear Petri dish and transmittance readings were taken further triplicate readings were taken for each sample.

2.14 Preparation of solvent extracts

The fruit samples of both tray dried and freeze-dried (Red and Green) were taken for two different methods of extraction using three different solvents, *viz*., methanolic freeze-dried red fruits (MFR), methanolic freeze-dried green fruits (MFG), ethanolic freeze-dried red fruits (EtOHFR), ethanolic freeze-dried green fruits (EtOHFG), ethlyacetate freeze-dried red fruits (EAFR), ethlyacetate freeze-dried green fruits (EAFG). Methanolic tray dried red fruits (MTR), methanolic tray dried green fruits (MTG), ethanol tray dried red fruits (EtOHTR), ethanol tray dried green fruits (EtOHTG), ethlyacetate tray dried red fruits (EATR), ethlyacetate tray green fruits (EATG), extractions were carried out according to Harborne 1973.

2.15 Soxhlet extraction

Hot continuous extraction: The finely ground crude powder is placed in a porous bag or "thimble'' which is placed in the Soxhlet apparatus. The extracting solvent in the flask is heated and the vapors condense in a condenser. The condensed extract drips into the thimble containing the crude drug when the level of the liquid chamber rises to the top of the siphon tube, the liquid contents of the chamber siphon into the flask. This process is continuous (6 to 8 h) and is carried out until a drop of solvent from the siphon tube does not leave the residue when evaporated. Then filter the extracts to allow drying with a flask evaporator then stored them at 4°C for further analysis.

2.16 Cold maceration

Around 5 g of dried fruit sample was taken into a conical flask and a known volume of solvent was added, kept for extraction in a rotary shaker (Thermo scientific model MAXQ 4000) at 37°C temperature, 150-200 rpm for 24 h. Then filtered extracts were dried then stored at 4°C for further experiments.

2.17 Percentage yield

The dried fruit powder was subjected to both extraction methods (Soxhlet and maceration), after the completion of extraction, the solvent extracts were allowed to evaporate at room temperature to get dry material. The percentage of yield was calculated by using the following formula:

$$\% \text{ of Yield (g/100 g)} = (W1/W2) \times 100$$

W1- Weight of the dried extract, W2- Weight of the initial dried fruit sample.

2.18 Estimation of total phenolic content (TPC)

The TPC was determined using Folin-Ciocalteu (FC) reagent according to the method with slight modification (Singleton and Rossi, 1965). Briefly, 10 µl of extract from (10 mg/ml) was mixed with 200 µl of FC reagent (1:10 folds) and incubated for 5 min at room temperature then 25 µl of 10% of sodium bicarbonate solution was and incubate at 37°C for 30 min, the absorbance was measured at 765 nm. The TPC was evaluated from the gallic acid standard curve (GAE µg/mg).

2.19 Estimation of total flavonoid content (TFC)

The TFC was determined by the method with slight modification (Sakanaka *et al*., 2005) Fruit extracts (50 µl) of each solvent was taken separately and add distilled water to make the volume 0.5 ml followed by the inclusion of 75 µl of 5% (w/v) sodium nitrite solution and incubate for about 6 min using water bath at 37°C, followed by addition of 150 µl of 10% (w/v) $AlCl_3$ and again incubate for 5 min at room temperature and finally add 0.5 mlof 1M NaOH, and addition of 100 µl distilled water to make up the volume to 2 ml, the absorbance was measured at 415 nm. The total flavonoid content was evaluated using (RE µg/mg) rutin standard curve (0.8 mg/ml) stock.

2.20 Antioxidant assays

Each extract was dissolved in respective solvents (methanol, ethanol, and ethyl acetate) to make a stock of 10 mg/ml (10,000 micrograms) from this required concentration used for assays. All the chemicals and reagents were used are laboratory grade, and reference chemicals were used for comparison in a few assays.

2.21 DPPH free radical scavenging activity (2, 2-diphenly-1-picrylhydrazyl)

The DPPH assay of the extract was followed according to the method with slight modification (Brand-Williams *et al*., 1995). By dissolving 6.3 mg DPPH in 100 ml methanol, the stock was prepared kept in a refrigerator until additional use. 10 µl (Concentration 100 µg/ml) of the extract was mixed with 100 µl of DPPH solution in a 96 well plate. The plate was incubated at 37°C for 60 min, and then read the absorbance at 517 nm. The percent of antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

where, Ac and As is the absorbance of control and sample, respectively. The control consists of 100 µl methanol in the position of plant samples.

2.22 $ABTS^{++}$ radical scavenging assay (2, 2-azino-bis (3-ethlybenzothiazoline-6-sulfonic acid)

$ABTS^{++}$ radical scavenging activity was carried out using the method (Sachindra *et al*., 2010) with slight modification. ABTS radical solution was prepared by mixing 5 ml of ready-to-use $ABTS^{++}$ solution with 100 ml of acetate buffer (0.05 M, pH 4.5) and 5 units of peroxidase and incubating at 37°C for 15 h. The decolorization of the $ABTS^{++}$ radical solution was started by mixing 1.9 ml of $ABTS^{++}$ solution with 15 µl (concentraion 150 µg/ml) of sample and incubating at 37°C for 1 h. The absorbance was measured at 734 nm. Scavenging activity (SA) % was calculated as follows:

$$SA, \% = [1 - (As \text{ sample} - As \text{ sample blank})/A \text{ control}] \times 100$$

2.23 Hydrogen peroxide scavenging assay

The activity was determined by the following method (Ruch *et al*., 1989) with minor modification. An aliquot of 0.6 ml of methanol for control and 50 µl (concentration 500 µg/ml) of different solvent extracts and made up to 0.6 ml with methanol, then add 0.6 ml 40 mM H_2O_2 and PO_4 buffer incubate for 10 min at room temperature and read the absorbance at 230 nm. The blank solution contains Na_3Po_4 buffer without H_2O_2. The scavenging of H_2O_2 (mM) in the assay was calculated using the following formula.

$$H_2O_2 \text{ Scavenging effect (\%)} = [(A0 - A1)/A0 \times 100]$$

A0= Absorbance of control, A1= Absorbance of the sample.

2.24 Superoxide anion radical scavenging activity (Nitroblue tetrazolium)

The assay was done based on the reduction of NBT in the presence of NADH and phenazine methosulfate according to the method prescribed by (Chou *et al*., 2009) with slight modification. The reaction mixture contains 100 µl of (156 µM) NBT, 100 µl of (468 µM) NADH, and 10 µl of extract. The reaction was commenced by adding up to 10 µl of (60 µM) phenazine methosulfate. All the reagents were prepared by using (0.1 M, pH 8.0) and the absorbance was recorded at 560 nm after 5 min incubation at room temperature. Percentage of inhibition was calculated against a control that contains all the reagent, and PO_4 buffer in place of the sample. To diminish in absorbance with plant extract and the reference compound illustrate their competence to quench superoxide radicals in the reaction mixture.

$$\% \text{ of Scavenging activity} = [(Ac - As)/Ac] \times 100$$

2.25 Reducing potential assay

The reducing power of all the extracts was determined according to the method developed by (Oyaizu, 1986) with a small alteration. A 50 µl of each extract was mixed with 150 µl of (0.2M, pH 6.6) and 200 µl of K_4 [$Fe(CN)_6$]. $3H_2O$ (1%, w/v) was added, mixed well, and incubated in a water bath for 20 min at 50°C. Then 200 µl of trichloroacetic acid (10% w/v in distilled water) was added and centrifuged at 8000 rpm for 10 min, and its 0.5 ml of supernatant was mixed with the corresponding volume of distilled water and 94 µl of 0.1% $Fecl_3$. It was further incubated at 20°C for 10 min and absorbance was read at 700 nm. The reducing potential of the sample was indicated as the variation in optical density from control as well as test, the highest the degree of absorbance the stronger the reducing power.

2.26 Statistical analysis

All experiments were carried out in triplicates (n = 3) and the results were expressed as mean values and standard error (SE). The differences between the tested extracts were analyzed using one-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference post hoc test with $p>0.05$. This treatment was carried out using SPSS v.14.0 program.

3. Results

3.1 Physical parameters

The obtained results of all the physical parameters were represented in Table 1. The size of the selected fruit's height, weight, and width was not significantly different. However, ripe red fruits measured slightly higher values (18.26 ± 1.27) over unripe green fruits (17.91 ± 0.73). There was a significant difference in °Brix value, the red fruit showed a higher value with 5.4, whereas a decline was noticed in green fruits with 2.1. The fruits with different colors after subjecting to different temperatures did not show any significant variations in the expression of color in powdered form. However, the green fruit showed slightly more lightness and yellowness compared to red fruits, redness was more towards red fruits.

Tray-dried and Freeze-dried both green and red fruits were analyzed for moisture, protein, ash, crude fat and carbohydrate content were given in Table 2. Tray-dried both the fruit samples exhibited almost similar percentage of moisture contents, but the Freeze-dried fruits showed little variation, the red fruits had retained moisture of 6.01 ± 0.56, and green fruits showed 4.20 ± 0.25 which is much low when compared with tray dried fruits. There was a difference in crude protein content between tray dried and freeze-dried, the highest percentage was observed in tray dried red fruits followed by freeze-dried 17.55 ± 0.48 and 14.79 ± 0.06, respectively. Green fruits showed little higher in freeze-dried with 9.75 ± 0.04, whereas lowest recorded in tray dried with 2.11 ± 0.09, indicating ripe fruits tray dried have more percentage of crude protein content. The result shows freeze-dried green fruits recorded the highest ash content (13.1 ± 0.41) and tray dried with low ash content (6.02 ± 0.87).

Table 1: Physical parameters of both red and green fruits of *D. palmatus*

Variable	Red	Green
Height (mm)	18.26 ± 1.27	17.91 ± 0.73
Weight (g)	3.26 ± 0.37	2.99 ± 0.267
Width (mm)	18.44 ± 0.70	17.69 ± 0.464
pH	6.13 ± 0.0058	5.643 ± 0.00578
°Brix	5.4	2.1
Color		
L*(lightness)	43.69	44.33
a*(redness)	4.04	-1.16
b*(yellowness)	12.96	18.45

Each value is the average of triplicate measurements. L-lightness (100, white; 0, black), a: redness (–, green; +, red), b: yellowness (-, blue; +, yellowness).

3.2 Yield

The highest yield (Table 3) was obtained from freeze-dried red fruits of methanolic Soxhlet extracts, and the lowest values were obtained from tray dried green fruits of ethyl acetate cold extracts.

3.3 Total phenolic content

The total phenolic content (TPC), in the present study, recorded the highest concentration from EtOHFR cold macerated fruits with 21.6 ± 0.17, followed by EtOHFG cold macerated with 19.5 ± 0.17, indicated that both red and green fruits have retained phenolic contents under freeze-dried temperature (Figure 6). The lowest concentration recorded was EATG Soxhlet extracted with 12.2 ± 0.06. Phenolic compounds are treated to be the primary antioxidants because they are the chain-breaking molecules that react with free radicals to stabilize them.

3.4 Total flavonoid content

Total flavonoid concentration (TFC) was noted highest from EATR Soxhlet extracts with 11.9 ± 0.5 and EtOHTR Soxhlet with 11.3 ± 0.2. The lowest concentration was recorded from MFG cold extracts with 1.1 ± 0.1 which is indicative that fully ripe fruits have a high concentration of flavonoids (Figure 7).

3.5 Antioxidant assay by DPPH

The obtained experimental results of both TPC and TFC have been further approved for their biological activity when assessed for antioxidant assay with DPPH. Both EtOHTR and EtOHFR Soxhlet

extracts have shown the highest percentage of antioxidant activity with 95.21 ± 1.95 and 93.63 ± 0.05 respectively, (Figure 8). The lowest recorded was 12.12 ± 4.92 from the EAFR cold extracts; reveals that red fruits have maximum antioxidant potency in both the temperature indicating temperature favored for the release of bound form of phenolics enhanced the reactivity. Phytoconstituents of plants have antioxidant properties which can prevent various biochemical processes.

Table 2: Proximate analysis red and green fruits of *D. palmatus*

Sample	Moisture (%) (%) N×6.25)	Crude protein	Ash (%)	Crude fat (%)	Carbohydrates (%)
Red fruit (TD)	9.40 ± 0.28	17.55 ± 0.483	8.02 ± 0.32	3.19 ± 0.01	61.72 ± 0.16
Green fruit (TD)	9.45 ± 0.32	2.11 ± 0.098658	6.02 ± 0.87	2.69 ± 0.11	79.41 ± 0.28
Red fruit (FD)	6.01 ± 0.56	14.79 ± 0.064	12.9 ± 0.1	5.8 ± 0.06	60.37 ± 0.11
Green fruit (FD)	4.20 ± 0.25	9.75 ± 0.043589	13.1 ± 0.41	2.9 ± 0.064	70.03 ± 0.02

The standard deviation for three replicate (n=3) determinations TD= Tray dry FD= Freeze dry; GF-Green fruit, RF- Red fruit.

Table 3: Percentage yield of both TD and FD DP fruits powder with cold and Soxhlet extraction using different solvents

	TD (Cold)		TD (Soxhlet)		FD (Cold)		FD (Soxhlet)	
Extracts	**GF**	**RF**	**GF**	**RF**	**GF**	**RF**	**GF**	**RF**
Methanol	32.87 ± 0.13	22.14 ± 0.01	31.11 ± 0.02	22.56 ± 0.015	14.89 ± 0.006	15.96 ± 0.01	34.68 ± 0.01	35.25 ± 0.015
Ethanol	5.592 ± 0.01	7.04 ± 0.006	17.55 ± 0.01	17.695 ± 0.015	5.263 ± 0.001	6.68 ± 0.005	5.72 ± 0.006	19.17 ± 0.006
Ethyl acetate	1.52 ± 0.01	2.23 ± 0.012	3.12 ± 0.017	4.08 ± 0.012	1.97 ± 0.012	2.16 ± 0.005	2.239 ± 0.00	18.99 ± 0.012

The value expressed are means ± SD of three measurements.TD = Tray dry FD = Freeze dry; GF-Green fruit, RF- Red fruit.

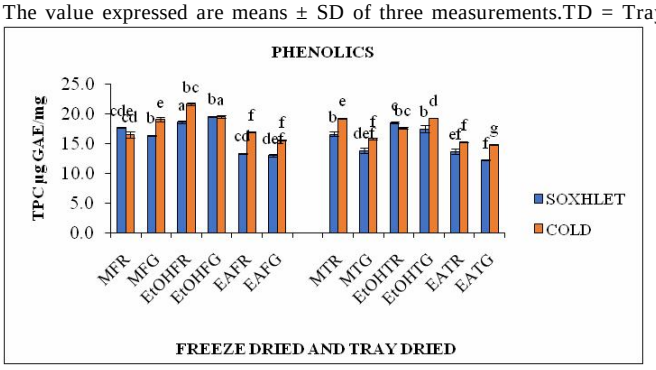

Figure 6: Total phenolic content of *D. palmatus* fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-g) in the same column and row differ significantly (p<0.05). M-Methanol, EtOH-Ethanol, EA-Ethyl acetate, F-Freeze dried, T-Tray dried, R-Red fruit, G-Green fruit.

3.6 ABTS++ radical scavenging assay

This particular assay is to find out the total antioxidant activity about the extract and it was observed that both the EAFR and EAFG Soxhlet extractions exhibited maximum activity with 60.88 ± 1.9 and 55.3 ± 0.3, respectively, lowest activity recorded from MTG cold macerated with 4.2 ± 0.8, indicating low temperature has not favored for the release of radical scavenging phytoconstituents (Figure 9).

3.7 Reducing power assay

Reducing power activity of compounds specify that they can donate an electron and without difficulty reduce the oxidized intermediates of lipid peroxidases, thus phytoconstituents act as strong antioxidants. The highest reducing potentiality has been recorded from the EtOHFG cold macerated with 1.5 ± 0.001, followed by similar potency from MFG Soxhlet extract and EAFR cold macerated with 1.3 ± 0.021 and 1.3 ± 0.014, respectively (Figure 10).

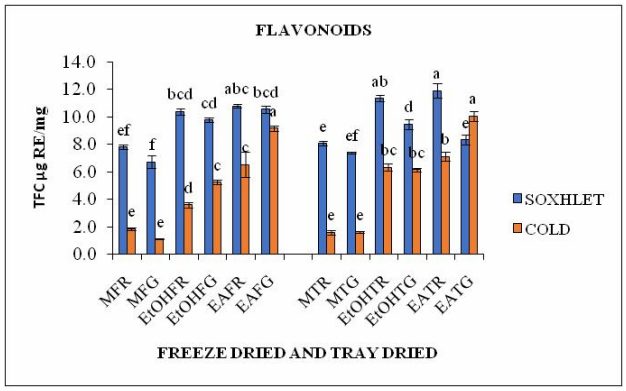

Figure 7: Total flavonoid content of D.palmatus fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-f) in the same column and row differ significantly (p<0.05). M-methanol, EtOH-ethanol, EA-ethyl acetate, F-freeze dried, T-tray dried, R-red fruit, G-green fruit.

3.8 Hydrogen peroxide assay

While dealing with reactive oxygen species, according to literature singlet oxygen or hydroxyl ions cannot cross the cell membranes but hydrogen peroxide can easily cross the membranes, thus it is an essential parameter to be evaluated. The obtained result is indicative that EAFG and EAFR Soxhlet have shown highest percentage with 99.8 ± 0.02 and 99.2 ± 0.023 (Figure 11). Thus, both the fruits have exhibited the highest scavenging activities, lowest recorded is somewhat difficult to assess with a 2.1 ± 0.07 from MFR cold

macerated, may be both freeze-dried and cold maceration has not favored for the release of important compounds.

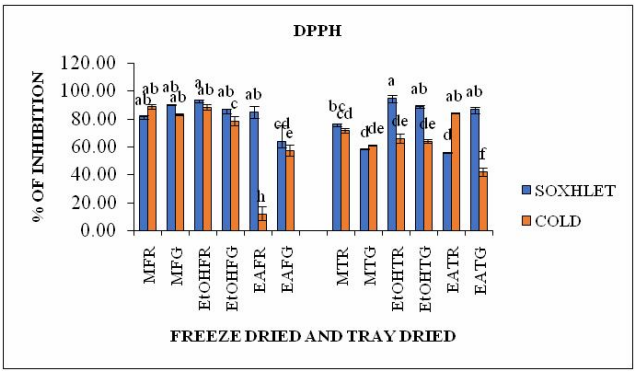

Figure 8: DPPH assay of *D. palmatus* fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-h) in the same column and row differ significantly (p<0.05). M-methanol, EtOH-ethanol, EA-ethyl acetate, F-freeze dried, T-tray dried, R-red fruit, G-green fruit.

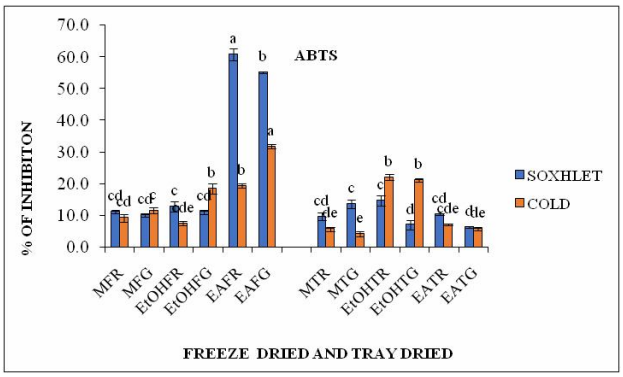

Figure 9: ABTS assay of *D. palmatus* fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-e) in the same column and row differ significantly (p<0.05). M-methanol, EtOH-ethanol, EA-ethyl acetate, F-freeze dried, T-tray dried, R-Red fruit, G-green fruit.

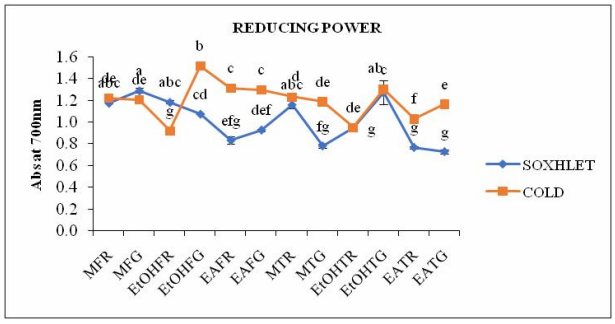

Figure10: Reducing power assay of D.palmatus fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-g) in the same column and row differ significantly (p<0.05). M-methanol, EtOH-ethanol, EA-ethyl acetate, F-freeze dried, T-tray dried, R-red fruit, G-green fruit.

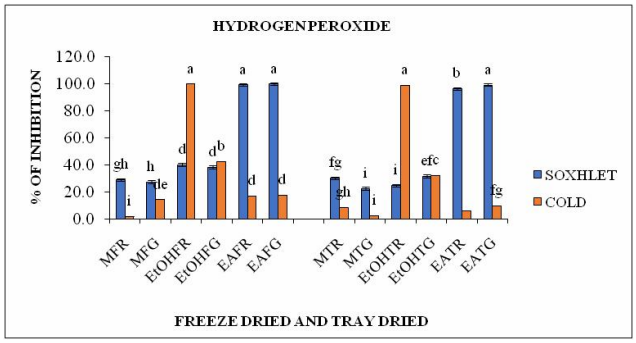

Figure 11: Hydrogen peroxide assay of D.palmatus fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-i) in the same column and row differ significantly (p<0.05). M-methanol, EtOH-ethanol, EA-ethyl acetate; F-freeze dried, T-tray dried, R-red fruit, G-Green fruit.

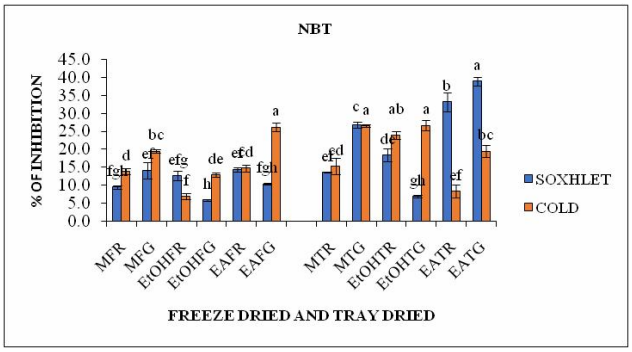

Figure 12: NBT assay of *D. palmatus* fruit extracts. Values are means of ± standard error of triplicate samples. Mean values bearing different superscripts (a-h) in the same column and row differ significantly (p<0. 05). M-methanol, EtOH-ethanol, EA-ethyl acetate; F-freeze dried, T-tray dried, R-red fruit, G-green fruit.

3.9 NBT assay

The present experimental results recorded showed the highest activity from EATG soxhlet extract with 38.9 ±1.2 followed by 33.2 ± 2.6 from the similar treatment of EATR indicating that temperature has an impact for the scavenging activity, the lowest recorded is from EtOHFG soxhlet extract with 5.9 ± 0.2and EtOHFR cold macerated with 6.9 ± 0.8 (Figure 12). Overall Green fruits have retained a stable activity with 26.2 ± 1.1 to 26.6 ± 0.4 with ethanol, methanol, and ethyl acetate cold macerated extracts has shown an intermediate antioxidant capacity for NBT analysis.

4. Discussion

The present results revealed the slight variations between the two fruits are a natural process as the fruit has attained its maximum size with its physiological developmental processes; a similar opinion has been reported (Hayat *et al.*, 2014). The succulent fresh red fruits showed higher pH 6.13 ± 0.005 when compared with raw green fruits with a value of 5.6 ± 0.005 showing slightly towards alkaline nature but both are not significantly different, during the process of development accumulation of pigments and enzymatic conversions naturally contribute to the increase in ^{o}Brix

value (Purvis, 1993; Gil *et al*., 2002). There is a marginal difference between pH and °Brix of ripe red fruits is an important property utilized for nutritional labeling and food quality (Marielli *et al*., 2015).

The moisture content of any food product is an index of its water-holding activity (Frazier and Westhoff, 1978) and is used as a measure of stability and susceptibility to microbial contamination (Davey,1989), extremely lower than the range value of some Nigerian pumpkins (Cucurbit species) seeds has been reported (Blessing, 2011). The high amount of moisture is also disadvantageous leads to spoilage (Desai and Salunkhe, 1991).

Nutritional proteins play a significant role in synthesis in addition to the maintenance of tissues, enzymes, and hormones required for healthy functioning (Hayat *et al*., 2014). Food manufacturing factories are primarily focusing on finding new raw materials to obtain the superior quality of that product (Ambawat and Khetarpaul, 2018). The cabinet dry was found to be the best method of drying for retention of phytochemicals (Thorat *et al*., 2018).

Fruits are generally not an extremely superior source of fats and are generally suggested as an element of a weight-reducing diet. The crude fat in the present study highest percentage recorded from freeze-dried red fruits with 5.8 % and lowest from tray dried green fruits with 2.82 %. earlier reports suggest that similar low-fat percentages from different fruit samples *viz*., banana (0.3%), apple (0.4%), grape, mango, and papaya with trace (Ogbuagu and Agu, 2008). Passion fruit with 1.20% and highest recorded 17% from avocado fruits. These observations are under *Syzgium caryophyllatum* (Kasunmala *et al*., 2021).

The extensive contributors to the antioxidant capacity of grains, fruits, and vegetables are due to the scientific interest in phenolic compounds as chemo-precautionary and therapeutic agents against several chronic diseases was stimulated in the late 1990s (Sánchez-Rangel *et al*., 2013), these results are in agreements with (Lopez-Vidana *et al*., 2017). Isoquercetin-a flavonoid glycoside was successfully isolated from methanolic stem and leaf extract of *Diplocyclos palmatus* and it has been under evaluation for the treatment of thromboembolism of vein in colorectal cancer, renal cell carcinoma and kidney cancer (Shashikala *et al*., 2019).

Drying methods influence qualitative and quantitative aspects of phytoconstituents for better retention of their activity (Irondi *et al*., 2013; Devi *et al*., 2019). Antioxidant properties are also used for potential therapeutic effects (Modi *et al*., 2018). Natural antioxidant phytoconstituents have a variety of *in vivo* effects. They may be responsible for the *de novo* synthesis of antioxidants or may also generate new mechanisms of antioxidants (Gadade and Patil, 2019). In contrast, earlier reports have shown that hot air oven-dried at 50^{0}C for 72 h, powdered methanolic Soxhlet extracts have shown highest radical scavenging activity, suggesting temperature has favored for effective radical scavenging activity (Attar and Ghnae, 2017). Here, the superoxide anion is a reduced form that begins oxidation but scavenges gradually, and several pathophysiological processes (Chou *et al*., 2009).

5. Conclusion

The present study is an effort to find out the impact of temperature on nutritional quality and bioactivity of phytoconstutients of the selected fruits; two different drying methods have been used. The difference in the physicochemical distinction is considerably diverse due to agro-climatic condition, species, and time of final harvesting stages. The pH of the fruit was acidic, color development in fruit is a result of anthocyanins, which have a high antioxidant capacity, °Brix levels of fruit could have been influenced by storage temperature.

Ethanolic cold macerated with a slight difference in case of similar treatments both with Soxhlet extracts. This indicates that the selected samples have a good quantity of phenolics both bound and free forms. The obtained total protein content also supports the presence of total phenolic content. In comparison with results of TFC also supports that some of the bound forms of phenolics have been released at the time of estimation. Temperature and solvents have been favored for obtaining the highest percentage. For the first time bioactivity, assay *viz*., hydrogen peroxide, reducing potential and NBT assay for selected fruits have been tested and overall bioactivity assay results reveal that TFC, ABTS,$^{++}$ H_2O_2, and NBT assay, ethylacetate Soxhlet extracts of both the fruits have shown very good antioxidant potency. The selected plant fruits possess a good amount of nutrients as well as showed bioactivity, however; further experiments are ongoing for the estimation of minerals, other phytoconstituents, isolation, and characterization of the specific bioactive compound responsible for the confirmation of bioactivity.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Ramya Banave Rajashekar, Nakkarike Manjubatta Sachindra, Naveen Shivanna and Leelavathi Sakriyanaik (2021). Effect of drying methods on proximate analysis and antioxidant activities of ripe and unripe fruits of *Diplocyclos palmatus* (L.) C. Jeffery. Ann. Phytomed., 10(2):398-408. http://dx.doi.org/10.21276/ap.2021.10.2.53**