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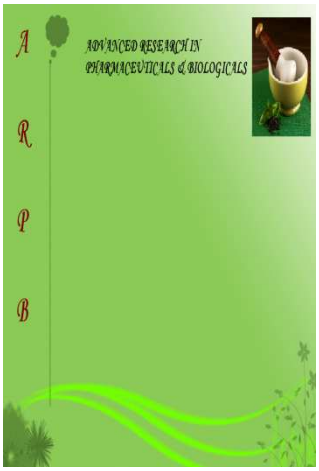

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EVALUATION OF ANTIOXIDANT POTENTIAL OF A FUNCTIONAL FOOD FORMULATION COMPRISING *PSIDIUM GUAJAVA* FRUIT, *JUGLANS REGIA L.* FRUIT AND WHEY

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ABSTRACT:

In vitro antioxidant activity of *Psidium guajava*, *Juglans regia* and Whey has been investigated by DPPH (1,1-dipheny-l,2-picryl hydrazyl) free radical, inhibition of lipid peroxidation and hydroxyl radical scavenging activity. The equiproportion methanolic extracts of *Psidium guajava*, *Juglans regia* fruit and aqueous extract of Whey showed a concentration dependent inhibition of lipid peroxidation and a significant free radical scavenging action against DPPH free radical, hydroxyl radicals and by lipid peroxidation which was evaluated by comparing with standard apigenin, catechin and beta-lactoglobulin.

KEYWORDS: *Psidium guajava*, *Juglans regia*, Antioxidant activity, DPPH free radical, Hydroxy radical.

INTRODUCTION

Functional foods are defined as the foods that in addition to nutrients supply with components that contribute to cure or to reduce the risk of developing chronic diseases. They are foods like the conventional ones, consumed as part of daily diet but able to produce proved metabolic or physiological effects useful in the maintenance of sound physical and mental health.[1] *Psidium guajava* (pink fleshed variety) fruit has high vitamin C content and lycopene constituent which prevents skin damage from UV rays and also offers protection from prostate cancer, besides giving a very good antioxidant activity. *Juglans regia* fruit contains significant amounts of polyunsaturated fatty acids specifically α-linolenic acid an essential plant based omega-3 fatty acid, L-arginine and is a good source of manganese, they help to reduce the risk of cancer and reduce severity of cardiovascular and neurodegenerative diseases. Whey provides essential and non-essential amino acids in biologically active form namely β-lactoglobulin, α-lactoalbumin and cysteine which enhances endogenous glutathione production which helps to increase body immune system[2,3].

EXPERIMENTAL

Collection and extraction: Fresh fruits of *Psidium guajava* (Guava) and *Juglans regia* (Walnut) were collected from local market and authentication was carried out from Blatter Herbarium, St. Xaviers institute, Mumbai and their identity was confirmed to be *Psidium guajava* (family-Myrtaceae) and *Juglans regia* (family-Juglandaceae). Whey was obtained as a by-product of cheese from local market. The pulp and peel of *P. guajava* and seed of *J. regia* were dried, powdered and extraction was carried out with soxhlet apparatus using methanol as solvent. The equiproportion combined extracts of *Psidium guajava, Juglans regia* (methanolic) and Whey (aqueous) (GWW extract) were concentrated on a boiling water bath to thickness and used for antioxidant assays.

Materials: DPPH, methanol, apigenin, catechin, beta-lactoglobulin, ethylene diamine tetraacetic acid (EDTA), ferric chloride, hydrogen peroxide, deoxyribose, phosphate buffer, trichloroacetic acid, thiobarbituric acid.

Working standard and Test solutions

a) Test solutions: 50 mg of equiproportion i.e. 1:1:1 methanolic extracts of *Psidium guajava*, *Juglans regia* and aqueous extract of Whey was dissolved in 10ml of methanol and volume was made upto 50 ml to give 1mg/ml stock solution. The above stock solution was

further diluted to get the working test solution in the concentration range of 10-100µg/ml[4].

b) Standard solution: Apigenin was used as positive standard with respect to Guava, Catechin with respect to Walnut and Beta-lactoglobulin for Whey. The respective standard solutions (10-100µg/ml) were prepared in methanol as similar to test solutions.

Procedure for Determination of Hydroxy radical scavenging activity: Stock solutions of all reagents were prepared in double distilled water. The assay was performed by adding 0.1ml of EDTA, 0.01 ml of $FeCl_3$, 0.1ml of hydrogen peroxide, 0.35ml of deoxyribose, 1ml of extract/working standard solution (10-200µg/ml), 0.3ml of phosphate buffer (pH-7.4, 50mM) and 0.1ml of ascorbic acid in sequential order of addition. The mixture was then incubated at 37^0c for 60 min. 1ml of the of the incubated reaction mixture was mixed with 1ml of 1% thiobarbituric acid (TBA) and 1ml of trichloroacetic acid in NaOH (0.025M) to develop the pink color chromogen and absorbance was measured at 532 nm. Deoxyribose degradation was measured as TBARS and % inhibition was calculated.

$$\% \text{ RSC} = \frac{A_{blank} - A_{sample}}{A_{sample}} \times 100$$

where,

% RSC = Radical scavenging capacity

A_{blank} = Absorbance of reagent blank

A_{sample} = Absorbance of sample

From RSC values, the IC_{50} values was calculated which represents the concentration of scavenging compound that was 50% neutralization. IC_{50} values were obtained by linear regression method using % activity in y-axis. RSC of methanolic extracts was determined and the activity was compared with the standards[5].

Procedure for Determination of Antioxidant activity by DPPH method: A commercially available and stable free radical DPPH (sigma chemicals, USA) soluble in methanol was used. DPPH in its radical form has an absorbance peak at 517nm, which disappears on reduction by an antioxidant compound. 1ml of different concentrations of the extract/standard (10-100µg/ml) was added to 2ml of freshly prepared methanolic solution of 90µM DPPH and volume was made upto 10 ml with methanol. The reaction mixture was kept at room temperature in the dark and after 1hr the absorbance was measured at 517nm using spectrophotometer. A blank was performed excluding the extract. The optical density of the sample and the blank was measured by comparing the methanol.

The % inhibition of DPPH in reaction mixture was calculated by comparing with the blank[6].

% RSC was calculated by the following formula:

$$\% \text{ RSC} = \frac{A_{blank} - A_{sample}}{A_{sample}} \times 100$$

where,

% RSC = Radical scavenging capacity

A_{blank} = Absorbance of reagent blank

A_{sample}= Absorbance of reagent sample

From RSC values, the IC_{50} values was calculated which represents the concentration of scavenging compound that was 50% neutralization. IC_{50} values were obtained by linear regression method using % activity in y-axis. RSC of methanolic extracts was determined and the activity was compared with the standards[6].

Procedure for Determination of Antioxidant activity by Inhibition of Lipid peroxidation method:

a) Preparation of liver homogenate: A 10% homogenate of the liver tissue in mice were prepared in 0.5M phosphate buffer at pH-7.4 using a Teflon homogenizer in ice cold condition. The homogenate was centrifuged at 5000 rpm for 10 mins. The supernatants were then used for further determination.

b) Lipid peroxidation Malonidialdehyde): The lipid peroxidation product (malonidialdehyde) was determined by thiobarbituric acid reaction. 0.1ml of liver homogenate, 2ml of 20% TCA were added. The contents were mixed well and centrifuged at 4000 rpm for 20 min in a boiling water bath. The tubes were cooled at room temperature and the absorbance was read at 532nm in a Shimadzu UV 160A (UV-Visible) recording spectrophotometer. The reaction between lipid peroxides and TBA is used as sensitive method for lipid peroxidation in animal tissues[7,8].

RESULTS

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity of methanolic extract of *P.guajava* fruit, *J.regia* fruit and Whey (GWW) was given in the **Table 1, 2 and 3**:

DISCUSSION

The methanolic extract of GWW showed promising free radical scavenging effect on DPPH radical in concentration dependant manner and the IC_{50} value GWW was found 49.81µg/ml. It also significantly inhibited degradation of deoxy-ribose mediated by hydroxyl radicals compared to that of standard scavenger Apigenin, catechin and beta-lactoglobulin. The concentration of methanolic extract of GWW needed for 50% inhibition was 76.45µg/ml for 50% inhibition. The antioxidant activity evaluation using lipid peroxidation method reveals that methanolic extract of GWW

Table 1

Material	Concentration	% Inhibition	IC_{50} Value (µg/ml)
GWW (Methanolic Extract)	10	17.07	49.81
	20	33.56	
	40	40.19	
	60	60.12	
	80	74.56	
	100	82.63	
Apigenin standard	10	29.3	38.93
	20	36.5	
	40	49.98	
	60	66.64	
	80	81.45	
	100	89.45	
Catechin standard	10	40.08	33.32
	20	42.32	
	40	52.17	
	60	56.39	
	80	63.52	
	100	77.73	
Beta-lactoglobulin standard	10	17.31	49.73
	20	30.6	
	40	46.7	
	60	52.34	
	80	60.49	
	100	72.74	

Table 2: Hydroxyl radical scavenging activity of methanolic extract of GWW

Material	Concentration	% Inhibition	IC_{50} Value (µg/ml)
GWW (Methanolic Extract)	10	15.45	76.45
	20	23.41	
	40	34.21	
	60	44.56	
	80	51.43	
	100	59.6	
Apigenin standard	10	19.52	43.51
	20	23.41	
	40	34.21	
	60	44.56	
	80	51.43	
	100	59.6	
Catechin standard	10	26.45	41.01
	20	39.65	
	40	48.12	
	60	64.23	
	80	74.78	
	100	89.46	
Beta-lactoglobulin standard	10	22.34	60.86
	20	28.45	
	40	39.45	
	60	45.67	
	80	64.12	
	100	70.23	

Table 3: Lipid peroxidation inhibition activity of methanolic extract of GWW

Material	Concentration	% Inhibition	IC_{50} Value (µg/ml)
GWW (Methanolic Extract)	10	31.94	71.17
	20	35.94	
	40	42.27	
	60	45.45	
	80	52.61	
	100	68.21	
Apigenin standard	10	40.08	50.68
	20	42.32	
	40	52.17	
	60	56.39	
	80	69.86	
	100	77.73	
Catechin standard	10	18.32	49.70
	20	20.40	
	40	39.39	
	60	62.0	
	80	81.46	
	100	97.32	
Beta-lactoglobulin standard	10	20.38	66.30
	20	26.23	
	40	36.74	
	60	50.89	
	80	55.72	
	100	65.69	

has good activity when compared to standard antioxidants Apigenin, catechin and beta-lactoglobulin. The concentration of methanolic extract of GWW needed for 50% inhibition lipid peroxidation was 71.17µg/ml. Hence, the equiproportion combined methonolic extracts of *Psidium guajava, Juglans regia* and aqueous Whey showed potent antioxidant activity and the results i.e IC_{50} values obtained were comparable to their respective standards. The methanolic extract of *P.guajava* and *J.regia* fruit revealed the presence of phenolic compound, tannins and flavonoids in phytochemical screening. The presence of flavonoids and phenolic compounds may be responsible for antioxidant activity. Plant polyphenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants. Flavonoids and phenolic compounds act as good antioxidants because they possess redox properties. They show antioxidant activity because they may act as reducing agents, Hydrogen donors, Singlet oxygen quenchers or metal chelators. The results of antioxidant activity suggest that methanol extract of *P.guajava* and *J.regia* fruit has significant antioxidant activity and that activity may be attributed to the presence of phenolic compounds, tannins and flavonoids in the extract of *P.guajava* and *J.regia* fruit. Also Whey consists of large number of amino

acids of which cysteine in particular contribute to its antioxidant activity. Antioxidant activity of the compound like tannins, flavonoids and phenolic compounds may play vital role in prevention of diseases like Diabetes, Aging, Cancer and Acute lung injury acute respiratory distress, inflammation, skin damage, Hyperpigmentation and in boosting Immunity.

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