Screening of *Michelia champacca* and *Muntingia calabura* extracts for potential Bioactives

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Abstract - The present study was carried out to evaluate the antioxidant activity of *Michelia champacca* (Seed and Fruit) and *Muntingia calabura* (Raw Fruit) extracts. 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 Chloroform extracts of *Michelia champacca* seed exhibited Highest Radical Scavenging Effect with 88.9% at 100µg/ml.The Chloroform extract of *Michelia champacca* fruit exhibited significant Antioxidant activity with Scavenging effect of 80% and the raw fruits of *Muntingia calabura* exhibited a Radical Scavenging Effect of 70% at the same concentration. Total phenolic content of the extracts of *Michelia champacca* and *Muntingia calabura* 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 *Michelia champacca* and *Muntingia calabura* in food will increase the Antioxidant content and may have a potential as a natural Antioxidant.

Keywords: *Michelia champacca*, *Muntingia calabura*, Antioxidant activity, Total phenol content.

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 anti-inflammatory, 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 anti-oxidation potential have strong protective effectsagainst major disease risks including cancer andcardiovascular diseases (Steinberg, 1991; Blocket 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 vegetableshas been attributed to the presence of anti-oxidants, especially anti-oxidant 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 conclusivelyshown that the majority of the anti-oxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from Vitamin C, E andβ-carotene (Wang et al., 1996; Kahkonen et al.,

Michelia champacca

SYSTEMIC CLASSIFICATION

Kingdom*: Plantae*

Order*: Magnoliales*

Family*: Magnoliaceae*

Genus*: Magnolia*

Species*: M.Champaca*

Michelia is a genus of flowering plants belonging to the Magnolia family (*Magnoliaceae*). The genus includes about 50 species of evergreen trees and shrubs. It is native to tropical and subtropical southeast Asia (Indomalaya) and southern China. The *Magnoliaceae* are an ancient family and the characteristic feature of the Magnolia family is that their large, cup-shaped flowers lack distinct petals or sepals. The leaves, flowers, and form of *Michelia* resemble *Magnolia,* but the blossoms of *Michelia* generally form clusters among the leaves. It is found in Tropical and subtropical moist broadleaf forests ecoregions at elevations of 200–1,600 metres (660–5,250 ft). It is native toBangladesh, China, India, Indonesia, Malaysia, Myanmar, Nepal, Thailand, and Vietnam.

Medicinal Uses

Bark -fevers, relives burning and in treating skin diseases, Root bark- amenorrhoea, Flowers - dyspepsia, nausea, fever, diuretic in renal diseases, Flower oil- cephalalgia and diuretic.

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.

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 *Michelia champacca*(fruit and seed) and *Muntingia calabura*(raw fruit)

MATERIALS AND METHODS

Plant material Collection

Michelia champacca (seed and fruit) from Prajyoti Hostel campus, K.Narayanpura, Bangalore-77.*Muntingia calabura* (raw fruit) from Kristu Jayanti College ,K.Narayanpura, Bangalore at an altitude of 949m.The collected plant samples were shade dried, powdered and stored in air tight containers. Plant samples were authenticated by Botanist Dr.Deepa. M.A., and procured in Herbarium, Department of Lifesciences, KristuJayanti College(Autonomous), Bangalore.

Crude Extraction

Fresh plant material was collected, shade dried and powdered in a mixer grinder.10g of each plant material (*Michelia champacca* and *Muntingia calabura)* were put into 50ml of different solvents such as Ethanol, Methanol, Chloroform and Water 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 50ml 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 theStandard method described by Trease& Evans (1989).

a)Test for Terpenoids (Salkowski Test)

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

b)Test for Flavonoids

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

c)Test for Saponins:

To 0.5ml of extract, 5ml 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.

d)Test for Tannins

About 0.5ml of the extract was boiled in 10ml 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.

e)Test for Alkaloids

0.5ml of the extract was diluted to 10ml with acidified alcohol and boiled . To 5ml of this diluted extract, add 2ml of dilute ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml 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.

f)Test for Reducing Sugars (Fehling's Test)

To 0.5ml 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.

g)Test for Anthraquinones

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

h)Test for Cardiac Glycosides (Keller-Killiani Test)

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

i)Test for Steroids:

2ml of acetic anhydride was added to 0.5ml of the extracts. To this , 2ml of concentrated sulphuric acid was added. The colour changed from violet to blue or green indicated the presence of steroids.

j)Test for Phenols (Ferric Chloride test)

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

k)Test for Carbohydrates

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

l)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 *Michelia champaca*(seed and fruit), *Muntingia calabura* plant extracts were determined by using Folin-Ciocalteu method (Demiray et al., 2009). Samples absorbance were measured at 650 nm. Results were expressed as catechol equivalents (µg/mg)

Evaluation of Antioxidant activity

The antioxidant activity of the *Michelia champaca*(seed and fruit) and the raw fruit extracts of *Muntingia calabura* 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 : 0.2ml -1ml of the standard was pipetted out into clean dry test tubes.0.2ml of extract was added to test tubes labelled as Test . Then 3.8ml of FRAP reagent [83.3ml of 0.1mM acetate buffer pH 3.6, 8.3ml of 0.3mM of 2,4,6-tripyridyl-s-triazine(TPTZ) solution and 8.3ml of 10mM of $FeCl_3.6H_20$] was added to all the tubes.The above reaction mixture was incubated for 30minutes at 37°C .After incubation, the absorbance was measured at 570nm against a blank using ascorbic acid as standard.

CUPRAC Assay: 0.2- 1ml of working standard Ascorbic acid was pipetted out into test tubes labelled as S_1-S_5. 1ml of the plant extract was added to the test tube labelled as Test.1ml of 0.01M $CuCl_2$ was added into all the tubes followed by the addition of 1ml of 7.5mM neocuproine alcohol solution and 1ml of ammonium acetate buffer of pH 7.The above reaction mixture was mixed well. Make up the volume to 4.1ml 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 450nm against a blank using ascorbic acid as standard.

RESULTS AND DISCUSSION

Percentage yield of plant extracts

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

Table No. 1: Yield percentage of *Michelia champacca* and *Muntingia calabura*

Sl.No.	Solvents	*Yield percentage(%)*		
		***Michelia champacca* seed**	***Michelia champacca* Fruit**	***Muntingia calabura* Raw Fruit**
1.	Ethanol	89.4	88.75	86.16
2.	Methanol	48.1	83.91	83.37
3.	Chloroform	79.6	85.48	**90.35**
4.	Aqueous	45.7	81.64	87.27

The chloroform extract of *Muntingia calabura* produced the highest yield (90.35%) and the aqueous extract of *Michelia champacca* seed produced the lowest yield (45.7%).

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 and Phenols. The results of phytochemical screening of three plant extracts were shown in Table No.2.

Table No. 2: Phytochemical Constituents of *Micheliachampacca* and *Muntingiacalabura*

Phytochemical tests	***M.champacca***								***M. calabura* raw fruit**			
	Seeds				**Fruit**							
	E	**M**	**C**	**W**	**E**	**M**	**C**	**W**	**E**	**M**	**C**	**W**
Terpenoids	+	+	+	+	+	+	+	+	-	-	-	+
Flavonoids	-	-	-	-	+	+	+	-	+	+	-	+
Saponins	-	-	-	-	+	-	-	-	-	-	+	-
Tannins	-	-	-	-	+	+	-	+	+	-	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Reducing sugars	-	-	+	-	-	+	+	-	+	-	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	-	-	-	+	+	-	+
Oils and Resins	-	-	-	-	-	-	-	-	-	-	-	-

*(+) indicates presence of the Phytochemical constituent.

*(-) 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 and Reducing sugars in the extracts of *Michelia champacca* Seed. The presence of Terpenoids, Flavonoids, Saponins Tannins and Reducing sugars in the extracts of *Michelia champacca* Fruit. It showed the presence of Flavonoids, Saponins, Tannins, Reducing sugars and Phenols in the extracts of *Muntingia calabura* .

Total phenol content

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

Table 3: The Total Phenol Content of the Chloroform extracts of *Michelia champacca* and *Muntingia calabura*

Sl.No.	**Solvent**	**Test Samples**	**Total Phenolic content (µg)**
1.	Chloroform	*Michelia champacca* Seed	12.74
2.	Chloroform	*Michelia champacca* Fruit	**20.51**
3.	Chloroform	*Muntingia calabura* Raw Fruit	3.8

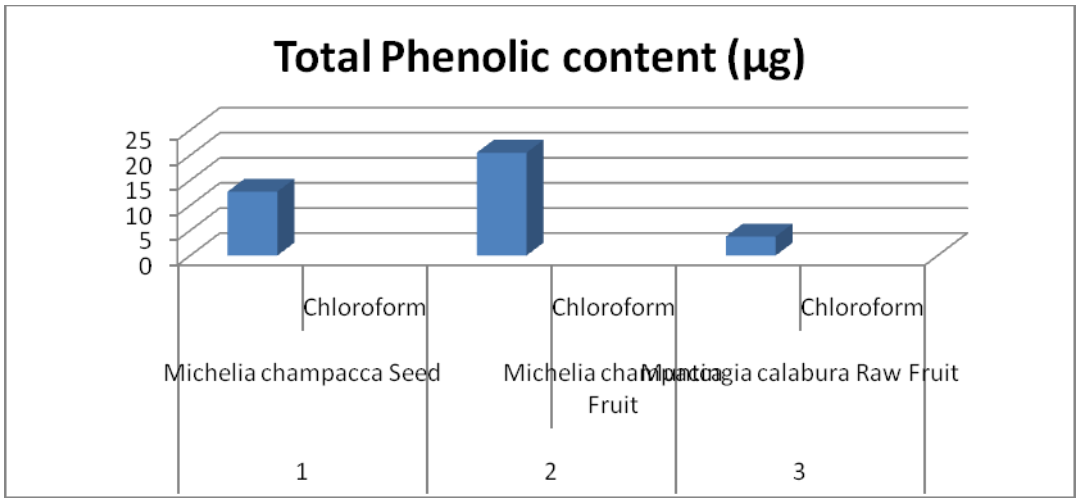

Fig.1: The Total Phenolic Content of Chloroform Extracts of *Michelia champacca, Muntingia calabura*

The TPC was found to be higher in *Michelia champacca* Fruit extract (20.51µg) than the extracts of *Michelia champacca* Seed (12.74 µg). The antioxidant activity of *Michelia champacca* and *Muntingia calabura* 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 extracts of *Michelia champacca* (seed and fruit) and *Muntingia calabura* (raw fruit) possess significant antioxidant activity (Table.No 4)

Table.No 4: FRAP and CUPRAC assay of *Michelia champacca and Muntingia calabura*

Sl.No.	Samples	Solvents	Scavenging Effect(%)	
			FRAP ASSAY	CUPRAC ASSAY
1.	*Michelia champacca* Seeds	Ethanol	10	11.1
		Methanol	10	44.4
		Chloroform	70	88.9
		Water	60	55.6
2.	*Michelia champacca* Fruits	Ethanol	20	44.4
		Methanol	40	55.6
		Chloroform	80	66.7
		Water	50	11.1
3.	*Muntingia calabura* Raw Fruit	Ethanol	30	55.6
		Methanol	50	44.4
		Chloroform	70	33.3
		Water	20	11.1

By comparing the FRAP and CUPRAC assay, it was observed that *Michelia champacca* Seeds had the Highest Radical Scavenging Activity (88.9%) and *Michelia champacca* Fruit had moderate Radical Scavenging activity(80%) .

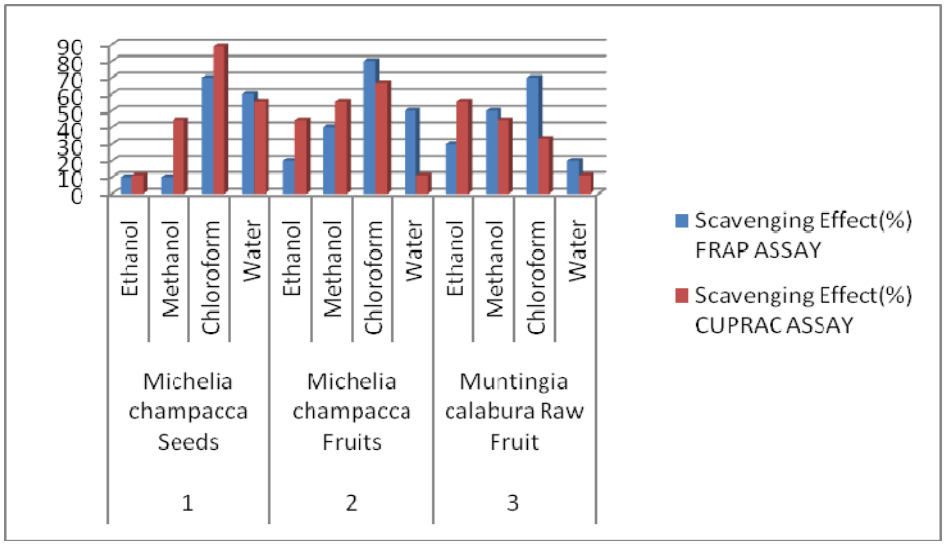

Fig. 2.:Comparison of Antioxidant activity of extracts of *Micheliachampacca*(seed and fruit), *Muntingiacalabura*(raw fruit)

The Chloroform extracts of *Michelia champacca* seed has the highest Radical Scavenging activity compared to the other extracts. The Chloroform extracts of *Michelia champacca* Seed possess radical Scavenging activity(88.9%) 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 *Michelia champacca* and *Muntingia calabura* extracts using different solvents revealed the presence of six different phytochemicals that include Terpenoids, Flavonoids, Saponins, Tanins, Reducing sugars and Phenols. The extracts were further analysed for total phenolic content (TPC), the results varied significantly between the different solvent extracts and the plants also possess significant in vitro antioxidant activity. The results of these investigations indicated that the *Michelia champacca* (Seed and Fruit) and *Muntingia calabura* (Raw Fruit) possess a potent antioxidant activity. The varied significant results revealed that the Chloroform 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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