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Research Article

SCREENING OF MUNTINGIA CALABURA AND THEOBROMA CACAO FOR POTENTIAL BIOACTIVES

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ABSTRACT

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Key Words:

Muntingia calabura, Theobroma cacao, Antioxidant activity, Total phenol content.

The present study was carried out to evaluate the antioxidant activity of Raw Fruit extracts of *Muntingia calabura* and *Theobroma cacao*. The antioxidant effect were evaluated for Radical Scavenging activity using FRAP (Benzei and Strain, 1996) and CUPRAC assay with certain modifications (Apak *et al.*, 2004). The raw fruits of *Theobroma cacao* exhibited the highest Antioxidant activity with a Radical Scavenging effect of 90% at 100 μ g/ml. The raw fruits of *Muntingia calabura* exhibited a Radical Scavenging Effect of 70% at the same concentration. Total phenolic content of the extracts of *Muntingia calabura* and *Theobroma cacao* were determined by Follins Ciocalteau method (Demray *et al.*, 2009) with certain modifications. Positive correlations were found between Total Phenolic Content of the extracts and Antioxidant activity. The Phytochemical screening suggests that phenols and flavonoids of these extracts might provide a considerable Antioxidant potential. Addition of *Muntingia calabura* and *Theobroma cacao* in food will increase the Antioxidant content and may have a potential as a natural Antioxidant.

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INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons which can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid (vitamin C), or polyphenols. Substituted phenols and derivatives of phenylenediamine are common antioxidants used to inhibit gum formation in gasoline (petrol).

Bioactive compounds, such as polyphenols and antioxidants, present in plant-based foods provide several health benefits beyond basic nutrition and are positively involved in the prevention of chronic diseases. Many studies found several interesting biological properties ofplant foods, such as antiinflammatory, antioxidant, ant mutagenic, antiviral, antimicrobial and antiquorum sensing activities. Cruciferous vegetables act as a precious source of natural antioxidants, which contribute in protecting the human body against damages due to the oxidative processes and represent a rich source of antimicrobial compounds (Florinda Fratianni et al., 2013).

Fruits and vegetables have had conferred on them the status of functional foods (Hasler, 1998), they seem to be capable of delivering health benefits besides fulfilling physiological needs. Routine or habitual consumption of fruits and vegetables confers significant benefits to human health (Steinmetz & Potter, 1996).

Epidemiological data as well as in vitro studies strongly suggest that foods containing phytochemicals with antioxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Steinberg, 1991; Block *et al.*, 1992; Ames *et al.*, 1993; Hertog *et al.*, 1993; Byers & Guerrero, 1995; Knekt *et al.*, 1997; Elliot, 1999; Kaur & Kapoor, 2001).

The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, especially antioxidant vitamins including ascorbic acid, α -tocopherol and β carotene (Gey *et al.*, 1991; Willet, 1994; Kalt & Kushad, 2000; Prior & Cao, 2000). However numerous studies have conclusively shown that the majority of the anti-oxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin

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rather than from Vitamin C, E and β -carotene (Wang *et al.*, 1996; Kahkonen *et al*).



Muntingia calabura

Systemic Classification

Kingdom: Plantae Order: Malvales Family: Muntingiaceae Genus: Muntingia L. Species: M. Calabura

Muntingia calabura, the sole species in the genus *Muntingia*, is a flowering plant native to southern Mexico, the Caribbean, Central America, and western South America south to Peru and Bolivia. Jamaica Cherry is a very fast-growing tree of slender proportions, reaching 25 to 40 ft in height, with spreading, nearly horizontal branches. It has serrated leaves 2.5–15 cm long and 1–6.5 cm wide. The leaves are evergreen, alternate, lanceolate or ovate, long-pointed at the apex, oblique at the base. The flowers are small, white, and slightly malodorous. The flowers with 5 green sepals and 5 white petals and many prominent yellow stamens last only one day, the petals falling in the afternoon. Flowers resemble strawberry bloom, hence the common name, Strawberry tree.

Medicinal Uses

Antioxidant activity; improvement in endothelial function, vascular function, and insulin sensitivity; as well as attenuation of platelet reactivity and reduction in blood pressure.



Theobroma cacao

Systemic Classification

Kingdom: Plantae Order: Malvales Family: Malvaceae Genus: Theobroma Species: T. Cacao

Theobroma cacao is the taxonomic classification for the plant also called the cacao tree and the cocoa tree, which is a 13–26ft tall evergreen tree in the family *Malvaceae*. It is a native to the deep tropical regions of Central and South America. Leaves are alternate, entire, unlobed, 10–40 cm (3.9–15.7 in) long and 5– 20 cm (2.0–7.9 in) broad. The flowers are produced in clusters directly on the trunk and older branches. The flowers are small, 1–2 cm in diameter, with a pink calyx. The fruit, called a cacao pod is ovoid, 15–30 cm long and 8–10 cm wide. On ripening, the fruit changes colour from yellow to orange, and weighs about 500 g . The pod contains 20 to 60 seeds, usually called "beans" which are embedded in a white pulp. The seeds are the main ingredient of chocolate, while the pulp is used in some countries to prepare refreshing juice, smoothies, jelly, and nata.

Medicinal Uses

Improvement in endothelial function, vascular function and insulin sensitivity, and reduction in blood pressure.

Moreover proper scientific screening of potential bio actives of these plants followed by chemical investigations is necessary to make these herbal remedies more viable. In this context, the present study was undertaken to evaluate the antioxidant of raw fruits of *Muntingia calabura* and *Theobroma cacao*.

MATERIALS AND METHODS

Plant material Collection

Muntingia calabura (raw fruit) from Kristu Jayanti College, K.Narayanpura, Bangalore at an altitude of 949m. *Theobroma cacao* (raw fruit) from Mutholy, Pala city, Kottayam, Kerala at an altitude of 29 m. The collected plant samples were shade dried, powdered and stored in air tight containers. Plant samples were authenticated by Dr.Deepa. M.A., Botanist, Department of Lifesciences, Kristu Jayanti College (Autonomous), Bangalore.

Crude Extraction

Fresh plant material was collected, shade dried and powdered in a mixer grinder. A 10 g of *Muntingia calabura* was put into 50 ml of different solvents such as Ethanol, Methanol, Chloroform and Water and *Theobroma cacao* was put into 50 ml of different solvents such as Ethanol, Methanol and Chloroform respectively, then covered and kept standing for 48 hours for extraction at room temperature. The solvent was removed from the sample by evaporating at 65°C using a waterbath. Then 50 ml of the respective solvents were added into each extract in the beaker and filtered using sterile cotton gauze. The extract was stored in a air tight container and used for further studies.(Susy Tjahjani *et al.*,2014) *Phytochemical Screening*

The extracts of different plant materials were subjected to phytochemical studies using the Standard method described by Trease & Evans (1989).

Test for Terpenoids (Salkowski Test)

To 0.5ml of the extract, add 2 ml of chloroform. Then 3 ml of Concentrated H_2SO_4 was carefully added to form a layer . A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for Flavonoids

5 ml of dilute ammonia was added to 0.5 ml of the extract. To that 1 ml of Concentrated sulphuric acid was added. A yellow colouration that disappeared on standing indicates the presence of flavonoids.

Test for Saponins

To 0.5 ml of extract, 5 ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion, presence of an emulsion indicates the presence of saponins.

Test for Tannins

About 0.5 ml of the extract was boiled in 10 ml of water in a test tube. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue or black colouration. This indicates the presence of tannins.

Test for Alkaloids

0.5 ml of the extract was diluted to 10 ml with acidified alcohol and boiled. To 5 ml of this diluted extract, add 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. To this, Mayer's reagent was added. The formation of a cream precipitate was regarded as positive for the presence of alkaloids.

Test for Reducing Sugars (Fehling's Test)

To 0.5 ml of aqueous extract in a test tube, Fehling's Solution A and B was added and then kept in a boiling waterbath. The reddish brown colouration indicated the presence of reducing sugars.

Test for Anthraquinones

0.5 ml of the extract was boiled with 10 ml of sulphuric acid. 5 ml of chloroform was added and shaken well. The chloroform layer was pipetted into another test tube and 1 ml of 10% dilute ammonia was added. The resulting solution was observed for colour changes as an indication for the presence of Anthraquinones.

Test for Cardiac Glycosides (Keller-Killiani Test)

To 0.5 ml of extract which was diluted with 5 ml of distilled water, add 2 ml of glacial acetic acid containing one drop of 0.5% ferric chloride solution. This was mixed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

Test for Steroids

2 ml of acetic anhydride was added to 0.5 ml of the extracts. To this, 2 ml of concentrated sulphuric acid was added. The colour

changed from violet to blue or green indicated the presence of steroids.

Test for Phenols (Ferric Chloride test)

0.5 ml of extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Test for Carbohydrates

0.5 ml of extracts were dissolved individually in 5 ml of distilled water and 2% anthrone reagent was added followed by concentrated sulphuric acid. A dark green colour indicated the presence of carbohydrates.

Tests for Oils and Resins

The extract was applied on a whatsmann filter paper. The development of a transparent appearance on the filter paper indicated the presence of oils and resins.

Determination of Total phenolic content (TPC)

The total phenolic content (TPC) of ethanol, chloroform, methanol and aqueous extract of *Muntingia calabura* (raw fruit) and *Theobroma cacao* (raw fruit) plant extracts were determined by using Folin-Ciocalteau method (Demiray *et al.*, 2009). Samples absorbance were measured at 650 nm. Results were expressed as catechol equivalents (μ g/mg)

Evaluation of Antioxidant activity

The antioxidant activity of the raw fruit extracts of *Muntingia* calabura and *Theobroma* cacao on the basis of the scavenging activity was determined according to the FRAP and CUPRAC assay method described by Benzie *et al.*,1996 & Apak *et al.*,2004 with certain modifications.

FRAP Assay: 0.2 ml to 1 ml of the standard was pipetted out into clean dry test tubes.0.2 ml of extract was added to test tubes labelled as Test. Then 3.8 ml of FRAP reagent [83.3 ml of 0.1 mM acetate buffer pH 3.6, 8.3 ml of 0.3 mM of 2,4,6tripyridyl-s-triazine (TPTZ) solution and 8.3 ml of 10 mM of FeCl₃.6H₂0] was added to all the tubes. The above reaction mixture was incubated for 30 minutes at 37°C. After incubation, the absorbance was measured at 570 nm against a blank using ascorbic acid as standard.

CUPRAC Assay: 0.2-1 ml of working standard Ascorbic acid was pipetted out into test tubes labelled as S_1 - S_5 . 1 ml of the plant extract was added to the test tube labelled as Test.1ml of 0.01M CuCl₂ was added into all the tubes followed by the addition of 1 ml of 7.5 mM neocuproine alcohol solution and 1 ml of ammonium acetate buffer of pH 7. The above reaction mixture was mixed well. Make up the volume to 4.1 ml using distilled water in all the tubes. The above reaction mixture was mixed well and incubated for 30 minutes under room temperature. The absorbance was measured at 450 nm against a blank using ascorbic acid as standard.

RESULTS AND DISCUSSION

Percentage yield of plant extracts

Fruits of *Muntingia calabura* and *Theobroma cacao* were extracted with different solvents and percentage yield was shown in Table No. 1.

	Solvents	Yield percentage (%)			
Sl.No.		<i>Muntingia calabura</i> Raw Fruit	<i>Theobroma cacao</i> Raw Fruit		
1.	Ethanol	86.16	89.34		
2.	Methanol	83.37	78.26		
3.	Chloroform	90.35	85.15		
4.	Aqueous	87.27	-		

Table No. 1	Yield percentage of Muntingia calabura and	1
	Theobroma cacao	

The chloroform extract of *Muntingia calabura* produced the highest yield (90.35%) and the ethanol extract of *Theobroma cacao* produced an yield of 89.34%.

Phytochemical Screening

The preliminary phytochemical studies were performed to screen the presence of different phytoconstituents in different solvent extracts. The results revealed the presence of six different phytochemicals which includes Terpenoids, Flavonoids, Saponins, Tanins, Reducing sugars, Phenols and Carbohydrates. The results of phytochemical screening of two plant extracts were shown in Table No.2.

 Table No 2 Phytochemical Constituents of Michelia

 champacca and Theobroma cacao

<i>Muntingia calabura</i> raw fruit			<i>Theobroma cacao</i> raw fruit			
Е	Μ	С	W	Е	М	С
-	-	-	+	+	-	+
+	+	-	+	+	+	-
-	-	+	-	-	-	+
+	-	+	+	-	-	-
-	-	-	-	-	-	-
+	-	+	+	+	+	+
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
+	+	-	+	-	-	-
-	-	-	-	+	-	+
-	-	-	-	-	-	-
	Mur E + + - + - + - - -	Muntingio raw E M - - + + - - + - - - + - - - + - - - - - - - - - + + - - - - - - - -	Muntingia cala raw fruit E M C - - - + + - - + + - - + + - + - - + - - - + - - - - - + - - - - - - - - - - - - - - - - - - - - - - - - - -	Muntingia calabura raw fruit E M C W - - + + + + - + - - + + - + + + - - + + - - - - + - + + + - - - - - - - - - - - + - - - - - - - <	Muntingia calabura Theorem raw fruit raw E M C W E - - + + + + - - + + + + - - + + + + - - + + + - + - + + + - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -	Muntingia calabura Theobroma raw fruit raw fruit E M C W E M - - + + -

*(-) indicates absence of the Phytochemical constituent

*E denotes the Ethanol extract of the respective sample, *M denotes the Methanol extract of the respective sample, *C denotes the Chloroform extract of the respective sample and *W denotes the Water extract of the respective sample.

The Phytochemical Screening showed the presence of Terpenoids, Flavonoids, Saponins, Tannins, Reducing sugars and Phenols in the extracts of *Muntingia calabura*. It showed the presence of Terpenoids, Flavonoids, Saponins, Reducing sugars and Carbohydrates in the extracts of *Theobroma cacao*.

Total phenol content

TPC varied significantly between chloroform extracts of *Muntingia calabura* (raw fruit) and *Theobroma cacao* (raw fruit). The results of TPC contents were tabulated in Table No.3.

 Table 3 The Total Phenol Content of the Chloroform extracts of Muntingia calabura and Theobroma cacao

SI.No.	Solvent	Test Samples	Total Phenolic content (μg)
1.	Chloroform	<i>Muntingia calabura</i> Raw Fruit	48.79
2.	Chloroform	<i>Theobroma cacao</i> Raw Fruit	3.8



Fig 1 The Total Phenolic Content of Chloroform Extracts of Muntingia calabura and Theobroma cacao

The TPC was found to be higher in *Muntingia calabura* Fruit extract (48.79 μ g) than the extracts of *Theobroma cacao* fruit (3.8 μ g). The antioxidant activity of *Muntingia calabura* and *Theobroma cacao* extracts may be due to the presence of significant amount of polyphenolic content.

Antioxidant activity

It shows the results of the FRAP and CUPRAC assay of the raw fruit extracts of *Muntingia calabura* and *Theobroma cacao* possess significant antioxidant activity (Table.No 4).

	Samples		Scavenging Effect(%)			
Sl.No.		Solvents	FRAP ASSAY	CUPRAC ASSAY		
1.	Theobroma	Ethanol	30%	44.4%		
	cacao Raw	Methanol	10%	2.2%		
	Fruit	Chloroform	90%	11.1%		
	<i>Muntingia</i> <i>calabura</i> Raw Fruit	Ethanol	30%	55.6%		
2		Methanol	50%	44.4%		
2.		Chloroform	70%	33.3%		
		Water	20%	11.1%		



Fig 2 Comparison of Antioxidant activity of extracts of *Muntingia* calabura (raw fruit) and *Theobroma cacao* (raw fruit)

The Chloroform extracts of *Theobroma cacao* has the highest Radical Scavenging activity compared to the other extracts. The Chloroform extracts of *Theobroma cacao* fruit possess the highest radical Scavenging activity (90%) compared to the Ethanol, Methanol or aqueous extracts. The Chloroform extracts of *Muntingia calabura* also showed significant Radical Scavenging Activity (70%) compared to the Ethanol, Methanol or aqueous extracts.

CONCLUSION

Qualitative phytochemical analysis of Muntingia calabura and Theobroma cacao extracts revealed the presence of seven different phytochemicals that include Terpenoids, Reducing Flavonoids, Saponins, Tannins, Phenols sugars. and Carbohydrates. The extracts were further analysed for total phenolic content (TPC), the results varied significantly between the extracts and the plants also possess significant in vitro antioxidant activity. The results of these investigations indicated that the Muntingia calabura (Raw Fruit) and Theobroma cacao (Raw Fruit) possess a potent antioxidant activity. The varied significant results revealed that the extracts possess significant invitro antioxidant activity. These medicinal plants may be used as a rich antioxidant potential to develop new source of drugs.

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