

## Evaluation of Antioxidant Potential and Functional Properties of Complementary Dishes Using Selected Green Leafy Vegetables



### Food Science

**KEYWORDS :** Complimentary dish, Functional properties, Green leafy vegetables, Phytochemicals.

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### ABSTRACT

Green leafy vegetables can contribute significantly to human nutrition and health, because they contain almost all essential nutrients as well as functional properties. Processing of leafy vegetables in the form of complementary dish could extend their shelf life and also improve its consumption. Five different locally available leafy vegetables were chosen to formulate and evaluate ready to eat complementary dish. Pre-processed (cleaning, washing, cutting and steam blanching 30Sec;95°C) leafy vegetables were developed into chutney by adding other ingredients like green chilli, ginger, tamarind, fenugreek and cumin seed were packed in LDPE pouch and stored in room temperature for further analysis. The phytochemical and antioxidant properties of the developed products were evaluated. Study revealed that among the selected leafy vegetables used in the development of functional complementary dish, highest percentage of inhibition of free radicals was exhibited by functional complementary dish made from *Amaranthus tristis*. Antioxidant activity of functional complementary dish prepared from *Mentha spicata* found to be significantly higher than its counter parts. It is concluded that the functional complementary dish obtained from green leafy vegetables with minimal processing attributed to the enrichment of nutrients and other bio-active compounds which are important in the prevention and control of degenerative diseases. Hence the developed product would be recommended as practical food-based strategy to combat malnutrition in India and other developing countries.

### INTRODUCTION

Green leafy vegetables (GLV's) are treasure trove of various vitamins such as  $\beta$ -carotene, Vitamin - C, folic acid and riboflavin as well as minerals such as phosphorous, calcium and iron. Functional elements like antioxidants and phytochemicals which are considered as an anticancer agent are abundant in green leafy vegetables (Shibata et al., 1992). Green leafy vegetables have long been known as good sources of vitamins and minerals (Aletor et al., 2002; Shukla et al., 2006). Various leafy vegetables especially, amaranth, mint, palak and curry leaves has achieved marketable position and wide spread production in India. Due to their surplus production and low cost of production they are termed as 'poor man's vegetables'. But the constraint falls in proper utilization and consumption of it. In ancient days people consuming leafy vegetables in the form of chutney which is technically re-

ferred as complementary dish. At present due to time pressures people dodge the domestic preparation of complementary dish and looking forward convenience food. Hence the present study was aimed to formulate a ready to eat complementary dish incorporated with selected green leafy vegetables and to evaluate its phytochemical and antioxidant properties.

A complementary dish is defined as something which mates a dish complete or perfect. The complementary dish should be nutritive, taste and easy to cook. without a complementary dish the food item cannot be fulfilled. In this juncture, the study was intended to formulate a ready to eat functional complementary dish using selected green leafy vegetables (Table 1) and to evaluate its phytochemical and antioxidant properties.

**Table 1 Botanical Information and Nutritional properties of Green Leafy Vegetables used in the study**

Botanical Name	Family	Common Name	Local Name	Parts used	Nutritional & Medicinal Properties
<i>Amaranthus tristis</i>	Amaranthaceae	Amaranth	Ara keerai	Leaf	menorrhagia, leucorrhoea, dysentery, diarrhea.
<i>Cardiospermum halicacabum</i>	Sapindaceae	Baloon Vine	Mudakkatran	Leaf	arthritis, inflammations, constipation
<i>Cissus quadrangularis</i>	Vitaceae	Veldt Grape	Pirandai	Leaf	Osteoporosis, Ulcer, Anaemia, piles.
<i>Mentha spicata</i>	Lamiaceae	Spearmint	Pudhina	Leaf	Fever, lesions, inflamed glands, indigestion cough, asthma, cold.

### Materials and Methods

#### Procurement and processing of leaves.

Fresh edible leaves of *Amaranthus tristis*, *Mentha spicata*, *Cissus quadrangularis* and *Cardiospermum halicacabum* were collected from horticulture in Pondicherry University, Puducherry. Leaves free from pests and mechanical damage were sorted for further processing. Then the leaves were splashed with normal water and twice in distilled water. Cleansed leaves were blanched by steam for 30 Sec at 95°C and it was sautéed with tamarind, red chilly, garlic, cumin seed, fenugreek seed and salt at different proportions (Table 2) and it was packed with aluminum foil.

#### Formulation and optimization of functional complimentary dishes

Other than the processed leaves, ingredients such as tamarind,

red chilly, garlic, cumin seed, fenugreek seed etc. were added. Amount of ingredients was optimized according to the sensory evaluation.

**Table : 2 Formulation and optimization of functional complimentary dishes**

Ingredients (g)	Amaranthus tristis	Cardiospermum halicacabum	Cissus quadrangularis	Mentha spicata
Sample	50	50	50	50
Tamarind	15	15	15	15
Red chilly	5	5	5	5
Garlic	15	15	15	15
Cumin seed	6.5	6.5	6.5	6.5

Fenugreek seed	6.5	6.5	6.5	6.5
Salt	2	2	2	2

### Evaluation of quality characteristics of functional complementary dish

#### Alkaloids

A portion of the extract was treated with wagner's reagent and detected for the development of reddish brown precipitate.

#### Flavonoids

To a few drops of the extract, added 5ml of sodium hydroxide solution. Strong yellow colour is appeared which turns to colourless on addition of few drops of dilute acid indicated the presence of flavanoids.

#### Phenols

- (a) To 1 ml of the extract in water or alcohol added few drops of 10% aqueous ferric chloride solution. Formation of blue or violet colour indicated the presence of phenols.
- (b) To 2ml of extract was diluted with 3ml of distilled water and a few drops of 1% aqueous solution of lead acetate was added. Formation of yellow precipitate showed presence of phenols.

#### Tannins

- (a) To 1-2 ml of the extract few drops of 5% aqueous ferric chloride was added. A bluish black colour which disappear on addition of few ml of dilute sulphuric acid forming yellowish brown precipitate which indicated the presence of tannins.
- (b) To 5ml of the extract a few drops of 1% solution of lead acetate was added. A yellow or red precipitate was formed indicating the presence of tannins.

#### Saponins

- (a) To 0.5 ml of extract a drop of 5% sodium bicarbonate was added. The blend was agitated dynamically and set aside for 5 minutes. Development of honey comb like froth was indicated the occurrence of saponins.
- (b) 1ml of solution of the extract was diluted with distilled water to 20ml and it was shaken for 15 mins. Appearance of permanent froth indicated the presence of saponins.

#### Free radical scavenging activity (DPPH)

0.4 mM of methanol solution of DPPH was added with 20µl of different solvent extracts of different concentrations ranging from 100µg to 500µg. The mix was permitted to stand at room temperature for about thirty min, ethanol treated as blank. And DPPH in ethanol without the tuber extracts served as positive control. After 30 minutes, the staining of the purple colour was read at 518nm in a visible spectrophotometer.

#### Super-oxide radical scavenging activity

The scavenging activity towards the superoxide radical was measured in term of generation of O<sub>2</sub> the reaction mixture consisted of phosphate buffer (50mM, pH 7.6), riboflavin, EDTA, Nitroblue tetrazolium (NBT) And sodium cyanide Test compounds at various concentration of 100µg to 500µg/ml were added to make a final volume of 3 ml The absorbance was read at 530 nm before and after illumination under UV lamp for 15 minutes against a control instead of sample.

#### Total Antioxidant Capacity

0.3 ml extract was added with 3 ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated for 90 min at 95 ° C. Then the mixture was subjected to cool, the absorbance of the solution was read at 695 nm against a blank. The antioxidant activity is denoted as the number of equivalent of ascorbic acid and gallic acid.

### FTIR (Fourier transform infrared spectroscopy) (Thermo Nicolet Model:6700)

A thin pellet was prepared by compressing the fine powder obtained from solid sample with potassium bromide (KBr). The sample is placed on a stage in the instrument. Infrared waves at different wavelengths were passed through the sample. The amount of light passed through the sample is measured by the detector which is present in the instrument. These values are noted on a graph and this chart acts as an infrared spectrum that can be associated to drug standards or a library database.

### RESULT AND DISCUSSION

#### Evaluation of functional properties of the developed product

The therapeutic plants are of abundant importance to the well-being of people. Phytochemicals are the bioactive substances adds value to medicinal plants. In our human body, certain physiological actions are facilitated by these phytochemicals (Vanitha and Thangarasu, 2013). The results of phytochemical screening provide an empirical foundation for the practice of traditional medicinal plants. The preliminary phytochemical screening of plants leaf shown presence of phenols, flavonoids, alkaloids, saponins and tannins and the results were depicted in the table 3.

**Table 3 Screening of Phytochemical properties of selected plant leaves**

Phyto-chemicals	Tests	Amaranthus tristis	Cardiospermum halicababum	Cissus quadrangularis	Mentha spicata
Alkaloid	Wagner	++	--	++	+
Flavonoid	Alkaline reagent	++	Trace	+	trace
Phenols	FeCl <sub>3</sub>	--	++	--	trace
	Lead acetate	Trace	+	++	+
Tannins	FeCl <sub>3</sub>	Trace	+	--	trace
	Lead acetate	Trace	+	++	+
Saponins	NaHCO <sub>3</sub>	+	--	+	--
	Water	--	--	--	--

#### DPPH

##### % of Inhibition

DPPH result showed that highest % of inhibition was found in *Amaranthus tristis* (96.06%), which shows the highest free radical scavenging activity followed by *Cardiospermum halicababum* (86.40%), *Mentha spicata* (60.64%). Lowest % of inhibition showed *Cissus quadrangularis* (60.42%), which shows the least free radical scavenging activity. Biswa *et al.*, (2012) reported that *Mentha spicata* shows high DPPH activity. Kannat *et al.*, (2007) reported that *Mentha spicata* shows antioxidant activity and it is effective for radiation processed lamb meat.

**Table 4 Percentage of Inhibition of functional complementary dishes**

FCD incorporated with leafy vegetables	% of inhibition
FCD 1	96.06
FCD 2	86.40
FCD 3	60.42
FCD 4	60.64

FCD 1 - *Amaranthus tristis*, FCD 2 - *Cardiospermum halicababum*  
FCD 3 - *Cissus quadrangularis*, FCD 4 - *Mentha spicata*

#### Total antioxidant activity

Total antioxidant activity showed the free radical scavenging activity was found to be significantly highest in *Mentha spicata* (25.64mg) followed by *Amaranthus tristis* (22.70mg), *Cardiospermum halicababum* (8.58mg). Lowest activity was shown by *Cissus quadrangularis* (3.17mg). The finding of the present study is

in agreement with the Mohsen et al., (2014) stated that mint is rich in phenolic compounds and has natural antioxidant properties. Huang et al., (2011) reported the antioxidant activity of *Cardiospermum halicacabum* and it inhibits the development of paw edema. Chidambaram et al., (2010) reported the free-radical scavenging property of *Cissus quadrangularis*. Jainu et al., (2008) reported that *Cissus quadrangularis* shows antioxidant activity and prevents gastric ulcer.

**Table 5 Total antioxidant activity of functional complementary dishes**

FCD incorporated with leafy vegetables	Total antioxidant activity (mg)
FCD 1	22.70
FCD 2	8.58
FCD 3	3.17
FCD 4	25.64

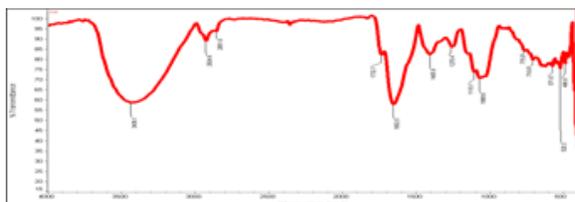
FCD 1 - *Amaranthus tristis*, FCD 2 - *Cardiospermum halicababum*  
FCD 3 - *Cissus quadrangularis*, FCD 4 - *Mentha spicata*

**FTIR studies on *Amaranthus tristis***

FTIR studies revealed the presence of functional group O-H, C-H, C=O in *Amaranthus tristis* plant leaves. It suggested that it might be presence of the Flavonoids, Tannins, Saponin, and Phenols. Velu et al., (2012) reported phenolic and flavonoid contents of the plant helps in the prevention of cardiovascular diseases.

**Table 6 FTIR studies on *Amaranthus tristis***

S. N.	Reference Wavelength cm <sup>-1</sup>	Wave-length cm <sup>-1</sup>	Functional group	Compound	Bond nature
1	3200-3600	3476	O-H	H-bonded Alcohol	Broad
2	2850-3000	2922	C-H	Alcohol	Strong
3	1670-1820	1687	C=O	Carbonyl compound	Strong
4	1350-1480	1382	-C-H	Alkane bending	Variable
5	1210-1320	1321	C-O	Acid	Strong
6	1050-1150	1066	C-O	Alcohol	Strong
7	1000-1400	1023	C-F	Alkyl halide	Strong



**Fig 1 FTIR studies on *Amaranthus tristis***

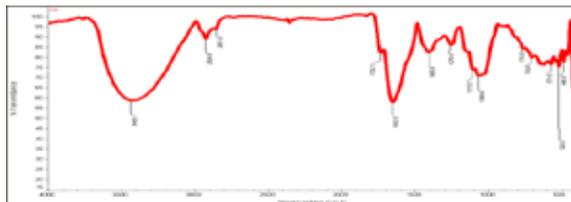
**FTIR studies on *Cardiospermum halicababum***

FTIR studies revealed the presence of O-H, C-H, C=O, C=C, C-N, C-O functional group in the *Cardiospermum halicacabum* plant leaves suggested the presence of Phenols, Flavonoids, Saponins. C=O of Amide suggested the presence of Alkaloids. Govindrajana et al (2012) and Waako et al (2005) reported the antimalarial activity of the plant.

**Table 7 FTIR studies on *Cardiospermum halicababum***

S. N.	Reference Wavelength cm <sup>-1</sup>	Wave-length cm <sup>-1</sup>	Functional group	Compound	Bond nature
1	3200-3600	3436	O-H	Alcohol	Broad
2	2850-3000	2924	C-H	Alkane	Strong
3	2850-3000	2851	C-H	Alkane	Strong

4	1670-1820	1732	C=O	Carbonyl	Strong
5	1640-1690	1652	C=O	Amide	Strong
6	1400-1600	1405	C=C	Aromatic	Medium-weak
7	1080-1360	1270	C-N	Amine	Medium-weak
8	1050-1150	1068	C-O	Alcohol	Strong
9	600-800	710	C-Cl	Alkyl halide	Strong



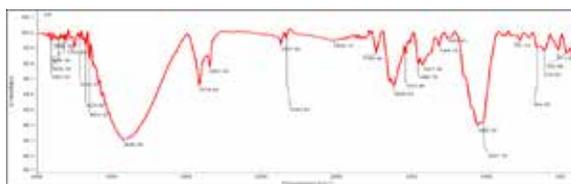
**Fig 2 FTIR studies on *Cardiospermum halicababum***

**FTIR studies on *Cissus quadrangularis***

FTIR studies revealed the presence of O-H, C-H, C=O, C=C, C-O, N-H functional group in the *Cissus quadrangularis* plant leaves suggested the presence of Phenols, Flavonoids, Saponins. Amine compounds suggested the presence of Alkaloids. Srisook et al., (2011) reported that it shows anti-inflammatory activity. Panthong et al., (2007) reported that it shows the analgesic as well as venotonic activity.

**Table 8 FTIR studies on *Cissus quadrangularis***

S. N.	Reference Wavelength cm <sup>-1</sup>	Wave-length cm <sup>-1</sup>	Functional group	Compound	Bond nature
1	3300-3500	3426	N-H	Amines	Medium
2	2850-3000	2919	C-H	Alkane	Strong
3	2850-3000	2851	C-H	Alkane	Strong
4	1740-1720	1740	C=O	Aldehyde	Strong
5	1620-1680	1620	C=C	Alkane	Variable
6	1400-1600	1543	C=C	Aromatic	Medium-weak
7	1050-1150	1062	C-O	Alcohol	Strong
8	600-800	619	C-Cl	Alkyl halide	Strong



**Fig 3 FTIR studies on *Cissus quadrangularis***

**FTIR studies on *Mentha spicata***

FTIR studies revealed the presence of functional group O-H, C-H, C=O, C=C in *Mentha spicata* plant leaves suggested that it might be present Phenol, Flavonoids and Saponins. C=O of amide suggested the presence of alkaloids. Bimakr et al., (2011) reported seven bioactive flavonoids in the plant. Yamamura et al., (1998) reported the presence of flavonoids, glucosides of lower alcohols in the plant which shows inhibitory activity on exocytosis in antigen stimulated rat basophils.

**Table 9 FTIR studies on *Mentha spicata***

S. No	Reference Wave-length cm <sup>-1</sup>	Wave-length cm <sup>-1</sup>	Functional group	Compound	Bond nature
1	3200-3600	3519	O-H	H-bonded Alcohol	Broad
2	2850-3000	2920	C-H	Alkane	Strong

3	1740-1720	1735	C=O	Aldehyde	Strong
4	1640-1690	1663	C=O	Amide	Strong
5	1400-1600	1436	C=C	Aromatic Compound	Medium-weak
6	1000-1400	1022	C-F	Alkyl Halide	Strong
7	500-600	572	C-Br	Alkyl Halide	Strong

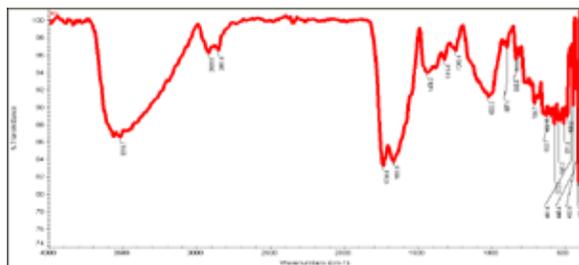


Fig 4 FTIR studies on *Mentha spicata*

## CONCLUSION

Among the selected leafy vegetables used in the development of functional complementary dish, highest percentage of inhibition of free radicals was exhibited by functional complementary dish made from *Amaranthus tristis*. Antioxidant activity of functional complementary dish prepared from *Mentha spicata* found to be significantly higher than its counter parts. It is concluded that the functional complementary dish obtained from green leafy vegetables with minimal processing attributed to the enrichment of nutrients and other bio-active compounds which are important in the prevention and control of degenerative diseases. Hence the developed product would be recommended as practical food-based strategy to combat malnutrition in India and other developing countries.

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