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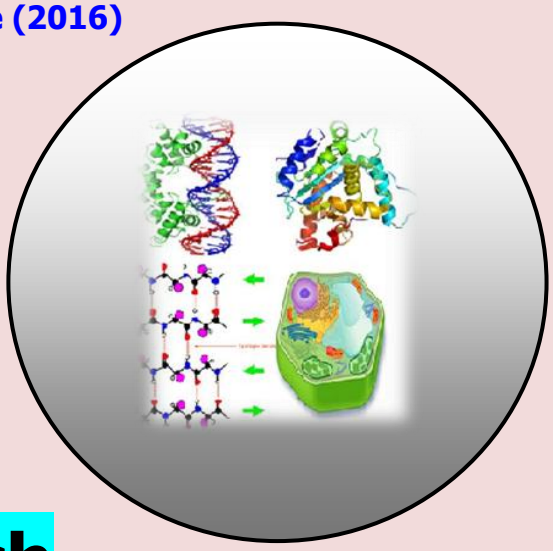

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A Comparative Study on Evaluation of Antioxidant Properties of *Anacardium occidentale* Fruit Juices Collected from Neyveli and Pandruti, Tamil Nadu-India

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ABSTRACT

The goal of this study was to determine the antioxidant properties of Anacardium occidentale fruit juices collected from Neyveli and Pandruti. They may vary in relating the antioxidant constituents corresponding to the region from which they were collected. Here, a comparative study of cashew apples was carried out for their anti-oxidant potentials on enzymatic and non-enzymatic anti-oxidant studies. In DPPH inhibitory and the Panruti shown high inhibitory comparable to the standard vitamin C as in the case of ferric thiocyanate method (lipid peroxidation) Neyveli respectively. But in the enzymatic and non-enzymatic inhibitory the Panruti produced maximum activity (GSH estimation 38.5 ± 0.01 Nano mole /ml,38.5:01.01 µg / ml in the glutathione peroxidise than the Neyveli. focusing the orientation of containing secondary active metabolites leading to act against free radicals.
Keywords: Anacardium occidentale , Cashew, non-enzymatic and ferric thiocyanate.

INTRODUCTION

Cashew tree is a tropical and subtropical tree belonging to the family Anacardiaceae, the genus *Anacardium* Linn, and the species *Anacardium occidentale* Linn var. nanum (Trevisan *et al*., 2006; Santos *et al*., 2007; Asogwa et *al*., 2008). Cashew apple is not a true fruit but a swollen peduncle to which the cashew nut is attached which is rich in reducing sugars (fructose and glucose), vitamins, minerals, and some amino acids. Based on external colour of the fruit, cashew apple can be broadly classified into red and yellow varieties (Awe and Tuned *et al*., 2010). Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years (Tamizhazhagan *et al*., 2017).

In Brazil and in other countries, is an agricultural activity that aims mainly the production of cashew nuts. The nuts represent only 10% of the total fruit weight and large amounts of cashew apples are lost in the field after the removal of the nut (Campos *et al*., 2002). Cashew apples possess anti-bacterial properties and have been proven to be effective in treating stomach ulcers and gastritis. Its juice is rich in vitamin C and has an anti-scurvy effect. Cashew apple juice is widely utilized in the cosmetic industry due to the presence of antioxidants and is used in the preparation of various creams and shampoos. Cashew extract contains anacardic acid which is an antioxidant and has been shown to limit the pigmentation effects of aging and to eradicate the cancer cells (Suneetha Runjala and LakshmanKella., 2017). Quality can be defined as the status of a drug that is

determined by identity, purity, content and other chemical, physical, or biological properties, or by the manufacturing processes (Tamizhazhagan *et al*., 2017a). The cashew apples are rich in volatile compounds and several studies were carried out by different researchers to reveal that. Forty eight volatile compounds were identified in the cashew apple juice, the volatile compounds being predominantly esters (42% of total compounds), followed by aldehyde (14%) (Garruti *et al*., 2003; Franco *et al*., 2005). Similarly, antioxidant, considering the activity in comparison to quercetin, has been described else whereby fruits, pseudofruis and roots (Oliveira *et al*., 2011), but not for the flowers. With respect to the chemical components found in the aerial parts of the cashew tree, phenolic compounds such as anacardic acids, cardanols, cardols and 2-methycardols have been isolated from the cashes nut shell liquid (Sujatha *et al.,*2011). Natural antioxidants play an important role as an endogenous plant defence system. Moreover, their consumption confers protection against some degenerative human diseases, as the generation of oxidant radicals is a normal phenomenon in biological systems, such as animals and plants (Suja *et al*., 2004; Espinet *et al*., 2000). Evidence suggests that the high content of fiber and antioxidants (e.g., ascorbic acid and polyphenols), of diets rich in fruits and vegetables may decrease the risk of chronic diseases (World Health Organization - WHO, 2003).This beneficial effect is due to the action of antioxidant compounds, which are capable of neutralizing free radicals and reduce oxidative damage in the body (Clifford, 1995). For this reason, the interest in the evaluation of antioxidant activity of fruits and vegetables has substantially increased and numerous studies have been performed (Thili *et al*., 2011; Mezadri*et al*., 2008; Ilahy *et al*., 2011; Park *et al*., 2011).

MATERIALS AND METHODS

Sample collection

The well matured cashew apples (*Anacardium occidentale L*.) were collected on June 2017 from taluk of Neyveli (11°33'15.8"N 79°24'29.8"E) and Panruti (11°44'47.6"N 79°33'23.7"E) also well-known places for cashew apple and Jackfruit and rich in soil nutrients (Yoganathan *et al*., 2017), located in Cuddalore district, Tamil Nadu, India. The collected cashew apples are immediately placed in plastic boxes only one layer of cashew apples protected from mechanical injury (Alves *et al*., 2008).

Juice extraction

The collected cashew fruits were transported to the laboratory, where the nuts were detached. The apples were washed thoroughly with distilled water. Then, they were cut and the juice obtained by pressing the mash through a muslin cloth was used for various clarification assays using clarifying agent as gelatine 0.3% to remove tannin content and resulted in clear solution. The solution was used for further evaluation parameter (Dedehou *et al*., 2015).

Evaluation of antioxidant activity

DPPH free Radical Scavenging Activity

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method previously reported by Shen *et al*., (2010) Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3mL of cashew apple juice of all extracts at different concentration (10- 50 µl/ml).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). The capability to scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{(A0-A1)}{A0} X100$$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples.

Ferric thiocyanate (FTC) method

The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The FTC assay was carried out as described by Kikuzaki and Nakatani (1993) with slight modification. A mixture of 4 ml of cashew apple juice extracts (final concentration 0.02% w/v) in 4 ml of 99.5% methanol, 4.1 ml of 2.51% linoleic acid in 99.5% methanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water, contained in a screw-cap vial was placed in an oven at 40^{o} C and incubated in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred into a test tube then, 9.7 ml of 75% (v/v) aqueous methanol was added to it, followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid.

Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance of red colour was measured at 500 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). The measurement was taken 24 hours until the absorbance of the control reached its maximum value. Vitamin E was used as a positive control. The percent inhibition was calculated on the final day by the following formula.

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} X\ 100$$

Assay of Glutathione peroxidase (Gpx)

Glutathione peroxidase was assayed according to the method of Rotruck *et al.* (1973) with slight modifications. The reaction mixture consisting of 0.4 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of water and 0.5 ml of cashew apple juice was incubated at 0, 30, 60, 90 seconds respectively. The reaction was terminated with 0.5 ml of 10% TCA and after centrifugation; 2 ml of the supernatant was added to 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). The colour developed was read at 412 nm and the protein. Enzyme activity is expressed in terms of µg of glutathione utilized/min/mg.

Estimation of Reduced glutathione (Rg)

The amount of reduced glutathione in the samples was estimated by the method of (Boyne and Ellman, 1972). 1ml of the cashew apple juice was treated with 4.0 ml of metaphosphoric acid precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g EDTA and 30 g NaCl dissolved in 100ml water). After centrifugation, 2.0 ml of the protein-free supernatant was mixed with 0.2 ml of 0.4 M Na_2HPO_4 and 1.0 ml of DTNB reagent (40 mg DTNB in 100 mlof aqueous 1% tri sodium citrate). Absorbance was read at 412 nm within 2 minutes. GSH concentration was expressed as nmol/mg protein.

RESULTS AND DISCUSSION

The present study was to investigate the antioxidant activity of two varieties (red and yellow) of cashew apples.1, 1-diphenyl-2picryl hydrazyl radical (DPPH) was used to determine the free radical-scavenging activity of cashew apple juice. Vitamin C (Sigma, USA) was used as standard control. In the DPPH scavenging test the result showed that both samples collected from Neyveli and Panrutimay contain similar bioactive compounds which contribute to their antioxidant effect in same way which explored in Table. 1 however the Juice of red verity cashew apple collected Panruti showed slight increase in inhibition of DPPH moreover both regions of the cashew apple juice dominated to produce potency µl/ml level against free radicals scavenging higher than the previous study using different fruit juices that exhibited inhibition in mg /ml dosage level (Lek Kia Beh *et al*., 2012). The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability. DPPH is stable free radical and accepts an electron or hydrogen radical to become a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecules (Siddaraju and Dharmesh., 2007) it was reported that decrease in the absorbance of DPPH radical caused by phenolic compound was the reaction between antioxidant molecules and radicals, resulting in the scavenging of radical by hydrogen donation, and visualized as a discolouration from purple and yellow (Meir *et al*.,1995).

Table 1. DPPH assay.

S.No	% inhibition of DPPH activity					
	Concentration µl/ml	Neyveli		panruti		Vit C (Std)
		CAJ-Y	**CAJ-R**	CAJ-Y	**CAJ-R**	
1	10	74.35±3.39	65.38±3.4	79.38±3.39	80.06±0.00	91.23±0.01
2	20	76.41±3.39	67.41±3.4	80.41±3.39	81.44±3.39	92.50±0.01
3	30	77.57±0.00	69.68±0.00	81.78±0.00	81.44±3.39	92.12±0.01
4	40	79.37±0.01	71.45±0.01	82.47±0.01	83.84±0.00	94.05±0.01
5	50	79.52±0.00	75.43±0.00	83.84±0.00	85.56±0.00	96.01±0.01

Values are given as mean ± SD

The percentage inhibition of linoleic acid of fruit juices collected from Neyveli and Panruti samples using ferric thiocyanate method was summarized in (table 2) and correlated with standard vitamin C at µl/ml concentrations. Ferric thiocyanate method estimates the ability of the antioxidant compounds to suppress pro-oxidant and oxidant activities in oxidizing systems.

Based on this, inhibitory potency of fruit juices was determined. Neyveli and Panruti (red and yellow) samples delivered the simultaneous results but Panruti yellow verity sample was to produce higher inhibitory activity against ferric thiocyanate inhibition in comparison with standard vitamin C. Previous study (Nadezhda *et al.,* 2014) has found those juices from monists (79.0%) and raspberry (87%) inhibition on oxidation of linoleic acid in the dose composed at grams in weight of raw juices which were lesser than these cashew apple juices in the inhibitory tendency against oxidant free radicles.

Table 2. FTC assay.

S. No	% inhibition of FTC assay					
	Concentration µl/ml	Neyvali		Panruti		Vit C (Std)
		CAJ-Y	CAJ-R	CAJ-Y	CAJ-R	
1	100	58.12±0.01	67.61±0.00	62.12±0.01	58.71±0.00	67.21±0.40
2	200	68.76±0.01	73.11±0.00	73.86±0.01	71.21±0.00	71.89±0.34
3	300	75.04±0.01	79.01±0.01	81.06±0.01	78.03±0.01	93.12±0.01
4	400	86.43±0.00	85.13±0.05	90.53±0.00	91.66±0.05	94.15±0.01
5	500	90.63±0.00	88.21±0.00	97.72±0.00	94.69±0.00	95.73±0.01

Values are given as mean ± SD

The most important H_20_2-removing enzymes in human cells are glutathione peroxidises (GSHPX), enzymes that require selenium (has selenocysteine at the active site) for their action. GSHPX enzymes remove H_20_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, an FAD containing enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power [Flohe. 1982]. By comparison of the activity produced by the cashew juices on enzymatic and non enzymatic oxidants the Panruti (red and yellow) samples exhibited maximum activity than the Neyvali (red and yellow) samples (table 3). The non-enzymatic antioxidant system (GSH) has a cellular pool of compounds such as phenolic which are capable of quenching the ROS (Noctor and Foyer, 1998; Apel and Hirt 2004). Here the Panruti (red and yellow) samples showed the antioxidant activity in GSH estimation 48.5±0.01 Nano mole /mland 38.5±0.01 Nano mole /ml. Thenon-enzymatic antioxidant evaluation also as 17.5±0.01µg /ml and38.5±0.01 µg /ml in the glutathione peroxidise content. This is because of the presence of non-enzymatic antioxidants such as flavones, anthocyanin, carotenoids and ascorbic acid in the fruit juices (Renata Araújo *et al*., 2015). The cashew apple as medium andioxidant among the 18 Brazilian fruits analyzed (Rufino *et al*., 2010). Glutathione (GSH) and AA remove free radicals by reacting directly with them non catalytically (Halliwell and Gutteridge.1999).

Table 3. Estimation of Reduced Glutathione (RG) and Glutathione peroxidase (GP).

Sample	**Reduced Glutathione antioxidant (GSH)** Nano mole /ml	**glutathione peroxidase (**µg /ml)
NCAJ-Y	24.3±0.01	11.3±0.01
NCAJ-R	35.6±0.01	29.3±0.01
PCAJ-Y	38.5±0.01	17.5±0.01
PCAJ-R	48.5±0.01	38.5±0.01

Values are given as mean ± SD

CONCLUSION

The results from the study demonstrated that juice extracted from *Anacardium occidentale* could be considered a potential source of bio active components with potential anti-oxidant activity. The sample collected from the Pantruti was found to have high quality properties affirmed in the anti-oxidant evaluation than the fruits collected from Neyveli. Hence the *Anacardium occidentale* juice may provide best nutrients in the production of well quality foods and become to provide bio active metabolites for incoming investigations.

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