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Short Communication

Development of moringa infusion for green tea and its evaluation

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ABSTRACT

***Moringa oleifera* leaves are known for its high nutritional quality. Its leaves are commonly used for culinary purposes and it was explored as a potential nutraceutical in recent decades. Tea or herbal infusions have become an integral part of daily diet for a population who concerned about a healthy lifestyle. Many herbs or plant parts have been used as infusions which provide health promoting phytochemicals to the consumers. Therefore moringa infusions were prepared along with some herbs/flavouring agents such as tulsi, ginger and lemon grass. Total polyphenol content in the infusions ranged between 685 and 1567 mg GAE/100 mL. Among phenolic acids detected, gallic acid was highest in all the treatments. Infusion containing moringa and tulsi scored high in organoleptic evaluation. Thus, moringa infusion can become an add-on variety to the tea/herbal infusion consumers.**

Keywords: *Moringa, herbs, infusion, polyphenols*

INTRODUCTION

Herbal infusion has become an integral part of urban populations' diet today. Health conscious people prefer nutritious and refreshing drink which relaxes them and relieves. A study by CBI (Centre for Promotion of Imports from Developing Countries), Ministry of Foreign Affairs, Netherlands, showed that in Europe, tea consumers are increasingly looking for high value specialty tea which are unique in flavour (www.cbi.eu/market-information/tea/trends). Green tea, black tea, infusions with herbs/fruits; fruit /herbal teas are becoming important premium products. Tea and herbal infusions are the major contributors for phenolic acids and other antioxidants in today's diet (Atoui *et al.,* 2005; Horzic *et al*., 2009; McAlpine *et al.*, 2016; Shahidi, 2000). Consumption of tea is linked to reduce risk in development of chronic diseases such as CVD (Cardiovascular Disease), different types of cancer, diabetes mellitus and obesity. Kuriyama *et al*. (2006) studied the association between consumption of green tea and mortality for over 11 years. The results showed that green tea consumption is inversely associated with mortality due to all causes and due to CVD. Another study by Imai and Nakachi (1995) showed the association between green tea consumption and CVD and disorders of liver in Japanese population. Results indicated that increased green tea consumption (e"10 cups) has decreased serum concentrations, triglycerides and an increased HDL (High Density Lipoprotein) with decreased LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein). A study by Iso *et al*. (2006) reported a reduction of 33% risk for developing Type 2 diabetes in subjects who has consumed six or more cups of green tea per day, compared to those consuming less than one cup in a week. Apart from tea leaves, medicinal plants, herbs and spices have also been shown to have bioactive compounds which benefits health (Azeez *et al*., 2016; Vats and Gupta, 2017). Hence the present study was conducted to develop moringa infusion along with herbs and estimation of their bioactive compounds.

Fresh leaves of *Moringa oleifera* cv. Bhagya were harvested in early hours of morning from the field of ICAR-Indian Institute of Horticulture Research (ICAR-IIHR), Bengaluru. Leaves were separated from the twigs manually, washed with potable water, drained and subjected to solar-tunnel drying. The dried leaves were powdered using lab scale stainless steel blender. Leaves of tulsi (*Ocimum sanctum*) and lemon

grass (*Cymbopogon citratus*) were obtained from the field of medicinal and aromatic plants ICAR-IIHR. Ginger (*Zingiber officinale*) was procured from local market. Leaves were washed with potable water, drained and oven dried (55 °C). Ginger rhizome was washed with potable water, peeled, cut in to small pieces and dried in oven at 55 °C. All the samples were powdered using lab scale stainless steel blender. Standardization of addition of herbs along with moringa was done based on sensory analysis.

Tea bags were prepared from moringa (T1), moringa + tulsi (T2), moringa + ginger (T3) and moringa + lemon grass (T4) as detailed in **Table 1.** A sample of 0.5 g of each treatment was prepared in tea bags and dipped in 50 mL of hot distilled water (85-90 °C) for about two minutes, allowed to cool, and filtered using Whatman 1 filter paper.

Table 1: Treatments and ratio of ingredients used for the preparation of infusion.

Treatments	Ingredients	Ratio
T1	Moringa	100
T2	Moringa + Tulsi	100:40
T3	Moringa + Ginger	100:40
T4	Moringa + Lemon grass	100:60

Total phenol content was analyzed in aqueous infusion according to Folin-Ciocalteu procedure as described by Jayasekara *et al*. (2011). In brief, an aliquot of 0.25 mL of infusion extract was mixed with 5 mL of 2% Na_2CO_3, allowed to stand for five minutes. Then 0.25 mL of Folin-Ciocalteu reagent (50%) was added and incubated in dark for 30 min at room temperature. Absorbance was read at 650 nm. Calibration was achieved with an aqueous gallic acid solution and total phenol content was expressed as gallic acid equivalent.

Standard solutions of phenolic acids were prepared in 80% ethanol. Chromatographic/MS grade organic solvents were used as the mobile phase for liquid chromatography. All mobile phases were filtered through membranes (0.45 µm). Different concentrations of individual compounds were made to obtain standard curves for individual phenolic acids which were identified and quantified by their molecular weight (parent mass m/z) and most abundant fragmented daughters.

The mobile phase consisted of an aqueous phase of 0.1% formic acid in water (A) and organic phase of 0.2% formic acid in methanol (B). The initial gradient was composed of 90% aqueous phase and organic phase (10%), which was held for 2.5 min. After 4 min, the gradient was changed to aqueous phase (70%) and organic phase (30%), held for 1 min. At 5 min, linear gradient was followed after arriving at aqueous phase (60%) and organic phase (40%) for 5 min. At 10 min the gradient was again changed to aqueous phase (80%) and organic phase (20%) for 2 min. and final step with aqueous phase of 90% and organic phase of 10% for 2 min. This condition was held for 1 min for equilibrating before the next injection. The flow rate was maintained at 0.3 mL/min. The analytical column used was 2.1 X 50 mm UPLC BEH C_{18} column (Waters) with particles of 1.7 µm and a column temperature of 25 °C. Exactly 2 µL of sample was injected. The eluted metabolites from UPLC column effluent were monitored.

Sensory evaluation was done by a group of 10 semi trained panellists who were well acquainted with green tea consumption. Numerical scoring test was used to evaluate moringa infusion. Scorings were provided as 90 for excellent, 80 for good, 70 for fair and 60 for poor. A completely randomized experimental design was used for this study and data were analyzed using WASP statistical software (WASP 2.0, ICAR Research Complex for Goa, Ela, Goa, India.) (Jayade *et al.,* 2015).

Total phenolics and total flavonoids content is presented in **Table 2.** Total phenol content ranged between 685 and 1567 mg GAE/100 mL of infusion. The results revealed that total phenol content was high in infusion containing moringa and tulsi (T2) followed by T4 which contains lemon grass along with moringa, however no significant difference was observed. The least content of polyphenol was found in T3 which had ginger with moringa. But, Maraes-de-Souza et al. (2008) reported less phenolic content in the infusions prepared from fresh herbs. Total phenolic content in fresh leaves of tulsi was 1.25 mg GAE/g as observed by Sailaja *et al*. (2010) and in ginger it was 840 mg/

Table 2: Total polyohenol content of moringa infusions

Treatments	Total polyphenol (mg GAE/100 mL infusion)
T1 (Moringa)	1550[a]
T2 (Moringa + Tulsi)	1567[a]
T3 (Moringa + Ginger)	685[b]
T4 (Moringa + Lemon grass)	1559[a]

Values are the mean of three replications (n=3). Values with the same superscript in each column do not differ significantly.

100g DW in water extract (Shirin and Prakash, 2010). High phenol content in tea containing lemon grass and low phenol content in tea containing tulsi was observed by Naithani *et al*. (2006). Herbal tea containing two species of tulsi and lemon grass showed 786 and 2651 mg GAE/100g of sample, respectively (Naithani *et al*., 2006). However, author has not discussed about the variation in phenol content in tea prepared from different herbs. In the present study, the lesser content of phenols in treatments T3 compared to other treatments might be due to antagonistic or synergistic effects among compounds in the infusion (Baptistaa et al.1998).

Phenolic acid profile revealed that most of the phenolic acids were present in all the treatments (**Table 3**). Gallic acid was the major phenolic acid followed by *trans*-cinnamic acid in all the treatments. Horzic *et al*. (2009) found gallic acid in different types of tea but it was not detected in linden and chamomile infusion. Caffeic acid was found highest in infusion containing tulsi compared to other treatments. Sundaram *et al*. (2012) and Zgorka and Glowniak (2001) have also reported the occurrence of caffeic acid in tulsi leaves. Thus, presence of most of the phenolic acids in moringa infusion provides a variety and medicinal advantage to the herbal infusion consumers.

Table 3: Profile of phenolic acids in moringa infusions

Phenolic acids µg/100 mL	T1	T2	T3	T4
Salicylic acid	244.38	933.02	49.72	126.72
2,4-dihydrobenzoic acid	25.13	229.61	34.55	62.11
Gallic acid	1045.30	539.26	3516.51	17524.83
Ferulic acid	64.54	208.47	20.28	117.01
Gentisic acid	90.98	55.23	59.53	80.18
Chlorogenic acid	23.47	25.23	5.70	9.50
Ortho-coumaric acid	17.78	71.72	74.06	ND
Para-coumaric acid	94.47	14.60	28.40	ND
Procatechuic acid	104.28	53.23	90.53	84.11
Para-hydroxybenzoic acid	1.63	0.42	0.58	1.10
Syringic acid	ND	ND	0.44	ND
Trans-cinnamic acid	299.21	261.41	109.72	228.69
Caffeic acid	24.81	13662.80	56.29	58.96
3-hydroxy benzoic acid	1.47	1.31	1.25	0.24
Benzoic acid	76.77	5.28	13.58	33.70
Vanillic acid	132.36	17.77	45.19	31.73
Sinapic acid	19.97	ND	ND	29.16
Ellagic acid	57.03	ND	ND	ND

ND-not detected

Sensory evaluation by semi trained panellists revealed that among the treatments, T2 scored the highest (82.4) followed by T1 (77.5), T3 (77.5) and least by T4 (75). The high sensory score of T2 could be due to the fact that the evaluators had already developed taste for tulsi as it is being used for ethnomedicinal purposes from ancient times in India (Cochen, 2014). Since moringa infusion is a new product, it might be difficult for people to accept it as an infusion. Health conscious people have to slowly develop the taste for it because of its nutritional quality.

It could be concluded from the study that *Moringa oliefera*, being a rich source of bioactive compounds can also be used for infusion preparations as new product by adding variety to the green or black tea consumers. Even though moringa along with tulsi scored high organoleptically, moringa infusion will be best from the health point of view as it provides more total phenols.

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