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Effect of cooking on total phenol, total flavonoids and DPPH free radical scavenging potential of *Plectranthus amboinicus*

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Abstract
Most leafy vegetables undergo cooking before consumption. Therefore, this study was conducted to evaluate the effect of cooking on total phenolics, total flavonoids and DPPH free radical scavenging potential. Plant phenolics and flavonoids play a great role in scavenging free radicals in the body and act as antioxidants. Thus their determination is sometimes needed. *Plectranthus amboinicus* is a commonly available medicinal herb in India. *Plectranthus amboinicus* is an edible, nutritive plant. Comparative studies on total phenol, total flavonoid, and DPPH free radical scavenging potential was carried out on raw and cooked extracted of *P. amboinicus*. The result indicates cooking enhances the antioxidant properties of *Plectranthus amboinicus*.

Keywords: *Plectranthus amboinicus*, cooking, total phenolic, total flavonoid, DPPH scavenging

Introduction
P. amboinicus (Loureira) Sprengel is a member of the family, Lamiaceae. or mint family. The paleotropical oil-rich genus, *Plectranthus* belongs to the subfamily Nepetoideae. It comprises about 300 species of annual or perennial herbs or subshrubs which are often succulents [1]. It is synonymous to *Coleus aromaticus*, Benth and is commonly known as Cuban oregano, Spanish thyme, Indian Borage, Mexican mint, etc. The herb has green, thick, succulent, heart shaped, leathery and juicy leaves with scalloped edges [2]. The raw leaves emanate an oregano-like flavor and odour when cut or crushed. *Plectranthus amboinicus* is an edible, nutritive plant [2] and it is known to possess antimicrobial, antiepileptic and antioxidant properties [3-5].
Antioxidants are substances that significantly delay or inhibit oxidation of an oxidizable substrate when present at low concentrations in comparison with those of the substrate.(6) Endogenous antioxidants are synthesized within the system of living organisms and repair free radical damage internally by initiating cell regeneration while exogenous antioxidants which are derived from sources outside the living systems such as diets(7) stimulate cell repair externally [8]. At present, there are keen interests and widespread researches on exogenous antioxidants from natural sources perhaps, due to the fact that they are less expensive, readily available and believed to have lesser side effects when compared to their synthetic counterparts [9].
Green leafy vegetables, fruits and vegetable oils are excellent sources of antioxidant components. Most vegetables undergo a cooking process rather than being eaten raw. Cooking practices may affect the antioxidant content and properties in vegetables [10]; however, little is known in this regard. Traditionally, in folk medicine people also use leaves of *P. amboinicus* in their food [1]. So this prompted us to investigate leaves of plant to identify its nutritional value. Thus, the aim of this study was to evaluate the *in vitro* antioxidant activity of raw and cooked leaf of plant *P. amboinicus*.

Materials and Methods
Collection of plant material
The young leaves of *Plectranthus amboinicus* were collected from Surat, Gujarat in month of December 2016, and authenticated by expert. The fresh leaves of plant were thoroughly washed 2-3 times to remove adhering dust and impurities. The edible part were separated and blotted on filter paper, and use for raw and cooked extract of plant.

Sample preparation

Fresh green leafy vegetables were rinsed in water, dried on paper towel and the edible portions were separated from the inedible portion. The edible portions were chopped into almost equal small pieces or slices, mixed well and a portion (40 g) of the chopped vegetables was cooked by steaming in 200 ml of distilled water for 10 mins, while the other portion was not cooked. Fresh and cooked samples of the green leafy vegetables were then blended, centrifuged and filtered. The filtrates were stored in the refrigerator for subsequent analysis [11].

Determination of total phenol

The total phenolics of each fruit extract were determined by the Folin-Ciocalteu method [12] with some modifications. The diluted aqueous solution of each extract (0.5 ml) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 ml). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/l in water, 2 ml) was added. After 2 hours of incubation, the absorbance was measured at 760 nm against water blank. A standard calibration curve was plotted using Gallic acid. The results were expressed as mg/g Gallic acid equivalents (GAE)/ g of fruit weight.

Determination of total flavonoids

The total flavonoids were estimated by aluminium trichloride colorimetric method [13]. A solution (2ml)of Fraction(II,III) was mixed with a solution (2ml) of aluminum tri chloride ($AlCl_3$) in methanol (2 %).The absorbance was read at 415nm after 10 min against a blank sample consisting of amethanol (2ml) and with $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as mg of Quercetin equivalents (QE)/g of dry weight.

DPPH free radical scavenging assay

The DPPH antioxidant assay was determined. (14) Briefly, 1mM DPPH in 99.5% Ethanol. To 0.5ml of DPPH redical solution, Add 2 ml of the prepared solution of silver nano particles (whole leaf derived nano particle and flavonoids derived nanoparticles), and the reaction mixture is vortexed for 10s and allow to stand at room temperature for 30 min. The absorbance is recorded at 517 nm by using UV-Spectrophotometer. Compare with the 75% ethanol which act as control solution. Ascorbic acid is used as reference antioxidant compound. The percentage of DPPH redical scavenging activity is expressed as:

DPPH scavenging effect (%) = [1- (Test sample absorbance/blank sample absorbance)] ×100 (%).

Result and Discussion

In this study, the effect of cooking on total phenolics and antioxidant activity *P. amboinicus* of were assessed.

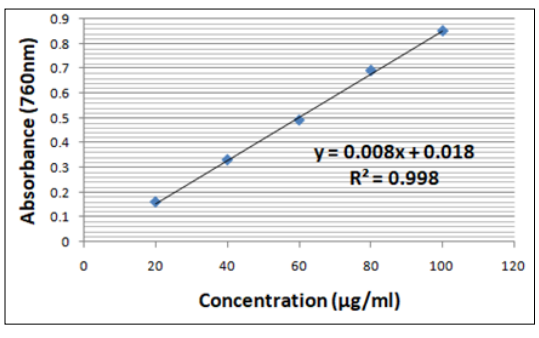

Fig 1: Standard curve of Gallic Acid

Gallic acid was used as a standard compound and the total phenols were expressed as mg/g Gallic acid equivalent (mg GAE/gm) using the standard curve equation: y = 0.008x + 0.018, R^2= 0.998, Where y is absorbance at 760 nm and x in total phenolic content. (Fig. 1)

According to Pietta 2000 [15], the antioxidant activity of phenolics is largely due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators. In present study, 8 mg GAE/gm TPC was obtain in raw sample while 10 mg GAE/gm was obtain in cooked leaf sample of activity *P. amboinicus*. This indicates that most of the phenolic compounds trapped in fibre of green leafy vegetables are actually more available in the cooked compared to the raw. The percent gain in the total phenol content during cooking may be due to the breakdown of tough cell walls and release of phenolic compounds trapped in the fibre of green leafy vegetables for easier absorption in the small intestine [16-17].

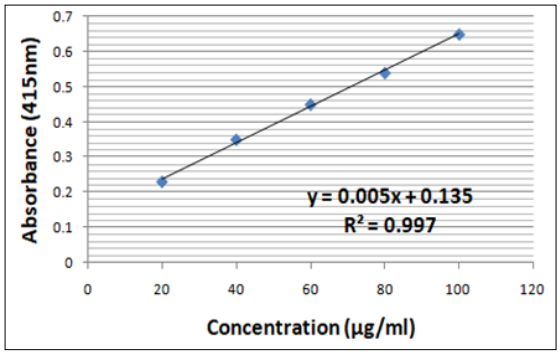

Fig 2: Standard curve of Quercetin

Quercetin was used as a standard compound and the total Flavonoids were expressed as mg/g Quercetin equivalent antioxidant capacity (mg QEAC/gm) using the standard curve equation: y = 0.005x + 0.135, R^2= 0.997, Where y is absorbance at 415 nm and x is total Flavonoid content. (Fig. 2)

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress, which has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [11]. The total flavonoid, reported as Quercetin equivalent antioxidant capacity (QEAC). In present study TFC was obtained 7.2 mg QEAC/g in raw sample while in cooked sample TFC was obtained 12.6mg QEAC/g. total flavonoids of cooked vegetables were higher than total flavonoids of raw vegetables, indicating a possible release of some flavonoids during the cooking of the green leafy vegetables [11].

The free radical scavenging activity of leaf of *P. amboinicus* was studied by its ability to reduce the DPPH. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [18]. A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant this colour disappears due to quenching of DPPH free radicals and convert them into a colorless product i.e. 2,2-diphenyl-1-hydrazine. Antioxidant mechanism performed by providing hydrogen atoms or electron [19]. The results revealed that DPPH radical scavenging ability significantly increased with cooking. DPPH radical scavenging ability was found 81% in raw sample while 90% in cooked sample. This increase in radical scavenging ability could be attributed to the increase in the total phenol and flavonoid content [11].

Table 1 Effect of cooking on total phenolic, total flavonoid and DPPH scavenging.

Sample	Total phenolic content mg GAE/g	Total flavonoid content mg QEAC/g	%DPPH free radical scavenging
Raw	8 mg GAE/g	7.2 mg QEAC/g	81%
Cooked	10 mg GAE/g	12.6 mg QEAC/g	90%

Conclusion

Cooking is a common practice to consume different leafy vegetables. Cooked vegetables have much better nutitional quality and due to chemical reactions during cooking, they are much better digestible and have an increased antioxidant value. In this study, total phenolic, total flavonoid, DPPH scavenging activity was enhanced while cooking. In conclusion, we suggest that it is better to consume leaf of *P. amboinicus* in their cooked forms. As observed in the present study that cooking enhances the antioxidant potential in *P. amboinicus* plant leaves as used in this study and so can be used as an easy and safe accessible source of natural antioxidants, as food supplements.

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