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DRYING EFFECT OF GREEN APPLE (*Malus pumila*) ON ITS PHYSICOCHEMICAL PROPERTIES, ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT

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ABSTRACT

Evidence suggests that a diet higher in fruits and vegetables may decrease the risk of Ceaseless diseases and thus the aim of this work is to compare the phytochemicals TPC and antioxidant activity of different crude extracts of freeze dried and tray dried green apple powder.(*Malus pumila). In vitro* phytochemical screening for all the crude extracts showed a positive result for alkaloid, flavonoid, saponin, tannin and terpenoids compounds. Thus acetone, hexane and ethanol extracts were used for further tests. The antioxidant activity of freeze dried crude extracts to DPPH was in the order of acetone> hexane > ethanol. However, the order of antioxidant activity for tray dried organic crude extracts to DPPH was in order of hexane > acetone > ethanol extract. The TPC of tray dried sample extract was found to be hexane>acetone>ethanol, similarly freeze dried sample extract was found to be acetone>hexane>ethanol. The result obtained in present study indicated *Malus pumila* as a rich source of natural antioxidants, and provides evidence that solvent extract of *Malus pumila* especially the hexane extracts contains medicinally important bioactive compounds and this justifies the use of the fruit as traditional medicine for the treatment of various diseases.

KEYWORDS: ***Antioxidant, antimicrobial, freeze dried, tray dried, DPPH, TPC.***

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INTRODUCTION

Fruit and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as cardiovascular diseases (CVDs) and certain cancers. Non-communicable diseases (NCDs), especially cardiovascular diseases (CVDs), cancer, obesity and diabetes, currently kill more people every year than any other cause of death. Vegetables and fruits are the main antioxidant suppliers in the human diet. Phenolic compounds, or polyphenols, constitute one of the most numerous and widely distributed group of sub- stances in the plant kingdom, with more than 8000 phenolic structures currently known.[1] Phenolic compounds structurally differ from simple molecules, such as phenolic acids, and from highly polymerized compounds, such as pro-anthocyanidins (tannins), which occur in plants and are common in many foods (fruits, vegetables, cereal grains) and beverages (wine, beer, teas)[2] Polyphenols provide health benefits by several mechanisms, including the elimination of free radicals, the protection and regeneration of other dietary antioxidants (e.g. vitamin E) and the chelation of pro-oxidant metals. The nature and content of phenolics varies dramatically among plants, which are mainly esterified or glycosylated.[3] They possess beneficial properties, such as immune modulatory actions, antioxidant and anti-cancer and antibacterial activity. Some studies evidenced an improvement of wound healing by these phyto-chemicals.[4] The current preoccupation about adverse effects of synthetic antioxidants should be considered and some foods, drinks and medicinal plant extracts may represent an alternative source of natural antioxidants. Plant extracts and essential oils can be used as an accessible source of natural antioxidants and a possible food supplement, or exploited for pharmaceutical applications Non-nutritive, health-promoting, bioactive components present in foods have the potential to exert beneficial effects against many chronic disease.[5] Besides their nutraceutical properties, polyphenols are indicative of the quality of fruits and vegetables. The effectiveness of the antioxidant action of these bioactive compounds depends on their chemical structure and concentration in foods[6] and many factors can influence the polyphenol content in plants. One of these is the type of cultivation, such as organic or conventional Phenolic compounds appear to influence the quality of fruits, contributing to their organoleptic and sensorial quality, in addition to improving fruit nutritional value.[7] Further studies have shown that polyphenolics have the ability to reduce cellular damage and therefore may be beneficial in promoting human health and protecting against numerous diseases linked to oxidative events, such as cardiovascular and respiratory disorders, cancers and diabetes. Many studies have shown a strong relationship between polyphenolic compounds, which have antioxidant activity, and reduced risk of various diseases.[8,9,10,11,12] Green apple (*Malus pumila*) is naturally delegated a herbaceous biennial from Rosaceae family and has a few assortments of apple reaches with skin hues going from red, green, yellow or pink and numerous bi-tri-shading varieties. [13] The substance of dietary fiber (DF) constitutes is on the normal half of dry weight, while phenolics may shift from 1200 to 4000 mg/kg dry weight.[14,15,16] Dark red shaded apple are the most famous for human utilization, both cooked and crude as serving of mixed greens or squeeze. There is developing enthusiasm for the utilization of characteristic sustenance hues, since engineered colors are turning out to be increasingly basically surveyed by the purchaser. Dietary fiber prolongs glucose assimilation flatting glucose profile by which insulin discharge is diminished. High insulin levels are associated with coronary heart ailments and diabetes.[17] Apple dietary fiber assumes essential part in counteractive action and treatment of heftiness, arthrosclerosis, coronary heart sicknesses, internal organ growth and diabetes.[18] Many vegetables and fruits are not eaten as raw. Some undergo special treatments, such as heating (jelly), freezing (fruit pulp), among others. Some studies showed that these treatments, used mainly by industry, tend to change the quality of polyphenols. The consumption of fruit pulp is widespread, due to the ease of preparation and seasonality. Frozen pulps are commonly used both for industrial purposes and domestic consumption. Some studies indicated that good conservation of polyphenol contents occurs upon freezing. Thus, this procedure appears an effective way for preserving antioxidant properties of fruits. Although, this process induces an irreversible physical destruction of cell walls and protoplasts, causing damage to fruit texture and therefore a loss of quality.[19] The freezing process slightly affects the content of extracted ellagic acid, total polyphenols and vitamin C, in raspberry cultivars.[20] This drying process is an economical method of drying for fruits or vegetables containing more than 70% moisture content (Ref?). Air drying is a basic method used to preserve food in which the solid to be dried is exposed to a continuously flowing hot air stream where moisture evaporates. Freeze-drying, also known as lyophilisation or cry desiccation is a process of removal of water and is used to preserve a material or make the material more convenient for transport (Ref?). The principal of freeze drying involves freezing the material and then reducing the surrounding pressure so as to allow the frozen water to sublimate directly from the solid phase to gaseous phase. Due to absence of liquid water and low temperature processing, most of the biochemical and microbiological reactions are inhibited which provides very good quality of the final product The preservation of foods as powder concentrate has received increased attention in recent years. A number of investigators have dedicated their attention towards preparing powders. Series of drying methods are recommended since bioactive compounds are highly sensitive to thermal degradation. The study was conducted to evaluate the effects of drying on the antioxidant, bioactive compounds and total phenolic content of *Malus pumila*.

MATERIALS AND METHODS

Collection of sample

The samples of green apple were collected from Wholesale fruit market, Koyambedu, Chennai, Tamil Nadu for the duration of the entire project 2016-2017 (year or eriod of study? Yes). Care was taken that fruits were of uniform size, undamaged and ripened.

Hot-Air Drying

200ml of semi-solid sample were subjected for drying at 50˚C.The oven was preheated to the required temperature and the samples were poured into plates and kept. The temperature was maintained and continued till the sample was completely dried. Drying was carried out till there is a constant loss of weight. The dried samples were powdered in a grinder and weighed. The pulp and peel powder was immediately packed and sealed in aluminium pouches and placed in the refrigerator for further analysis.

Freeze Drying

200g of green apple was ground in a mixer and poured into plates and kept for pre-freezing at 4 °C for 1 day. The plates were then freeze dried at -40°C at a pressure of -3mbar for 3 days to obtain freeze dried powder. The dried green apple was ground into powder and weighed. The pulp and peel powder was immediately packed and sealed in aluminium pouches and placed in the refrigerator for further analysis

Preparation of extract

The tray and freeze dried powder (mention quantity)2g were extracted for 24 h with 20 ml of solvent (hexane, acetone, ethanol, petroleum ether and water) at room temperature. After filtration through Whatman No 0.45 µm, the resulting solutions were evaporated under vacuum at 60°C by Buchi Rotavapor R-200 to dryness. The residues were weighed and preserved for further use.

Preliminary Phytochemical Screening

The extracts were subjected to different chemical tests for the detection of different phytoconstituents using standard procedures.[21,22,23]

Estimation of Moisture Content

The moisture content of the sample was determined using the forced air draft oven method (AOAC 2000). The samples were dried in the oven at 103°C. Readings were taken after 1 hour to determine weight loss in the samples.

Estimation of ash content

5g of the sample was weighed and taken in the crucible. The crucible was transferred to a muffle furnace maintained at around 550˚C and left for about 5-6 hours until white or light grey ash was formed. crucible was cooled and reweighed.[13]

Estimation of fat content

5 g of pre dried sample was weighed into an extraction thimble, with porosity permitting the rapid flow of solvent (n-hexane). The initial weight of the flat bottom boiling flask was noted. The Soxhlet apparatus was assembled along with round bottom flask, and the extraction was started by heating solvent in boiling flask for 4 hours. After extraction the boiling flask was dried with the extracted fat in a hot air oven at 60°C, cooled to room temperature in a desiccators and the final weight was noted.

$$\text{Fat}(\%) = \frac{\text{weight of fat (g)}}{\text{weight of sample (g)}} \text{ X } 100$$

Determination of Anti-Oxidant Activity –DPPH Assay

Antioxidant activity was determined by DPPH assay as described by Sarker *et al.*,[24]. 0.5g of sample was extracted with 10ml of 80% ethanol in a water bath for 3 hours at 45°C. The samples were then centrifuged at 5000rpm for 5min and the supernatant was separated. Varying concentrations of the sample were taken into tubes. 0.004% of DPPH solution was made and 6ml of this solution was added to each of the tubes. The tubes were incubated in dark for 30min and the OD at 517nm was taken.

$$\%\ \text{DPPH Scavenging Activity} = \frac{[A_{\text{Control}} - A_{\text{Test}}]}{A_{\text{Control}}} \text{ X } 100$$

Determination of Total Phenolic Content

Total phenolics were analysed spectrophotometrically using the modified Folin–Ciocalteu colorimetric method as described by Ching Hui chang *et al.,*[25]. Ethanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45˚C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Agar well diffusion method

The bacteria *Escherichia coli* and *Staphylococcus aureus* (Name of the organisms used? Source?) were inoculated into a sterile nutrient broth and incubated overnight in a rotator shaker. The culture was swabbed on the surface of sterile Nutrient agar (Hi-media) plate. Agar wells of radius 5mm were prepared and 20µl of the ethanolic green apple extract, acetonic green apple extracts and water green apple extract were added to the each well. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of zone of inhibition was measured in mm and the results were recorded. The extracts were examined for antimicrobial activity on *Escherichia coli, Staphylococcus aureus* which were the representatives.

RESULTS and DISCUSSION

Qualitative analysis phytochemical analysis

The result of the preliminary phytochemical screening was carried out on the extract of hot air and freeze dried powders which revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids supporting the reason for its wide range of biological activities as showed in Table.1. No difference was observed between tray and freeze dried extracts. It was observed that all the extracts of tray and freeze dried sample contain alkaloids, triterpenoids, polyphenols, Quinones, Flavonoids, Tannins and saponins. Among the 5 extracts acetone, ethanol, hexane and water was found have better phytoconstituents.

Table 1

Results of phytochemical screening of freeze and tray dried extracts of Malus pumila

Phytochemicals	Acetone	Ethanol	Petroleum ether	N-Hexane	Water
Phenols	+	+	+	+	+
Saponin	+	-	-	+	+
Tannins	+	-	-	-	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	-
Quinone	-	+	-	-	+
Terpenoids	+	+	-	+	-
Fixed oil	+	+	-	+	+

Determination of Moisture, Ash and Fat content

On comparison of the tray and freeze dried powder the moisture content in the freeze dried powder was high may be due to low temperature drying. Higher value of ash content in the tray dried sample emphasise that it have high minerals and a loss of minerals in freeze dried sample. The fat content was found to be similar for tray and freeze dried samples, and a reduction ash and fat is found on dehydration.

Table 2

Moisture, Ash and Fat content for Fresh, Freeze and Tray dried sample

Sl no.	Sample	Moisture content (wb%)	Ash Content %	Fat content %
1	Fresh green apple	83.66 ± 0.66	2.4±0.07	0.04±0.19
2	Freeze Dried green apple	7.33 ± 0.28	8.79±0.12	1.8±0.10
3	Tray Dried green apple	5.39±0.34	9.88±0.05	1.58±0.14

Anti-oxidant activity -DPPH radical scavenging activity

The DPPH radical scavenging activities of the green apple extracts were shown in Fig 2 & 3. The scavenging activity of the freeze dried green apple acetonic extract fraction (66%) was higher than those of all the other extracts. Freeze dried extracts of samples showed higher scavenging activities against DPPH radicals than the fresh extracts. However, fresh and freeze dried green apple pineapple (????? Ref?) extracts showed relatively lower scavenging activities compared to fresh and freeze dried green apple extract.

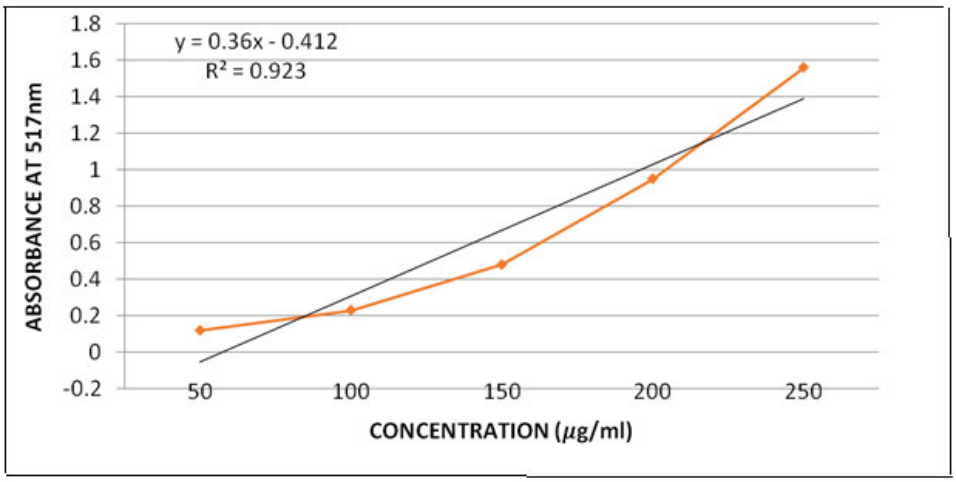

Figure 1

Gallic acid standard for DPPH Assay

A high scavenging activity was found in acetone extract of freeze dried sample and a low scavenging activity was found in petroleum ether. The hexane extract showed better results for tray dried sample and lower effects on water extracts of freeze and tray dried sample.

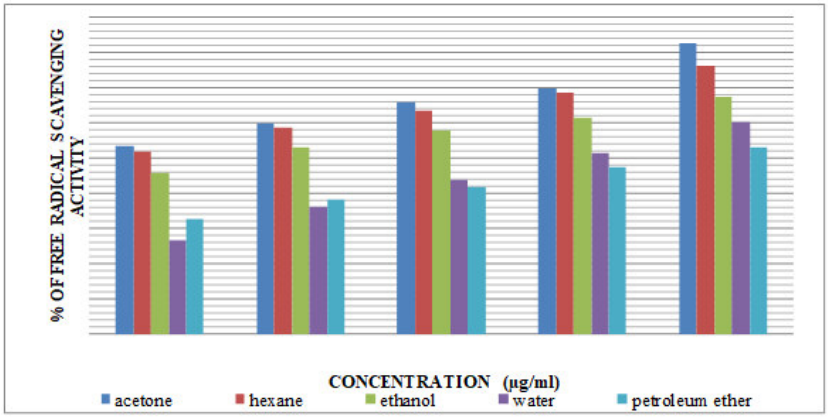

Figure 2
Effect of scavenging activity (DPPH) on freeze dried sample with different solvent extract.

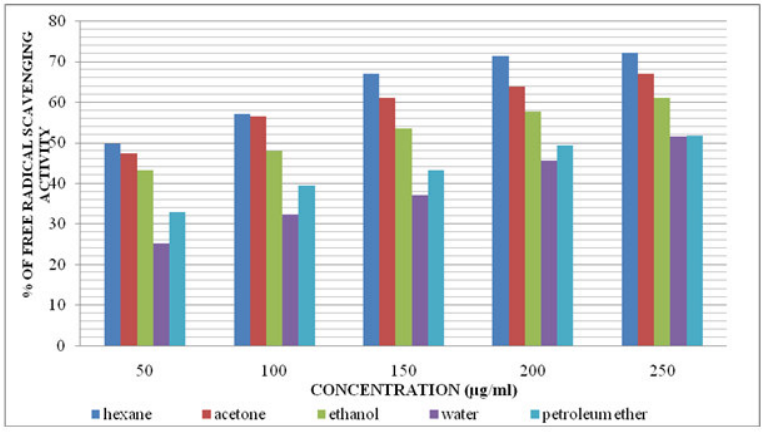

Figure 3
Effect of scavenging activity (DPPH) on Tray dried sample with different solvent extract.

Total phenolic content

Levels of phenolic content were expressed in terms of Gallic acid equivalent (GAE). The equation of the right-hand side of the proportioning of total phenolic content by the method of Folin- Ciocalteu gave y = 0.36x - 0.412 with R^2 = 0.9239 (Abdoullatif *et al.*, 2012). The total phenolic compound contents in the extracts are shown in table 2. It appeared that extract of freeze dried green apple powder had the highest content of phenolic compounds (6.62±0.01 mg GAE/100 g of extract).The acetone extracts showed better results for tray and freeze dried samples and water showed lower values in both samples.

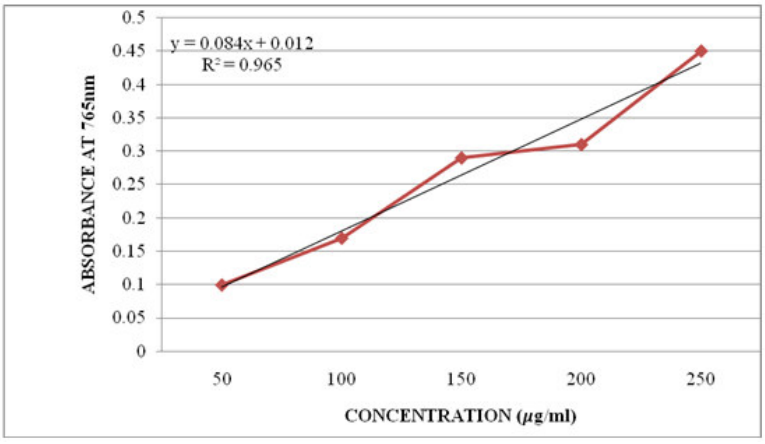

Figure 4
Gallic acid standard for TPC

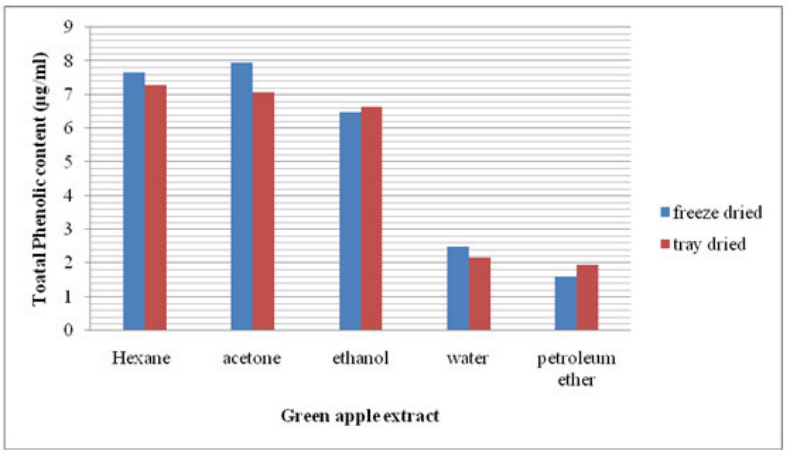

Figure 5
Total Phenolic content of freeze and tray dried sample with different solvent extract.

Screening for Antibacterial Activity Assay

Green apple have been to known to have great potential as antimicrobial agents against selected pathogens and they can be used as an alternative medicine in the treatment or control of bacterial infections. This study supports the use of these green apple not only as dietary supplements but also as agents to prevent bacterial infections. The result of the microbial assay against *Escherichia coli* and *Staphylococcus aureus* by agar well diffusion assay (Table3) indicates that the acetone extract of green apple showed better zone of inhibition compared to the water extract of green apple.

Table 3
Zone inhibition in Agar well diffusion assay

Sample	Bacteria	Ethanol extract	Aceton extract	Water extract	Hexane extract	Petroleum ether
		Zone of Inhibition (mm)				
Tray dried	*Escherichia coli*	15	18.5	7	13.7	11
	Staphylococcus aureus	9	19	5.4	14mm	10.5mm
Freeze dried	*Escherichia coli*	13.4mm	15.2mm	5mm	11.3mm	9mm
	Staphylococcus aureus	7mm	16mm	6.7mm	12mm	11.5mm

CONCLUSION

There has been an explosion of consumer interest in the health enhancing role of specific foods or physiologically active food components. This study was performed to compare the various properties of *Malus pumila* drying methods, extracts with different solvents, comparison of proximate, phytochemical analysis. On performing all the proximate and phytochemical tests, it was observed that freeze dried powders had an advantage over the tray dried samples. There is minimum loss of nutrients in the process of freeze drying and the food can be preserved for a longer time. Anti-oxidant activity was displayed by both the samples however, freeze dried showed better radical scavenging than the tray dried sample. The antioxidant activity was slightly less than the standard values. Both freeze dried and tray dried have been known to show antimicrobial activity against most microorganisms. On comparing the results of antimicrobial activity of the ten samples, acetone extract showed an overall better result compared to the freeze and tray dried samples. This study further encourages Nutraceutical, Pharmaceutical and functional food companies to come up with products produced from medicinal plant, fruits and vegetables that are not only costly but also have adverse effects.

CONFLICT OF INTEREST

Conflict of interest declared none.

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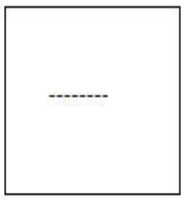

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