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Research Article

TOTAL PHENOL CONTENT ESTIMATION OF *CAMELLIA SINENSIS, CURCUMA LONGA, MORINGA OLEIFERA, MURRAYA KOENIGII* AND *PIPER BETEL* AQUEOUS EXTRACTS

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ABSTRACT
Plant phenols and polyphenols present in fruits and vegetables works as an antioxidant compounds when consumed and are thought to play important roles in long term health by reducing the risk of chronic and degenerative diseases. Accordingly various epidemiological and *in vitro* studies on medicinal plants, fruits and vegetables has proven that antioxidant plant metabolites provides protection against the oxidative stress caused by reactive free radicals, chelating metals and active oxygen hydroxyl radicals; and provides health benefits to the body. Noteworthy antioxidant, anti-tumor, antiviral and antimicrobial activities are also reported for plant phenols and they are considered as active principles of numerous folk herbal medicines. Thus the presence of these compounds in the regular diet might be beneficial to human health by lowering the incidence of cancer and cardiovascular diseases. Phenols and polyphenols possess diverse physiological roles in the body and are a key for good health. Thus considering the benefits exhibited by these natural products to human health has recommended regular intake of some typical plant-derived food and beverages in everyday diets. The present work was to identify the alternative natural and safe sources of food-based antioxidants especially of plant origin. As majority of the rich diversity of Indian medicinal plants are yet to be scientifically evaluated for such properties, there is an attempt made here to find the potential antioxidant plant sources. Five plants having ethnobotanical medicinal importance and are edible in nature, are selected for the present study. Aqueous extracts of selected plants were screened for Total Phenol content (TCP) using Folin-Ciocalteau Method.

Keywords: *Plant Phenols, Antioxidant Activity, Free Radicals, Degenerative Diseases, TCP*

INTRODUCTION
Plant-based Natural Antioxidants: Antioxidants of natural origin are a realistic and powerful tool in the dietary and natural products industry. Now a days, awareness is found to explore the therapeutic potential of medicinal plants as antioxidants (Khodaparast and Dezashibi, 2007; Nahak and Sahu, 2010; Saleh *et al.*, 2010).
Natural antioxidants play a key role in health maintenance and can combat chronic and degenerative diseases, due to their free radical scavenging activity. The therapeutic potential of medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful. Thus proper study and research on plants can find their potential utility for prevention of the disease (Moshahid *et al.*, 2009; Kumar *et al.*, 2010).
The plants do naturally synthesize phyto-chemicals or PSMs (Plant Secondary Metabolites) such as phenols, terpenoids, tannins, curcuminoids, alkaloids, flavonoids, anthocyanins and carotenoids etc., as a part of their defense system.
Variety of aromatic, medicinal and spice plants contain these phyto-chemicals or PSMs (Plant Secondary Metabolites), capable of exerting antioxidant effect by quenching various deleterious free radicals (Nahar *et al.*, 2009; Wu *et al.*, 2009; Patel and Jasrai, 2009).
Considering the common routine use of plants; in the present study five edible plants (Table 1) were selected for the antioxidant activity screening assay through detection of Total phenol content (TPC). The phenols are polar in nature and thus can be readily extracted using polar solvents like water, ethanol, methanol etc.

Table 1: Plants selected for the present study

Plants	Common name	Plant Habit	Plant Part Used	Family
Camellia sinensis (L) O.Kuntze	Tea (Assam variety)	Herb	Processed leaves	Theaceae
Curcuma longa L	Turmeric	Herb	Rhizome	Zingiberaceae
Moringa oleifera Lam	Drumstick tree	Tree	Twigs	Moringaceae
Murraya koenigii (L) Sprengel	Curry leaf Tree	Tree	Leaves	Rutaceae
Piper betel L	Betel vine	Climber	Leaves	Piperaceae

Among the selected plants; tea is routinely used as a beverage in many Asian countries and at other places too. The fermented tea leaves are processed and been sold in market as a ready to use form. Turmeric rhizome and curry leaves are a routine spices used in the flavoring of curries, food dishes etc. in Indian cooking. Moringa tree possess small and delicate leaves which are many times used as a vegetable and consumed after cooking. While betel vine possess heart-shaped very shiny leaves. They are often chewed as a refreshing agent in India. The leaves have a characteristic aroma and a pungent taste (Table 2; Figure 1). Thus based upon the routine use of mentioned plants, they are screened to find the presence of beneficial phenolics and to correlate their medicinal effects. These plants are also documented for the medicinal uses in traditional medicine system. The plant material was extracted in the universal solvent-water.

Table 2: Medicinal uses of selected plants

Plants	Medicinal importance
Camellia sinensis	Cures cold, dropsy, dysentery, diarrhoea, epilepsy, eruptions, fever, neuralgic headaches, hemoptysis, hemorrhage, malaria, ophthalmia, smallpox, sores, toxemia, tumors and hepatitis.
Curcuma longa	Relives Biliary disorders, anorexia, coryza, cough, sore throat, diabetic wounds, liver and gallbladder problems, amenorrhea, anemia, asthma, conjunctivitis, jaundice, viral hepatitis, gastrointestinal discomfort, alzheimer's disease, rheumatoid arthritis, urticaria and traumatic diseases.
Moringa oleifera	Treat headaches, anemia, scurvy, piles, catarrh, sore throat, fever, bronchitis, asthama, eye and ear infections, wounds, urinary tract infection, glandular swelling and rheumatism.
Murraya koenigii	Beneficial in piles, itching, fresh cuts, skin eruptions, vomiting, burses, dropsy, halitosis, diarrhea and dysentery.
Piper betel	Cures boils, abscesses, wound, itches, abrasion, cuts and injuries, ringworm, mastitis, mastoiditis, leucorrhoea, otorrhoea, conjunctivitis, headache, hysteria, cold and cough, dyspnoea, disease of throat, colic, dysentery, constipation, piles, swelling of gum, rheumatism and joint pain.

Phenols as an Antioxidant: Among the various phyto-chemicals found in plants, Phenolics are the most wide spread secondary metabolites in plant kingdom and many medicinal plants exhibit antioxidant activity due to their total phenolic compounds (TPC). More precisely, the group Phenolic compounds includes phenolic acids, polyphenols, flavonoids and catechins etc. and they are very essential for plant development and play a significant role in their defense mechanisms by providing defense against microbial attacks and act as a deterrent to herbivores (Apak *et al.*, 2007). When consumed, these phenolic compounds play an important role in inhibiting and scavenging free radicals and are responsible to prevent the deleterious consequences of oxidative stress and provide protection against infections and degenerative diseases to human. Flavonoids are very commonly found in ripened fruits, leafy vegetables,

herbs etc., and in general are highly effective free radical scavengers. Flavonoids are very effective scavengers of peroxyl radicals, active oxygen radicals and they are also chelators of metals and inhibit the Fenton and Haber-Weiss reactions (Rathee *et al.,* 2006; Ghasemi *et al.,* 2009). Phenolic antioxidants have the ability to reduce lipid peroxidation, prevent DNA oxidative damage and scavenge reactive oxygen species (ROS) like superoxide, hydrogen peroxide, and hydroxyl radicals in our body (Yoo *et al.,* 2008). The antioxidant activity of phenol is mainly due to their redox properties, active hydrogen donor ability of hydroxyl substitution (-OH) and singlet oxygen quenchers. Several studies are focused to find the relationship between antioxidant activities of phenolic compounds as hydrogen donating free radical scavengers and their chemical structures (Sreelatha and Padma, 2009; Agrawal *et al.,* 2010; Kumar *et al.,* 2010). According to Siddhuraju and Becker, 2003, higher amount of polyphenol content corresponds to the higher antioxidant activity. Moreover, the synergistic effects of polyphenols make the antioxidant activity of the crude extracts more effective than the isolated compounds. Bioactive compounds commonly found in fruits, vegetables, herbs, spices, condiments and medicinal plants are studied in various *in vitro* and *in vivo* systems and have shown to possess many beneficial pharmacological effects like antioxidative, anticarcinogenic, atherosclerosis, anti-inflammatory, angiogenesis inhibitor activities etc. (Yoo *et al.,* 2008; Wu *et al.,* 2009). Thus, in recent years increased interests in nutrition, health and fitness among the people, has greatly emphasized the positive aspects of diet/ functional foods responsible for providing additional physiological benefits such as preventing or alleviating chronic diseases together with the fulfillment of basic nutritional requirements (Kaur and Kapoor, 2001; Khodaparast and Dezashibi, 2007).

Measuring Total phenol content (TPC) or Total antioxidant capacity assay: The Phosphomolybdenum method or Folin-Ciocalteu method usually detects antioxidants such as ascorbic acid, some phenolics, polyphenols, aromatic amines, glutathione, cysteine, α-tocopherol and carotenoids by their hydrogen and electrons donating ability (Sangeetha *et al.,* 2010). These compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent (Ghafar *et al.,* 2010).

MATERIALS AND METHODS

Figure 1: Plants used for the study

(a) Selection of Plants: In the present investigation, as described earlier aqueous extracts of five edible plants belonging to various taxa were screened for antioxidant activity (Table 1; Figure 1). Plants namely *Camellia sinensis* (L) O. Kuntze, *Curcuma longa* L, *Moringa oleifera* Lam, *Murraya koenigii* (L) Sprengel and *Piper betel* L, were selected for the study. The plants *Moringa* and *Murraya* were collected from Vadodara, Gujarat area. While plant materials such as *Camellia, Curcuma, Piper,* were obtained from the local markets of Gujarat State, India.

(b) Plant material extraction:

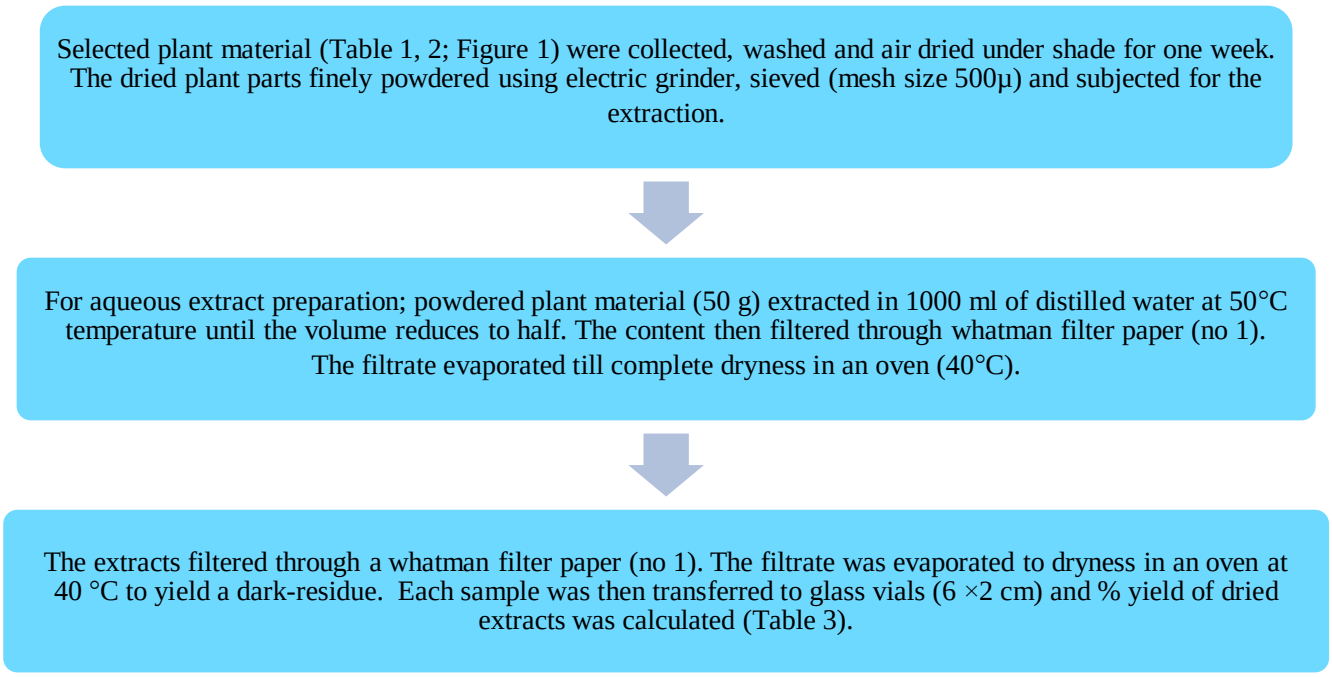

Figure 2: Extraction of Plant material

(c) Screening Antioxidant Activity: Aqueous extracts of selected plants were subjected for the screening of Total Phenol Content- Antioxidant activity following standardized protocols. The chemicals utilized were of pure and analytical grade. OD readings were taken using UV-VIS Spectrophotometer (Elico), in six replicates for each sample. The detailed procedure of the *in vitro* assay is mentioned as below. IC_{50} value was calculated for each standard, representing the concentration of the compounds that caused 50% free radical inhibition/ antioxidant activity.

Estimation of Total Phenol Content by Folin-Ciocalteau method: For the assay, to 6 ml of double dist. water added 2 mg dried aqueous extract, 0.5 ml Folin-Ciocalteau reagent and 1.5 ml 20% Na_2CO_3 solution. Then the total volume made up to 10 ml by addition of dist. water. The mixture incubated for 30 min. at 25ºC and then OD taken at 760 nm. Six replicates for each sample were tested and SE (Standard error) was calculated. The presence of antioxidant activity is indicated by a colour change from light yellow to blue. As per the assay, the intensity of blue colour is directly correlates with the extant of the antioxidant activity or the amount of phenol present in the sample (Figure 3). The IC_{50} value recorded for the Gallic acid standard was 0.50 mg/10ml. The % phenol content calculated using % extract yield with reference to Gallic acid standard curve and referred as Gallic acid equivalents (GAE) (Ghasemi *et al.*, 2009).

RESULTS AND DISCUSSION

Plant material Extraction and Extract yield: The fresh plant juices, organic solvent extracts and also oils of plants are routinely utilized by various researchers in various assays in search of newer antioxidant

compounds. In the present study, shade dried and powdered plant material was extracted in double distilled water. Based upon the % extract yield (Table 3) in water; plants can be arranged in the decreasing order as following. *Moringa oleifera* > *Murraya koenigii* > *Piper betel* > *Camellia sinensis* > *Curcuma longa*.

Table 3: % Yield of plant material

Plants	% Aqueous extract yield
Camellia sinensis	21.47
Curcuma longa	7.53
Moringa oleifera	34.83
Murraya koenigii	29.33
Piper betel	27.66

Antioxidant Activity by Selected Plant extracts: During the normal metabolism, oxidation reactions produce free radicals which start off chain reactions.

Biochemically antioxidants are the free radical scavengers and thereby they interrupt the harmful free radicals in the body and forms a stable radical by direct quenching of the hydroxyl radicals, singlet and triplet oxygen free radicals, chelating transition/ heavy metals, peroxide decomposers, acting as reducing agent, enzyme inhibitors and synergists (Aluyor and Ori-Jesu, 2008; Mandal *et al.,* 2009; Samojlik *et al.,* 2010).

Phenolics are the aromatic secondary plant metabolites widely spread throughout the plant kingdom and associated with colour, sensory qualities and nutritional and antioxidant properties of food. The key role of phenolic compounds in termination of free radical induced chain reactions, and a fair correlation between antioxidant and free radical scavenging activity and Phenolic content estimation assays is emphasized in several previously published reports (Aquil *et al.,* 2006; Singh *et al.,* 2009).

As per various studies; an antioxidant activity of traditionally used medicinal plants and herbal drugs is often correlated with its medicinal and disease curing ability in the body (Aquil *et al.,* 2006). The antioxidant property is due to the presence of their active secondary metabolites. To explore the use of plant extracts as antioxidants, the potential antioxidant activity can be evaluated and measured by its hydrogen donating potential.

Considering the highly significant therapeutic role of antioxidant phyto-chemicals; in the present investigation, efforts were made to find out the antioxidant capacity of selected edible plants using % Phenol quantification by Folin-Ciocalteau method. The results of the experiment is expressed in GAE (Gallic acid equivalent) has overall demonstrated presence of potential antioxidant activity in all the screened plant extracts (Table 4).

Table 4: % Total Phenol Content GAE recorded for the Aqueous extracts of selected Plant

Plants	% TPC GAE ± SE
Camellia sinensis	4.963 ± 0.053
Curcuma longa	0.132 ± 0.002
Moringa oleifera	0.662 ± 0.032
Murraya koenigii	3.041 ± 0.028
Piper betel	3.257 ± 0.039

The % Total Phenol Content of plant extracts were calculated in respect to the standard compound Gallic acid and the value thus defined as GAE (Gallic acid Equivalent) and % yield of plant extracts (Table 4; Figure 4). The standard compound Gallic acid at 0.01mg/ml conc. exhibits 0.00010% Phenol content estimated by Folin-Ciocalteau Method.

Figure 3: % Total Phenol Content as visible by the colour difference in test-tubes during assay

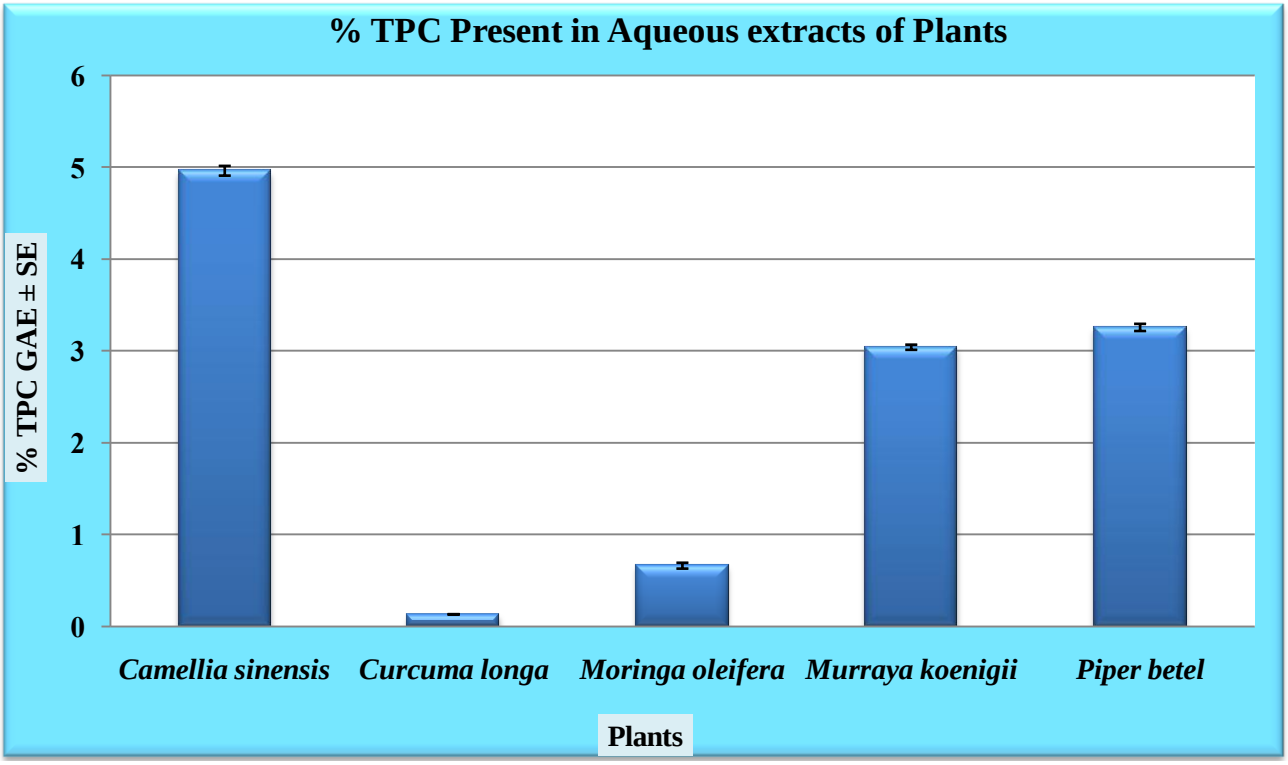

Figure 4: % Total Phenol Content present in aqueous extracts of studied plants

In the present Folin-Ciocalteau method, all the selected plant extracts demonstrated presence of potential antioxidant phenolic compounds. Among the aqueous extracts screened, an excellent amount of antioxidant activity in TPC assay were demonstrated by *Camellia sinensis* (4.963 ± 0.053), followed by extract of *Piper betel* (3.257 ± 0.039), *Murraya koenigii* (3.041 ± 0.028) % GAE Total Phenol Content (Table 4; Figure 3, 4). Also *Moringa oleifera* (0.662 ± 0.032) and *Curcuma longa* (0.132 ± 0.002) % GAE demonstrated moderate amount of phenol content. Phenols are polar in nature and can be better extracted in polar solvents like water, methanol, ethanol etc. According to Nahak and Sahu (2010) polar solvents like methanol and ethanol are helpful to better isolate the phenolic compounds. Bushra *et al.*, 2009 in their study found that, higher extract yields of phenolic contents and thereby high antioxidant

activity obtained using aqueous organic solvents like 80% methanol and 80% ethanol, as compared to the absolute organic solvents like ethanol and methanol extracts.

Conclusion

Many herbs, vegetables, fruits, spices and aromatic plants, are the primary source of natural antioxidant compounds and thus are highly useful in industry as a natural additives/ preservation agent in food and cosmetics because of their dual functionality in preventing lipid oxidation and a microbial spoilage. Apart from this, diet of antioxidant rich foods and beverages protects the body from the onset of various degenerative diseases due to their protective biochemical functions. In the present study, good amount of % Total phenol content is found within aqueous extracts of all selected plants. Thus inclusion of screened plants in our daily diet helps to prevent free radicals oxidative damage in the body and keep the body healthy in a natural way.

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