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A Study on Analysis and Applications of Phytochemicals and Antioxidants of *Centella Asiatica* in the Development of Food Product

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Abstract

Nature has blessed and gave our nation with a colossal abundance of medicinal plants. Hence, our nation, India has frequently been alluded to as the "medicinal garden" of the world. *Centellaasiatica*L. belongs to the family Apiaceae is an ancient Ayurvedic plant traditionally well-regarded for its nutritional and medicinal values.*Centella*is also known as Pennywort, Asiatic pennywort, Indian pennywort, Gotukola and Brahmi. *Centellaasiatica*contains various classes of phytonutrients such as triterpenes, carotenoids, glycosides, flavonoids, alkaloids, volatile oils and fatty oils which has wide range of pharmaceutical activities. The present work is carried out to perform antioxidant activity and phytochemical analysis of leaf powder of *Centellaasiatica*and its incorporation in formulating a new food product.Results of the current study revealed that *Centellaasiatica* leaf powder consisted high concentration of potent antioxidants and potentially good source of functional ingredients which can be used in design and development of herbal foods due to their stability even upon the drying treatment and also that can alleviate oxidative stress and related disorders.

Key words: Medicinal plant, *Centellaasiatica,* Antioxidants, Phytochemical analysis, New food product.

Introduction

Nature has blessed and gave our nation with a colossal abundance of medicinal plants. Hence, our nation, India has frequently been alluded to as the "medicinal garden" of the world. These medicinal plants assume a significant part in keeping up human wellbeing and furthermore help in improving the quality of human life. Medicinal plants have been utilized in numerous regions including medication, food and nutrition, seasoning agents, refreshments, colouring, bug anti-agents cosmetics and other significant industrial purposes. The preservative impact of numerous medicinal plants recommends because of the presence of antioxidants and antimicrobial constituents.

Centellaasiatica L. belongs to the family Apiaceae is an ancient Ayurvedic plant and traditionally well-regarded for its nutritional and medicinal values. It is found in damp and shady banks of water bodies and other moist places of tropical and subtropical India up to 1800 m in hills. *Centellaasiatica* has been used as medicinal herb for thousands of years in India, China, and Indonesia due to its beneficial functional properties. Its potential antioxidant, antimicrobial, cytotoxic, neuroprotective and other activities have been widely claimed in many reports and is very much related to its properties and mechanism of action of the plant‘s bioactive constituents, namely the triterpenic acid (asiatic acid madecassoside acid), triterpenicsaponin (madecassoside and asiaticoside), flavonoids and other phenolic compounds [14]. It also been called one of the “miracle elixirs of life” because legend has it that an ancient Chinese herbalist lived for more than 200 years of life as a result of using the herb.

Herbs/medicinal plants are valuable source of natural products with potential application in the protection and preservation of specific foods and have been used in the food industry mainly as antioxidants, antimicrobial and antifungal. There are few constraints have been facing by the food industry to incorporate herbs/medicinal plants as an ingredient into the food matrix. Because, each herb/medicinal plant has their characteristic aroma and taste and that will easily change the product unique characteristics. Ultimately it will show an impact on acceptability of the product. *Centella* herb, which has mildly bitter taste and acceptable aroma when compare with bacopa. The utilization of *Centellaasiatica* in daily cuisine as a leafy vegetable has increased in recent years. Demand is increasing rapidly for the processed and convenience foods because of the increasing awareness about the health, changing socio-economic needs, and insufficient time to cook food with the correct/ balanced amount of nutrition. One such nutritionally balanced convenience food is the nutritious granola bars, which are acquiring popularity in the worldwide market. Therefore, nutritionally, combining cereal grains with other ingredients are valuable to provide better nutrition. By changing and adding additional ingredients, nutritional value of granola bars can be altered. Along these lines, granola bar was chosen to provide value addition with the herb/medicinal plant i.e., *Centellaasiatica* in the current study.

Materials and Methods

Medicinal compounds can be present in different parts of the plant like root, stem, bark, heartwood, flower, fruit and leaf or plant extrudates. For medicinal purpose and further processing, only the leaves and the aerial part of the plants are used [2, 6, 18]. They found that the leaf tissues have higher antioxidants and phytochemical content than the callus and cell suspension. Hence the leaves of *Centellaasiatica*was chosen to analyze its antioxidant and phytochemicals.

Collection of the leaves

Whole plants of *Centellaasiatica* were collected from KrishiVignana Kendra, Tirupati. A voucher specimen was prepared with a sample of *CentellaAsiatica* species and submitted to the department of botany, SVUniversity, Tirupati to ensure the identification of the herb (Fig. 1). The taxonomic identities of the plant were confirmed by the experts and the collected leaves were further processed.

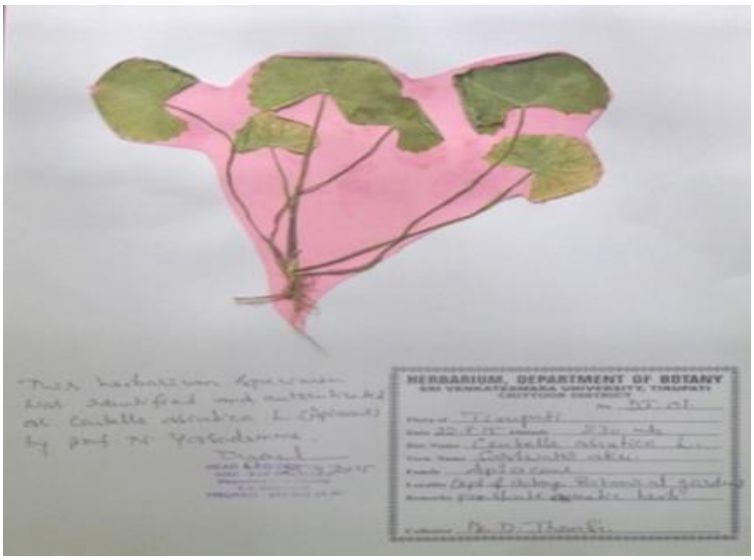

Figure 1: Voucher specimen of *CentellaAsiatica*

Pre-processing of the leaves

The collected leaves were washed thoroughly under running tap water and then with distilled water. Washing helps to remove the adherent dust particles and any other foreign material. The cleaned leaves were kept for shade drying about 2 hours to evaporate excess moisture in the leaves.

Drying

The *Centellaasiatica* is highly perishable due to their high water content. There is a need to preserve the nature‘s storehouse of nutrients through convenient processing techniques. Dehydration/drying seems to be the simplest technology for preserving herbs/medicinal plants, especially when they are abundantly available. To achieve the desired quality of dried products optimum conditions of drying time and temperature are necessary. Cabinet drier was used to decrease the moisture and to retain the maximum nutrients. The *Centellaasiatica* leaves were spread out thinly on the trays in which the drying takes place. The Heat was produced in cabinet drier by an air current sweeping across the trays, by conduction from heated trays to remove the moist vapours. The temperature was maintained about 40 ^{0} C and continuously heated for 24 hours. Then the dried herb/medicinal plant leaves were ground into a coarse powder with the help of mixer. The powder was stored in airtight container for quality analysis.

Screening of antioxidants and phytochemicals

Centellaasiatica contains several valuable functional compounds known as antioxidants, flavonoids, phenols and Vitamin-C. These biologically active compounds are responsible for a wide range of therapeutic activity. The functional compounds will give the benefits beyond basic nutrition. In the current study *Centellaasiatica* leaves powder was used to estimate Total antioxidant activity, total flavonoids, total phenols and vitamin-C using standard protocols.

- Total antioxidants were estimated in *Centellaasiatica* leaf powder by following DPPH assay testing method [1].
- The flavonoids content was determined by aluminiumtrichloride method using catechin as reference compound [19].
- The total phenolic content was estimated by FolinCiocalteu method [16]with slight modifications.
- Vitamin C was estimated in *Centellaasiatica* leaf powder by using the standard testing method [13].

Food Product development

Different kinds of granola bars are available in the market such as protein bar, yoga bar, meal replacement bar, fiber rich bars etc. But no granola bar was identified as memory enhancing and neuroprotective properties and such other benefits related to it. *Centellaasiatica* is a very important herb/medicinal plant which could be incorporated into food and beverages due to its pharmacological activities in brain improvement, memory enhancement, neuroprotection and antioxidant effect. In this context, in development of herbal foods, granola bar was chosen to give value addition with *Centellaasiatica* in the current study. By formulating different trials with selected ingredients the final product is standardized. The ingredients used to develop the granola bar includes Foxtail millet flakes, Bajra flakes, Soy chunks, Organic Rolled oats, Pumpkin seeds, Flax seeds, Sesame seeds, Chia seeds, Sugar, Skimmed Milk Powder, Cocoa powder, Fresh Dates, Raisins, Honey and *Centellaasiatica* leaves powder.

The procedure consists of- All ingredients were weighed proportionately and kept aside.The millet flakes, soy granules, oats and oilseeds were dry roasted separately.Binding syrup was prepared by using sugar, gum *acacia,* cocoa powder and skimmed milk powder with continuous heating and stirring.The dry roasted ingredients such as millet flakes, soy granules, oats and oil seeds were added along with *Centellaasiatica*to the binding syrup mixture and blended properly to obtain a uniform homogeneous mass.The mass was then spreaded on butter paper and pressed with a spatula for proper setting.The prepared mass was baked at 145ºC for 20 minutes in preheated oven (170ºC for 15 minutes). After baking it was left to rest for cooling and made into rectangular pieces.The prepared granola bar was packed in metallized aluminium pouches and labelled.

Results

The results obtained with regard to the current study are presented which are as follows.

Analysis of *Centellaasiatica*

Dried powder of *Centellaasiatica* leaves were analysed to know the availability of major functional components such as total antioxidant activity, total flavonoids, total phenols and vitamin-C. The obtained results are presented in tables.

Table 1: DPPH Assay of *CentellaAsiatica* leaves powder
The scavenging activity percentage (%)

***CentellaAsiatica* powder (%)**	**Mean±S.D**
100 µg	15.737±0.012
200 µg	21.880±0.020
300 µg	26.350±0.020

The table 1 shows antioxidant activity of *Centellaasiatica*. From the table 1, it was observed that the scavenging activity percentage of *Centellaasiatica*. At the low concentration, *Centellaasiatica* (100µg/ml) showed 15.737±0.012 of activity whereas at high concentration (300µg/ml) showed 26.350±0.020 activity.

Medicinal herbs contain many powerful components that can help in preventing/healing of various illnesses of humans. The functional components of *Centellaasiatica* is depicted in table 2.

Table 2: Functional components of *CentellaAsiatica*

Functional Components	**Mean±S.D**
Total Flavanoids (mg/g)	29.666±0.577
Total Phenols (mg/g)	69.666±0.577
Vitamin-C (mg/100g)	36.360±0.020

In the present study *Centellaasiatica* leaves powder has shown the flavonoids content about 29.666±0.577 as mentioned in table 2. *Centellaasiatica*consists high flavonoids content when compared with that of most commonly consumed fruits and vegetables [8].The results obtained in this study showed a significant level of phenolic compounds and assessed as catechol equivalent. Total phenolic compounds of *Centellaasiatica* leaves powder were found about 69.666±0.577.The *Centellaasiatica* herb powder contains vitamin-C about 36.360±0.020 which is mentioned in the table 2.

The data in table 3 shows the organoleptic evaluation scores which was conducted for each trial by selected semi trained panel members.

Table 3: Organoleptic evaluation of granola bar

Sensory attributes	Formulations	Mean±S.D	F-value	p-value
Appearance	F1	4.50±0.200	268.750	0.000*
	F2	5.00±0.200		
	F3	8.00±0.200		
Colour	F1	4.06 ±0.020	284.310	0.000*
	F2	5.73±0.231		
	F3	7.73 ±0.150		
Flavour	F1	3.60±0.200	331.000	0.000*
	F2	5.60 ±0.200		
	F3	7.80±0.100		
Texture	F1	4.50±0.200	273.323	0.000*
	F2	5.03±0.153		
	F3	7.80±0.200		
Mouth feel	F1	4.80 ± 0.100	0.047	0.954^{NS}
	F2	5.80±0.400		
	F3	7.40±0.100		
Taste	F1	4.13±0.153	357.346	0.000*
	F2	6.60±0.200		
	F3	7.76±0.153		
Overall Acceptability	F1	4.10±0.200	346.333	0.000*
	F2	5.60±0.200		
	F3	7.80±0.100		

F1-Formulation-1 (2g leaf powder), F2- Formulation-2 (4 g leaf powder), F3- Formualtion-3(6g leaf powder)

****** Significant at 0.01 level ($p<0.01$) * Significant at 0.05 level ($p<0.05$)

NS- Not Significant.

Discussion

The significant compounds which provide antioxidant activity in *Centellaasiatica* are Vitamin-C, Tannins, Carotenoids, Polyphenols and Flavonoids. Ascorbic acid is a naturally occurring antioxidant compound found in medicinal plants. Hence, in the current quality analysis, ascorbic acid was taken as standard and the results were compared with that. The reduction of DPPH may be due to the hydrogen donating or electron donating property of the plant extracts. DPPH is a free radical and accepts an electron or hydrogen radical to become stable molecules [17].

Antioxidants are free radical scavengers that provide protection to living organisms from damage caused by reactive oxygen species. Although almost all organisms possess antioxidant defence and repair systems, these systems are insufficient to cope over entire damage. Therefore, dietary antioxidant supplementation is promising in strengthening the antioxidant defence and repair systems. Polyphenol, flavonoid, βcarotene, tannin and vitamin C are readily found in *Centellaasiatica* contributing to significantly higher antioxidant activity in the herb. The total antioxidant activity of *Centellaasiatica* is presented in table 1.

Antioxidant activity of *Centellaasiatica* is comparable to the activity of rosemary and sage and has been identified with high potential to be explored as a source of natural antioxidants. A research carried out on antioxidativebehaviour of malaysian plant extracts in model food and oil systems[7]. They reported that antioxidant in *Centella* extract (84%) is comparable to vitamin C (88%) and grape seed extract (83%).*Centella* extract exhibited comparable activity with grape seed extract which is claimed to be a powerful antioxidant due to its proanthocyanidin content. A study conducted ontriterpene composition and bioactivities of *Centellaasiatica*, they evaluated the antioxidant activity of *Centella* extract by measuring its ability to scavenge DPPH free radicals while vitamin C, green tea and grape seed extract (1 mg/mL each) which were used as positive controls[5]. *Centella* extract profound free radical scavenging activity of 83% inhibition at a concentration of 1 mg/mL.Asiaticoside, which is present abundantly in *Centella*(1.97 mg/mL), may be a significant contributor to the observed antioxidant activity. The present research findings were also on par with this study. Another study which is conducted onasiaticoside-induced elevation of antioxidant levels in healing wounds, stated that the enhancement of antioxidant activity might due to *Centella*'sasiaticoside and flavonoid content[15].

Flavonoids are the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts. *Centellaasiatica* is reported to contain flavonoids, 3-glucosylquercetin, 3-glucosylkaemferol, and 7-glucosylkaemferol. Apart from these, two new flavonoids named castilliferol 1 and castillicetin 2 have been isolated from the whole plant recently. Presence of several flavonoid derivatives such as quercetin, kaempferol, patuletin, rutin, apigenin, castilliferolcastillicetin, and myricetin has been reported in *Centellaasiatica*[3]. The potent antioxidants catechin, quercetin and rutin are found to be suitable to be used as markers for standardizing *Centellaasiatica* extract to be incorporated as an ingredient in the development of functional foods, due to their stability upon the different drying treatments. The flavonoids have been analysed as a quercetinequivalent[11].

The results of the present study was on par with the study conducted on effect of different drying methods on the degradation of selected flavonoids in *Centellaasiatica*[10]. They studied the effect of different drying methods on flavonoids degradation. Their results revealed that leaf, root and petiole of *Centellaasiatica* consisted of high concentration of flavonoids, in particular, potent antioxidants rutin, quercetin and catechin. Nevertheless appreciable amount of the active compounds retained in all drying methods especially in vacuum drying, more retention was

observed. *Centellaasiatica* contains quercetin about 947-3501µg/100g and also they stated that the major flavonoids of *Centellaasiatica* were naringin followed by quercetin, catechin and rutin. According to their results, the concentration of the different flavonoids were found to be highest in the leaf of *Centellaasiatica* (164-4688 µg/100g), followed by root (45-3561 µg/100g) and petiole (72-978 µg/100g) respectively.

Phenolic compounds are ubiquitous secondary metabolites in plants and they are known to have antioxidant activity. The major active compounds of *Centellaasiatica* are polyphenols. *Centellaasiatica* leaves showed the highest antioxidant activity because it contains highest phenolic contents, when compared to other plant parts. The phenolic compounds are the major contributors to the antioxidative activities of *Centellaasiatica*. These activities scientifically proved, which indicates that *Centellaasiatica* has high potential for use in food, pharmaceutical and cosmetic applications. A study carried out on Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of HalimiumHalimifolium (Cistaceae) and they revealed that the content of phenolic compounds were greater in leaves than in other parts[12].

Vitamins play an important role in maintaining of human health. Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that is naturally present in some foods and also it can be added to others. Humans, unlike most animals, are unable to synthesize vitamin-C endogenously; hence it is considered to be an essential dietary component[9]. Vitamin C is easily destroyed by heat and oxygen. This might be because of the increased activity of ascorbic acid oxidizing enzymes due to drying of leaves, which led to destruction of ascorbic acid and leaching of vitamin-C.

The Vitamin-C is an important dietary antioxidant as it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including neurodegenerative diseases, cardiovascular disease, stroke, cancer, and cataract genesis [4].

The data in table 3 shows that there was a statistical significance ($p < 0.05$) for all sensory attributes i.e., appearance, colour, flavour, texture, taste and overall acceptability except mouth feel between three formulations of reference granola bar. The results revealed that the formulation-3 got highest mean scores for all sensory attributes followed by formulation-2 and formulation-1.

Conclusion

Results of the current study revealed that *Centellaasiatica* leaf powder consisted high concentration of potent antioxidants and potentially good source of functional ingredients which can be used in design and development of herbal foods due to their stability even upon the drying treatment and also that can alleviate oxidative stress and related disorders.Medicinal plants/herbs as potential source of many significant bioactive components aids a prominent role in maintaining for humans not only in the diseased condition but also as potential material for maintaining proper health. The current study concludes that foods are not intended to satisfy hunger and only provide essential macro- and micronutrients to the body but also to supply it with bioactive ingredients that aid to decrease nutrition-related diseases and ensure physical and mental well-being. With rapid advances in food technological research, active ingredients from herbs/medicinal plants served as key ingredients for the development of novel food products. Granola bars have been a good choice for research to test the novelty in the products because of increasing demand in global convenience health/functional food product market.

References

1. Braca, A., Sortino, C., Politi, M., Morelli, I., & Mendez, J. (2002). Antioxidant Activity of Flavonoids from LicaniaLicaniaeflora. Journal of Ethnopharmacology, 79(3), 379- 381.
2. Brinkhaus, B., Lindner, M., Schuppan, D., & Hahn, E. G. (2000). Chemical, Pharmacologicaland Clinical Profile of the East Asian Medical Plant CentellaAsiatica. Phytomedicine, 7(5), 427-448.
3. Das, L., Raychaudhuri, U., &Chakraborty, R. (2012). Supplementation of Common White Bread by Coriander Leaf Powder. Food Science and Biotechnology, 21(2), 425-433.
4. Halliwell, B., &Gutteridge, J. M. (2015). Free Radicals in Biology and Medicine. Oxford University Press, USA, 5th edition.
5. Hashim, P. (2011). CentellaAsiatica in Food and Beverage Applications and its Potential Antioxidant and Neuroprotective Effect. International Food Research Journal, 18(4), 1215.
6. James, J. T., &Dubery, I. A. (2009). PentacyclicTriterpenoids from the Medicinal Herb, CentellaAsiatica (L.) Urban. Molecules, 14(10), 3922-3941.
7. Jaswir, I., Hassan, T. H., & Said, M. Z. M. (2004). AntioxidativeBehaviour of Malaysian Plant Extracts in Model and Food Oil Systems. Asia Pacific Journal of ClinicalNutrition, 13, S72.
8. Justesen, U., Knuthsen, P. and Leth, T. 1998. Quantitative Analysis of Flavonols, Flavones, and Flavanones in Fruits, Vegetables and Beverages by High-Performance Liquid Chromatography with Photo-diode Array and Mass Spectrometric Detection. Journal of Chromatography, 799, 101–110.
9. Li, Y., &Schellhorn, H. E. (2007). New Developments and Novel Therapeutic Perspectives for Vitamin C. The Journal of Nutrition, 137(10), 2171-2184.
10. MohdZainol, M. K., Abdul-Hamid, A., Abu Bakar, F., & Pak Dek, S. (2009). Effect of Different Drying Methods on the Degradation of Selected Flavonoids in CentellaAsiatica. International Food Research Journal, 16(4), 531-537.
11. Rahman, M., Hossain, S., Rahaman, A., Fatima, N., Nahar, T., Uddin, B., (2013). Antioxidant Activity of CentellaAsiatica (Linn.) Urban: Impact of Extraction Solvent Polarity. Journal of Pharmacognosy and Phytochemistry, 1(6), 27–32.
12. Rebaya, A., Belghith, S. I., Baghdikian, B., Leddet, V. M., Mabrouki, F., Olivier, E., &Ayadi, M. T. (2014). Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of HalimiumHalimifolium (Cistaceae). Journal of Applied Pharmaceutical Science, 5(1), 52-57.
13. Sadasivam, S. (1996). Biochemical Methods. New Age International (P) Limited, New Delhi, 2, 124-126.
14. Seevaratnam, V., Banumathi, P., Premalatha, M. R., Sundaram, S. P., &Arumugam, T. (2012). Functional properties of Centellaasiatica (L.): a review. Int J Pharm Phar Sci, 4(5), 8-
15. Shukla, A., Rasik, A. M., &Dhawan, B. N. (1999). Asiaticoside-Induced Elevation of Antioxidant Levels in Healing Wounds. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 13(1), 50-54.
16. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16(3), 144-158.
17. Soare, J. R., Dinis, T. C., Cunha, A. P., & Almeida, L. (1997). Antioxidant Activities of some Extracts of Thymus Zygis. Free Radical Research, 26(5), 469-478.
18. Zainol, N. A., Voo, S. C., Sarmidi, M. R., & Aziz, R. A. (2008). Profiling of CentellaAsiatica (L.) Urban extract. Malaysian Journal of Analytical Sciences, 12(2), 322- 327.
19. Zhishen J., Mengcheng T., Jianming W. (1999). The determination of flavanoid contents in mulberry and their scavenging effects on superoxide radicals. Food chem. 64: 555-559.