

Studies on total phenolic content and antioxidant activities of aqueous acetone and methanolic extracts from raw and boiled rhizomes of *Maranta arundinacea* L. and *Curcuma amada* Roxb.

* Vazhayil Saipriya, Arumugam Abirami, Archana Krishnan, Perumal Siddhuraju

Department of Environmental Sciences, Bioresource Technology Lab, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Abstract

The present study is designed to investigate the effect of the domestic cooking (boiling) on bioactive molecules extracted using aqueous acetone and methanol from edible parts of raw and boiled rhizomes of *Maranta arundinacea* and *Curcuma amada* Roxb. and the change in biochemical composition when it was subjected to hydrothermal processing was determined by *In vitro* antioxidant activities. The present investigation indicates that the processed sample of acetone extract was more effective in extraction of bioactive compounds than the raw sample of both the extract. The results illustrated that these perennial herbs offer considerable potential as functional food ingredients owing to the presence of excellent source of starch and fibres, minerals and trace minerals, phenolic compounds etc, which produce different biological activities and also to their high antioxidant capacity. The proper conservation of these underutilized rhizomes in their natural habitat will improve the biodiversity enrichment of these rhizomes and consumption of it minimizes the degenerative diseases.

Keywords: *M. arundinacea*, *C. amada*, antioxidant assays, flavonoids, bioactive compounds

1. Introduction

Numerous epidemiological studies established a connection between phytochemicals and the assortment of biological activities that plays the role on health benefits in human beings. Naturally derived bioactive compounds with antioxidant activity and mineral component from fruits and vegetable sources offer an alternative source of dietary ingredients to promote healthy life. The underutilized root and tuber crops are unseen treasure of healthy nutritious food. These crops being rich in food, nutrition and pliant to biotic and abiotic stresses can address the issues like sustainable livelihood. In general, tropical root and tuber crops are considered as the third important crops after cereals and grain legumes. These crops played significant role in food security, nutrition since inception of human habitation in mother earth. Now in the perspective of weather change, they are gaining the status as best resource for adaptive food, nutrition and livelihood. These mainly include cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas*), West Indian arrowroot (*Maranta arundinacea*) and other minor root crops. These crops are important to agriculture, food security and income for people in the developing countries. They are also recognized as the most efficient transformers of solar energy into food energy. Apart from those major tropical tuber crops, there are some minor crops which are cultivated in small pockets in many parts of the world. Those are rich in functional food properties with nutritional potential and medicinal values. These underutilized root and tubers are rich in minerals, vitamins, antioxidants and dietary fibre. And their starch is used primarily in food although the speciality uses in the field of paper, pharmaceutical and cosmetic industries have been reported. They can play an important role in extenuating hidden starvation through diet diversification. Most of these crops are bestowed with resilience to global warming and climate

change. Arrowroot (*Maranta arundinacea*) is an herbaceous rhizomatous plant cultivated widely in tropical countries for its starchy roots. The West Indian Arrowroot (*Maranta arundinacea*), a native of tropical America, is widely cultivated in countries like India, Sri Lanka, Indonesia, Australia and Philippines. The edible tuberous rhizomes are rich in starch and are also a commercial source of fine grade starch used often in weaned foods and biscuits. The starch also is reported to have medicinal uses and is an important ingredient in the preparation of barium meals and tablets. The plant which grows under shade is resistant to many insects and pathogen attack. Traditionally the tuberous rhizomes are used in the treatment of diarrhoea (Nishaa. S. *et al.*, 2012)^[8]. Local indigenous people use the root of *Maranta* as a poultice for smallpox sores, and as an infusion for urinary infections. Root has a bitter sharp taste, diuretic, natural, emollient, expectorant, antipyretic, appetizer; useful in inflammations, troubles in the mouth and the ear, gleet, ulcers on penis, scabies, lumbago, stomatitis (Srivastava. A.K, 2006). Many of these underutilized species are able to cope with very harsh and difficult environments as some of them are grown wild in less favourable environments. As mentioned earlier, climate changes and global warming have led us to opt for underutilized crops to ensure food security. Underutilized crops are normally planted by local people in rural areas. A lack of competitiveness may be an important factor for underutilization. Mankind depends on a very limited number of crops to meet the needs of staple diets and on a very limited number of major non-food crops to meet associated needs. Nevertheless in the past human societies depended on a much wider range of species for food, fiber, health security and other needs. Hence, the present investigation is aimed at investigating the antioxidant potential of the selected underutilized samples through the *in vitro* antioxidant assays. The need exists for safe, economic,

powerful, and natural antioxidants to replace the synthetic ones. Many plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. Tropical roots and tuber crops are known as the energy banks of nature serving either as primary or secondary staple to meet the calorie needs of about one fifth of world's population. These crops have myriad and complex roles to play in the food security and reduction of hunger and elimination of poverty. Under angiosperms there are about 18 families which have more than 30 genera producing edible roots and tubers. Economically important tropical root and tuber producing species are commonly used for food, feed, medicine and in industries are enunciated in this compendium. And the sample for the study which belongs to the genus *Curcuma* in the family Zingiberaceae comprising more than 80 species of rhizomatous herbs. As the spices are considered to be the storehouse of active phytochemicals. The various spices belonging to the genus *Curcuma* are well known for their multiple uses as medicines, cosmetics, dyes, flavourings and nutraceuticals. Extensive work has been carried out on *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger), but *Curcuma amada* (mango ginger) is an untapped medicinal plant of the ginger family. They have originated in the Indo-Malayan region and are distributed widely in the tropics from Asia to Africa and Australia. Mango ginger (*Curcuma amada* Roxb.) is a unique species having mango flavour in its rhizomes and as the rhizomes being a storehouse of bioactive compounds which has the extensive use and is of high medicinal importance. It is medicinally used as coolant, aromatic and astringent and to promote digestion. A rhizome paste has traditionally been used for healing of wounds, cuts and itching and the external use of the rhizome paste for sprains and skin diseases is also an old practice in ancient days and the rhizome also possess carminative properties, as well as being useful as a stomachic. (R.S. Policegoudra et al., 2011)^[11]. Its shelf-life and quality is governed by storage temperature and time. Temperature is the most critical factor that alleviates or aggravates the physiology and bioactivity in mango ginger rhizomes after harvest. In areas where cold storage facilities are not available, it is a common practice to re-bury the rhizomes or leave them unharvested. Because of high medicinal properties and its importance in the food industry as a source of raw mango flavour, there is a need to retain quality after harvest. The subject of this study, is to evaluate the importance of the *Maranta arundinacea* and *Curcuma amada* Roxb. species in the Agro-eco system, analyze the Total phenolic content and antioxidant activities of aqueous acetone and methanolic extracts from raw and boiled rhizomes of *M. arundinacea* and *C. amada* Roxb. Of collected samples. The research can contribute to the health benefits for the society and helps for the awareness that the further utilization prospectus of the *Maranta arundinacea* and *Curcuma amada* Roxb. could be exploited and advocated for wider consumption and conservation in rural vicinity.

2. Materials and Methods

Chemicals

Ferric chloride, 2, 2'-diphenyl-1-picrylhydrazyl (DPPH),

potassium persulfate, 2,2-azino-bis (3-ethylbenzo-thiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra acetic acid (EDTA) disodium salt were obtained from Hi Media, Merck or Sigma. All chemicals were of analytical grade. All analysis was performed with UV-Visible spectrophotometer. (Cyber lab-UV 100.USA).

Plant Samples and processing

Rhizomes of *M. arundinacea*, and *C. amada* were collected from their natural vicinity at Palakkad district, Kerala, India, during Dec 2013. After procuring the samples, and upon the arrival of in the laboratory, the samples were cleaned by washing with water to remove the debris and the damaged portions. Then samples were divided into two equal portions. One portion was taken as raw and the other portion was taken for boiling. The samples were cut into pieces and boiled at 100°C for 15 min in the ratio 1:10 (w/v). The remaining water after boiling was discarded and the samples were dried in room temperature. After drying the raw and boiled samples were ground to fine powder and stored in screw capped bottles at room temperature for further analysis.



Plant

Rhizome

Fig 1: *Maranta arundinacea*



Plant

Rhizome

Fig 2: *Curcuma amada*

Solvent Extraction

Powdered samples of *M. arundinacea* and *C. amada* (each 15 g) were defatted by using petroleum ether in the ratio of 1:10 w/v with occasional shaking at room temperature for 24 h. then the samples were filtered through Whatmann No.1 filter paper. Then the samples were air-dried and extracted with 70% acetone and 80% methanol for all the samples in the ratio of 1:7 by occasional stirring at room temperature for 48 h and filtered through Whatmann No.1 filter paper. The residues were re-extracted with 70% acetone and 80% methanol in the ratio of 1:5 for another 24 h. The solvent extracts obtained were dried at 40 °C in an incubator (NSW. New Delhi) until sample getting a constant weight and recovered with the

respective solvents. The extract percentage recovery was calculated as equation;

$$\text{Recovery \%} = \frac{(\text{Extract} + \text{container (g)}) - (\text{Empty container (g)})}{\text{Sample weight (g)}} \times 100$$

Estimation of total phenolics and tannins

The total phenolic content was determined according to Folin-Ciocalteu method (FCM) described by (Siddhuraju and Becker, 2003)^[15]. FCM actually measures a sample's reducing capacity and can be considered as another antioxidant (electron transfer) capacity assay. For the assay, aliquots (100 µl) of extracts were taken in test tubes and the volume was made up to 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20 %) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE). Tannins in the extracts were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was weighed in a 100 mm × 12 mm test tube and to this 1.0 ml of distilled water and then 1.0 ml of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4° C for 4 h. Then the sample was centrifuged (3000×g for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenol content of the supernatant was measured, as mentioned above and expressed as the content of non-tannin phenolics on a dry matter basis (Siddhuraju and Manian, 2007)^[16]. From the above results, the tannin content of the sample was calculated as follows:

$$\text{Tannin (\%)} = \text{Total phenolics (\%)} - \text{Non-tannin phenolics (\%)}$$

Total flavonoids

The total flavonoid content was measured by a spectrophotometric assay (Zhishenet *al.*, 1999) outlined by Siddhuraju and Becker. A total of 0.1 ml aliquot of standard solution of Rutin at different concentrations (0–100 mg/l, external calibration with n = 6 concentrations) or sample was added to 10 ml volumetric flasks containing 4 ml water. At the onset of the experiment, 0.3 ml of 5 % NaNO₂ was added to the flask. After 5 min, 3 ml of 10 % AlCl₃ was added. At 6 min, 2 ml of 1M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 ml with water and mixed thoroughly. The absorbance of the mixture was determined at 510 nm versus the prepared blanks. Total flavonoid content was expressed as mg rutin equivalents (RUT) per g extract.

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacities of phenolic extracts of raw and processed *M. arudinacea* and *C.amada* were estimated according to the procedure described by Benzie and Strain, (1996) as modified by Pulido *et al.* (2000). FRAP reagent (900

µl), prepared freshly and incubated at 37°C, was mixed with 90 µl of distilled water and 30 µl of test sample, or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37° C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l FeCl₃.6H₂O and 25 ml of 0.3 mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). At the end of incubation the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Methanolic solutions of known Fe (II) concentration ranging from 100 to 2000 µmol/l (FeSO₄.7H₂O) were used for plotting the calibration curve. The parameter Equivalent Concentration (EC₁) was defined as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l FeSO₄.7H₂O. EC₁ was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

Metal chelating activity

The extracts (100 µl) were added to a solution of 2 mmol /l FeCl₂ (0.05 ml). The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The results were expressed as mg EDTA equivalent/g extract using the calibration curve of EDTA. Linearity range of the calibration curve was 0.5-2.5 µg (Diniset *al.* 1994)^[4].

Stable free radical scavenging activity using DPPH' method

The radical scavenging activity of sample extracts was measured using DPPH radical by the method of Brand-Williams *et al.* (1995)^[3] with slight modifications. Extract of 0.1 mL prepared in methanol was mixed with 3.9 mL of DPPH' (6×10⁻⁵ mol/l methanol) and the solution was incubated in room temperature at dark for 30 min and the decrease in absorbance at 515 nm was determined at the end of the incubation period with a spectrophotometer. The trolox standard was prepared in the range of 0-2.5 mmol/L. The concentration of DPPH was calculated from trolox standard graph and expressed as mmol trolox equivalents/g extract.

Total antioxidant activity assay by scavenging of (ABTS^{•+}) radical cation

ABTS was dissolved in water to a 7 mM concentration, ABTS radical cation (ABTS^{•+}) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30° C to give an absorbance at 734 nm of 0.700± 0.02 in a 1cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 µl aliquot of each dilution into the assay, they produced between 20-80 % inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 10µl of samples or Trolox standards (final concentration 0-15 µM) in ethanol OD (optical density)

was taken at 30° C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration (Re *et al.*, 1999) described by (Siddhuraju and Becker, 2003a). The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as $\mu\text{mol/g}$ sample extracts using the calibration curve of trolox. Linearity range of the calibration curve was 0.25-1.25 mm/l. The total antioxidant activity of ASC and BHA were also measured by ABTS method for comparison.

Nitric oxide scavenging activity assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured by the Griess reaction. Nitric oxide interacts with oxygen to produce nitrite ions that can be observed by Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci *et al.*, 1994)^[7]. Various concentrations (500 μg) of sample extracts and sodium nitroprusside (SNP, 5 mM final concentration) in phosphate buffer saline, pH 7.4, in a final volume of 1 ml were incubated at 25° C for 150 min. A control experiment without samples but with equivalent amount of vehicles was conducted in an identical manner of the sample. After incubation, the reaction mixtures were mixed with Griess reagent (1% sulfanilamide and 0.1 % naphthylethylenediamine dihydrochloride in 5 % H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine was measured at 540 nm. The total antioxidant activity of ASC (500 μg) and QUE (500 μg) were also measured by nitric oxide scavenging method for comparison. The % Nitric oxide scavenging activity was calculated by the following equation;

$$(\%) \text{ Nitric oxide scavenging activity} = (\text{Control OD} - \text{Sample OD}) / \text{Control OD} \times 100$$

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means were determined by Duncan's multiple-range test ($P < 0.05$) using SPSS (Version 13.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determinations \pm standard deviation. Pearson's correlation test was conducted to determine the linear correlations among variables.

3. Results and Discussion

Total Phenolics and tannins

The total phenolic and tannin content of the two different solvent extracts of raw and processed *M. arudinacea*, and *C.amada* were determined using FolinCiocalteu's reagent and the results are presented in Table 1. On comparing both extracts highest total phenolics and tannins were observed to be with acetone extracts which is in good agreement with the findings of Saravanan and Aradhya (2011). Total phenolic content (TPC) of the extract was found to be in the range of 93.39 \pm 1.36 (CPA) to 22.40 \pm 1.92 (MRM) mg TAE/g extract. The highest TPC was followed by other extracts such as MPA, CPM, MPM, MRA, CRM, CRA, MRM and the values are mentioned

in the Table 1. TPC was found to be lower than that of *C.aeruginosa* (700 mg GAE/100 g) and extract of Beetroot as 431.29 \pm 4.8 mg GAE/100 g and higher than that of *D.alata* varieties such as *Kimabajo* (69.9 \pm 1.3 mg GAE/100 g), *Samero* (70.2 \pm 1.2 mg GAE/100 g). As like total phenolic content (TPC), tannin content was found to be highest in *C.amada* tuber of processed acetone extract (39.2 \pm 0.42 mg TAE/100 g). Tannin is present in many different plant species and foods including tea, cocoa, beans, grapes, strawberry, etc. Tannin content present in all samples in the order of CPA > MPA > CRA > MPM > MRA > CRM > CPM > MRM. The lowest tannin content present in the processed methanol extract of *C.amada* may be due to leaching out of tannins in heating time. Among all extracts, maximum yield was obtained for processed acetone extracts of tuber samples. The extractable total phenolics and tannins of the acetone extracted samples were found to be higher than in other samples for each solvent that could be due to the solubility of phenolics and other aroma compounds. So, here the 70% acetone was found to be more efficient solvent for extracting phenolics and tannins. Although these compounds play an unknown role in nutrition (non-nutrients), many of them have properties including antioxidant, antimutagenic, anticarcinogenic, and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome (Ferguson, 2001).

Flavonoids

Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activities. Epidemiological studies suggest that Flavonoids in human diet may reduce the risks of various cancers as well as menopausal symptoms as they are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, inhibit hydrolytic and oxidative enzyme activity as well as antinociceptive and anti-inflammatory activities (Okonk *et al.*, 2012). The content of flavonoids in the raw and processed tubers were also estimated and given in table 1. The flavonoids present in the samples in the order of MPM > CPA > MRM > MPA > CRM > CRA > MRA. Among the extracts, MPM (65.22 \pm 3.33 mg TAE/g extract) showed highest content of flavonoids and the lowest was found to be in MRA (46.33 \pm 2.64 mg TAE/g extract). Methanol extract of *M.arudinacea* showed comparable content of (290 \pm 7 mg QE/100g extract) flavonoids (Nishaa *et al.*, 2013). According to the report of Souravh Bais (2011) the flavonoid content of *A.campanulatus* tuber of methanolic and acetone extract are 22.25 \pm 0.56 (QE equivalent) and 13.1 \pm 0.45 (QE equivalent) respectively. Most of the processed samples showed to contain more flavonoids in both tubers. It might be due to the structural modification of flavonoids like solubility, reduction in particle size etc., during steaming.

Table 1: Total Phenolics, Tannins and Flavonoid content of *Maranta arudinacea* and *Curcuma amada*.

Samples	Phenolics ^a	Tannins ^a	Flavonoids ^b
MRA	32.71 ^c \pm 1.71	24.71 ^d \pm 0.55	46.33 ^a \pm 2.64
MPA	59.73 ^a \pm 1.86	28.48 ^b \pm 0.46	52.77 ^{cd} \pm 3.0
MRM	22.40 ^e \pm 1.92	20.14 ^f \pm 0.31	53.55 ^{bc} \pm 3.68
MPM	32.95 ^c \pm 1.95	25.88 ^c \pm 0.37	65.22 ^a \pm 3.33

CRA	23.88 ^e ± 2.06	27.71 ^b ± 0.58	50 ^{cd} ± 4.04
CPA	93.39 ^a ± 1.36	39.2 ^a ± 0.42	59.55 ^{ab} ± 4.33
CRM	29.27 ^d ± 2.29	24.13 ^{de} ± 0.33	52.55 ^{bc} ± 3.68
CPM	33.83 ^c ± 1.97	3.77 ^e ± 0.46	62.22 ^a ± 3.3

Values are mean of triplicate determinations ± standard deviations. Mean values followed by different superscript letters in the same column are significantly ($p < 0.05$) different.

^a mg gallic acid equivalents/g extract.

^b mg rutin equivalents/g extract.

MRA- *M.arudinacea* Raw Acetone extract; MPA- *M. arudinacea* Process Methanol extract; CRA-*C.amada* Raw Acetone extract; CPA-*C.amada* Process Acetone extract; CRM-*C.amada* Raw Methanol extract; CPM-*C.amada* Process Methanol extract.

Free radical scavenging activity on DPPH

The DPPH assay is one of the widely accepted and most extensively used methods for estimating the free radical scavenging activities of antioxidants (Hu *et al.*, 2011; Wang *et al.*, 2012). When an electron or hydrogen atom donating antioxidant is added to DPPH system, a number of DPPH radicals get reduced equal to their number of available hydroxyl groups (Singh and Rajini, 2004; Wang *et al.*, 2012). At this stage of reaction, the stable yellow colored diphenylpicryl hydrazine (DPPH-H) is formed and the extent of reaction depends on the hydrogen donating ability of antioxidants, stable DPPH radical is widely used to evaluate antioxidant activities in a relatively short time when compared with the other methods. The DPPH radical scavenging activity of 80% methanol and 70% acetone are given in the table 2. The values of the samples were in the range of 10997.56 ± 156.09 (CRM) to 14272.50 ± 154.72 (CPA) extract/g of DPPH. When compared with standards such as BHA (841240.87 ± 18706.23) and ascorbic acid (814172.74 ± 18706.23) the samples showed lower activity. At 1000 µg/ml concentration the *M.arudinacea* exhibited 70.11% scavenging activity on DPPH (Agnel Ruba *et al.*, 2013)^[2]. The DPPH radical scavenging activity of *C.amada* showed 5 ± 0.8 EC₅₀ value (Policegoudra *et al.*, 2007)^[12]. According to the result of Agnel (2012) the rhizomes such as *C.malabarica* and *C.brog* showed the DPPH radical scavenging activity of 4.2 ± 0.20 and 1.9 ± 10 respectively. The highest activity observed in the processed acetone extract of *C.amada* against the stable lipophilic free radical DPPH, indirectly reveals their inhibiting capacity for lipid peroxidation. This also suggests its preventive ability against the deleterious free radical chain reactions in susceptible matrices such as biological membranes.

Antioxidant activity by ABTS⁺ assay

The reaction of ABTS radical cation has been widely used to measure the antioxidant capacity of natural extracts (Cai *et al.*, 2006; Adel *et al.*, 2011). The cationic radical scavenging ability

of methanol and acetone extracts of *M.arudinacea* and *C.amada* tubers were shown in the table 2. The scavenging ability of samples was in the range of 21313.85 ± 681.98 (MRA) to 27903.84 ± 971.78 (MPA) mMole trolox eqv/g extract. Samples of *M.arudinacea* and *C.amada* showed higher activity than *D.hamiltonii* as 10108.9 µmol/g (Ponnusamysamydurai *et al.*, 2012). According to the report of Agnel Ruba (2013)^[2] at 1000 µg/ml concentration of *M.arudinacea* possessed 57.28% scavenging activity on ABTS and the scavenging effect increases with the concentration of standard and samples.

Ferric reducing antioxidant power

Reducing power is a measure of ability of the extracts to reduce Fe³⁺ to Fe²⁺. Substances which have reduction potential reacts with potassium ferric-cyanide to form potassium ferrocyanide which then react with ferric chloride to form ferric ferrous complex that has become one of the antioxidant capacity indicators of medicinal plant (Halvorson *et al.*, 2002)^[6] as it may accord with overall antioxidant activity. This is because antioxidants are strong reducing agents and this is principally because of the redox properties of their hydroxyl groups and the structural relationships of any parts of their structure (Obboh and Rocha 2007; Eleazu *et al.*, 2011)^[9, 5]. The reducing power, shown by the ability of the extracts to reduce Fe³⁺ to Fe²⁺ was determined and the results were presented in Table 2. The ferric reducing antioxidant power of samples are in the order of CPA > MPM > CRA > MRM > CPM > CRM > MPA > MRA. Processed acetone extract of *C.amada* showed the highest reducing power as 7009.75 ± 137.17 and lowest activity showed MRA (2769.92 ± 50.13). The activity of Potato, Yam, Radish, and Beetroot were 472.45 ± 1.6, 5.34 ± 0.5, 78.54 ± 1.9, and 95.16 ± 1.0 µmol/TE/100g respectively. Here, the processed samples shows higher activity than the raw samples, it may be due to the leaching out of the antioxidants during the processing of the samples.

Metal chelating activity

The essay was employed to detect chelating activity of Fe²⁺. The chelating effect for all the extracts are evaluated and denoted in table 2. The activity was in the range of 0.83 ± 0.019 mg TAE/g extract (CaPA) to 1.54 ± 0.02 mg TAE/g extract (MaRA). The metal chelating activity of all the samples and standards are in the order of α-tocopherol > BHA > CRM > MPM > MRM > CRA > CPM > CPA > MPA > MRA. Metal chelating activity of samples are lower than standards. Chelating capacity of the *C.pictus* extract increased with increase in concentration. The metal chelating effect of extracts of rhizome of methanolic extract (74.6%) > ethanolic extract (52.2%) (Jayasri *et al.*, 2008). Processed samples have higher activity than raw samples due to increase in extractability of antioxidant compounds during thermal processing.

Table 2: DPPH[•], ABTS^{•+}, FRAP and metal chelating activity of raw and processed tubers of *M. arudinacea* and *C. amada*.

samples	IC ₅₀ of DPPH (g extract/g DPPH)	ABTS (mmol TE/ mg extract)	FRAP (mmol Fe(II)/g extract)	Metal chelating (mg EDTA/g extract)
MRA	11610.706 ^d ± 141.78	21313.85 ^c ± 681.98	2769.92 ^d ± 50.13	0.83 ^e ± 0.019
MPA	11167.88 ^e ± 172.11	27903.84 ^a ± 971.78	2893.64 ^d ± 51.47	1.044 ^d ± 0.019
MRM	11737.22 ^d ± 189.78	24608.84 ^b ± 715.17	3593.14 ^d ± 107.77	1.48 ^a ± 0.01
MPM	11576.64 ^d ± 162.56	27489.37 ^a ± 536.007	4068.99 ^d ± 118.86	1.49 ^a ± 0.016
CRA	10997.56 ^e ± 156.09	27592.5 ^a ± 687.62	3750.17 ^d ± 178.87	1.23 ^b ± 0.018

CPA	14272.50 ^a ± 154.72	27820.95 ^a ± 836.4	7009.75 ^d ± 137.17	1.05 ^{cd} ± 0.021
CRM	10997.56 ^e ± 156.09	27592.5 ^a ± 687.62	3302.87 ^d ± 210.45	1.54 ^a ± 0.02
CPM	13065.69 ^b ± 124.73	27779.5 ^a ± 715.17	3464.66 ^d ± 149.04	1.10 ^c ± 0.06
BHA	814172.7 ^a ± 18706.2	655137 ^a ± 61415.86	350760.4 ^c ± 72476.7	10.48 ^b ± 0.06
ASC	841240.8 ^b ± 2737.22	599419.7 ^a ± 104323.5	730073.8 ^a ± 89814.8	-
α-TF	-	-	-	12.67 ^a ± 0.25
TRO	-	-	637663.6 ^b ± 55147.37	-

Values are means of three triplicate determinations ± standard deviations. Mean values followed by different superscript in the same coloumn are significantly (P<0.05) different. BHA- butrylated hydroxyl anisole; ASC -ascorbic acid; α-TF- α-tocopherol; TRO-trolox; MRA - *M.arudinacea* Raw acetone extract; MPA- *M.arudinacea* Process acetone extract; MRM- *M.arudinacea* Raw Methanol extract; MPM- *M.arudinacea* Process Methanol extract; CRA- *C.amada* Raw Acetone extract; CPA- *C.amada* Process Acetone extract; CRM- *C.amada* Raw

Methanol extract; CPM - *C.amada* Process Methanol extract.

** - mmolTrolox equivalents/g extract

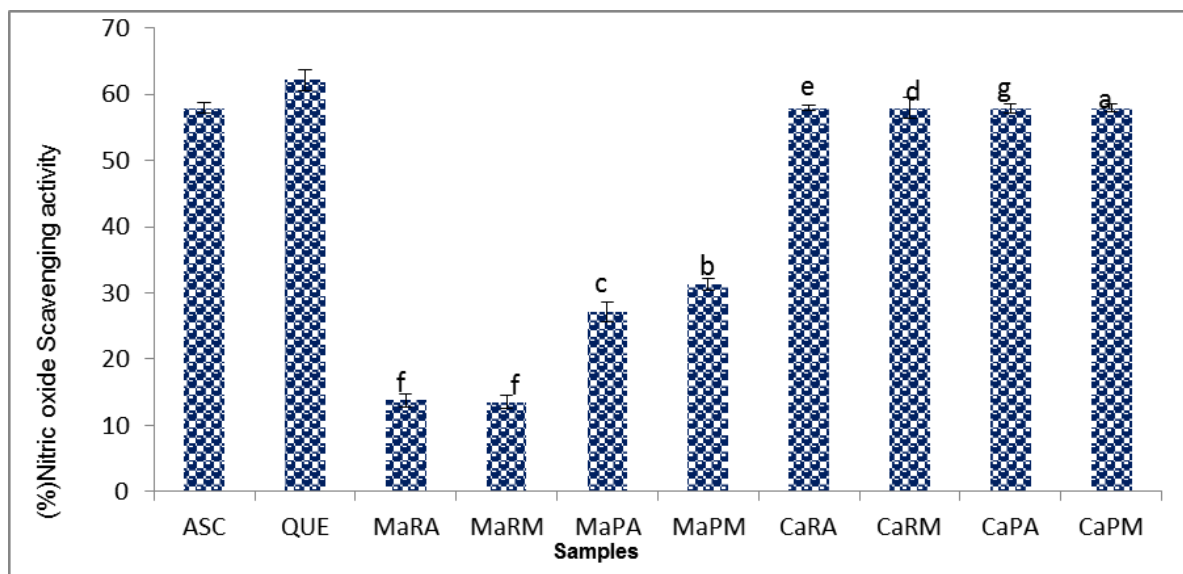
* - mM FeSO₄ equivalents/g extract;

** - mg EDTA equivalents/g extract

Nitric oxide radical scavenging activity

This scavenging effect against nitric oxide radical is also a wanted property of an antioxidant molecule. Hence this was measured and the results were shown in the figure 1. From the result it was found that the activities of samples were ranging from 13.8±0.95 (MRA) to 57.93±0.36% of (CRA). Raw and processed extract of *C.amada* tuber showed higher activity compared with the standards Quercetin (62.13±1.54) and Ascorbic acid (57.93±0.84). The inhibition % and IC₅₀ value of methanol extract of *A.campanulatus* tubers was 62.97% in 77.02 2g/ml (Sahu et al., 2009)^[19]. Raw and boiled extract of *C.amada* shows higher NO activity than *M.arudinacea* it clearly indicates *C.amada* contains more antioxidants.

Fig 3: Nitric oxide radical scavenging activity of aqueous Acetone and Methanol extract of *M.arudinacea* and *C.amada* rhizomes.



Nitric oxide radical scavenging activity of *Maranta arudinacea* and *Curcuma amada* extract. Values are means of triplicate determination ± standard deviations. Bars having different letters are significantly different (P<0.05). ASC- ascorbic acid; QUE- quercetin; MRA- *M.arudinacea* Raw Acetone extract; MRM- *M. arudinacea* Raw Methanol extract; MPA- *M. arudinacea* Process Acetone extract; MPM- *M. arudinacea* Process Methanol extract; CRA- *C. amada* Raw Acetone extract; CRM- *C. amada* Raw Methanol extract; CPA- *C. amada* Process Acetone extract; CPM - *C. amada* Process Methanol extract.

4. Conclusion

Neglected and underutilized food resources constitute the bedrock of the diversity in traditional and indigenous food systems of developing country communities. Traditional and indigenous foods are less deleterious to the environment and address cultural needs and preserve the cultural heritage of

local communities. Underutilized minor crop species are still a major source of nutrition for many indigenous communities. Thus, food and nutrition security of poor and marginal rural people is possible through the conservation and promotion of indigenous crop species that contain high nutrition. The present study paved the way to analyze and reveal the antioxidant potential of the two lesser utilized tubers *M.arudinacea* and *C.amada* and the investigation helps to encourage the proper conservation of these underutilized rhizomes in their natural habitat which will improve the biodiversity enrichment of these rhizomes and consumption of it minimizes the degenerative diseases. The antioxidant capacity was in positive correlation with presence of high amounts of phenols, tannins and flavonoids. Further investigations regarding *in vivo* studies and toxicity assessment to understand and promote it as healthy food ingredients are will be conducted. Based on the active profile exposed through various assays, it can be concluded that, among the extracts of two samples, the investigation helps

to predict that extract of the investigated samples showed promising levels of free radical scavenging activity and antioxidant potential which may be accounted for by the high phenolic content and it could be noticeable for better utilization as natural antioxidants in various food and pharmaceutical industries. The isolation of these bioactive components in the extracts would certainly help to ascertain the individual potency of the compounds which could be further exploited for use by the same industries. *Curcuma amada* is nutritionally rich when compared to *Maranta arundinacea*. Processing has enhanced the functionality and improved the availability of bioactive substances present in the *Maranta arundinacea* and *Curcuma amada*.

5. References

- 1 Angel GR, Vimala B, Bala Nambisan. Phenolic content and antioxidant activity in five underutilized starchy curcuma species. International Journal of Pharmacognosy and Phytochemical Research, 2012; 4(2):69-73.
- 2 Agnel Ruba A, Mohan VR. Evaluation of total phenolic and flavonoid contents and in vitro antioxidant activity of rhizome of *M. arundinacea*. An international journal of pharmaceutical sciences, 2013; 4:0976-7908.
- 3 Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT. Food Science and Technology. 1995; 28:25-30.
- 4 Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994; 315:161-169.
- 5 Eleazu CO, Okafor PN, Ahamefuna I. Total antioxidant capacity, Nutritional composition and Inhibitory activity of unripe plantain (*Musa paradisiaca*) on oxidative stress in alloxan induced diabetic rabbits. Pakistan Journal of Nutrition, 2010; 9:1052-1057.
- 6 Halvorson BL, Holte K, Myhrstad MCW, Baikmo I, Hvattum E, Remberg SF. A synthetic screening of total antioxidants in dietary plants. Journal of Nutrition. 2002; 132:461-471.
- 7 Marcocci L, Packer L, Droy-Lefaix MT. Antioxidant action of Ginkgo biloba extract EGB761. Methods in Enzymology, 1994; 234:462-475.
- 8 Nishaa S, Vishnupriya M, Sasikumar JM, Hephzibah P, Christabel, Gopalakrishnan VK. Antioxidant activity of ethanolic extract of *Maranta arundinacea* L. tuberous rhizome. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5:0974-2441.
- 9 Oboh G, Puntel RL, Rocha JWT. Hot pepper (*Capsicum annum*, Tepsin and Capsicum Chinese, Habenero) prevents Fe²⁺ induced lipid peroxidation in brain-in vitro. Food chemistry. 2007; 102:178-185.
- 10 Samyadurai P, Thangapandian V. Nutritional assessment, polyphenols evaluation and antioxidant activity of food resource plant *Decalepis hamiltoni* Wight and Arn. Journal of Applied Pharmaceutical Science. 2012; 05:106-110.
- 11 Policegoudra RS, Aradhya SM, Singh L. Mango ginger (*Curcuma amada* Roxb.)—A promising spice for phytochemicals and biological activities, 2011.
- 12 Policegoudra RS, Abiraj K, Channe Gowda D, Aradhya SM. Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome. Journal of Chromatography, 2007; 852:40-48.
- 13 Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/ antioxidant power assay. Journal of Agricultural and Food Chemistry, 2000; 48:3396-3402.
- 14 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 1999; 26:1231-1237.
- 15 Siddhuraju P, Becker K. Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var. *utilis*) extracts and certain non-protein amino acids through in vitro models. Journal of the Science of Food and Agriculture, 2003; 83:1517-1524.
- 16 Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) Seed extracts. Food Chemistry, 2007; 101:10-19.
- 17 Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chemistry. 2002; 79:61-67.
- 18 Siddhuraju P. The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (*Vigna aconitifolia*) (Jacq.) Marechal seed extracts. Food Chemistry, 2006; 99:149-157.
- 19 Sahu KG, Khadabadi SS, Bhide SS. Evaluation of *in vitro* antioxidant activity of *Amorphophallus campanulatus* (Roxb.) ex blume Decne, International Journal of Chemical Sciences. 2009; 7(3):1553-15.
- 20 Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radical Biology and Medicine. 1995; 18(2):321-36.
- 21 Bais S, Singh K, Bigoniya P, Rana AC. The *in vitro* antioxidant and free radical scavenging activities of suran (*Amorphophallus campanulatus* (Araceae) tubers extract. International Journal of Pharmacy and Life Sciences, 2011; 2(12):0976-7126.