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Phytochemical's Content of Herbal Teas Formulated from Dried Moringa (*Moringa Stenopetala*) and Stevia (*Stevia Rebaudiana Bertoni*) Leaves

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Abstract

Herbal teas that refer to any green or leafy part of a plant are gaining popularity because of their health benefits. The demand for Moringa and Stevia currently increased in Ethiopia due to their nutritional and medicinal values. However, there is no work on their herbal tea formulation and their phytochemicals content determination. The purpose of this study was to evaluate the phytochemical contents of herbal teas formulated from dried leaves of Moringa and Stevia. Seven Moringa-based herbal teas were brewed with Stevia ranging from 0 to 35% with five-level (5) and compared for their total phenolic, total flavonoid, and condensed tannin. The 100% Moringa tea infusion was considered as control. The results showed that *herbal tea brewed with 25-35% stevia in the formulation results in the* highest total phenolic, total flavonoid, and condensed tannin) values comparable to control. This study provides evidence that adding stevia to Moringa improves the phytochemicals content of moringa without compromising its health-promoting compounds.

Keywords: Herbal; Infusion; Moringa; Phytochemicals Content; Stevia

Introduction

Background of the Study

Herbal teas that refer to any green or leafy part of a plant are gaining popularity among consumers [1]. They have been extensively consumed because of their health benefits [2,3]. The phytochemicals are biologically active compounds naturally occurring compounds widely distributed in the plant kingdom and beneficial components of the human daily diet which contribute significantly to protect against degenerative disease [4]. Various studies have shown that many plants are rich sources of phytochemicals compounds such as flavonoids, tannins, and lignins, found in plants, all act as antioxidants [5,6]. These bioactive compounds vary in type, number, and position of functional groups [7]. Phenolic compounds among phytochemical substances including phenolic acids and flavonoids are the major groups of natural components in plants that have received increasing interest over the last decades due to free radical scavenging properties [4].

Herbal teas are made by infusion or decoction from fresh and dried leaves, seeds, grasses, nuts, barks, fruits, and flowers, or other botanical elements [8,9]. Herbal teas are commonly consumed for their therapeutic and energizing properties, since they can help to induce relaxation, being able to aid with stomach or digestive problems, provide cleansing properties to the body, and strengthen the immune system as well. It is important to note that different herbs

might have different medicinal properties, which thus allows us to make our herbal infusions according to how we want the cup of tea to benefit with properties known to improve certain health conditions [10,11].

Moringa Stenopetala is a tropical plant that belongs to the family Moringaceae which its family is represented by a single genus called Moringa. Its English names are Africa Moringa tree, Ben oil tree, Cabbage tree, Horse-radish tree. Besides, it is also known as Shiferaw in Amharic and Aleko, Aluko and Haleko in the Southern Rift Valley of Ethiopia, in Gamo Gofa Areas and named as African Moringa tree because of its native to southern Ethiopia, North Kenya, and Eastern Somalia. *M. stenopetala* is described as providing multifunction in nutrition through decrease hunger; improve health and human nutrition by suppling different food products as all parts of the tree are edible; it also described as Africa's solution to malnutrition and also called mothers' best friend [12,13]. The fresh and dried leaf of Moringa is widely sold and consumed in cities of Ethiopia, due to its perceived awareness of health and nutritional benefits such as hepatoprotective effect, antihypertensive, antihyperglycemic or antidiabetic, anticholesterol, antispasmodic, anti-cancer, anti-asthmatic, antioxidant, In ocular diseases and anti-aging [14]. Stevia rebaudiana (Bertoni) is an herb of the 950 genera of the Asteraceae family and it is native to Paraguay and widely used in many parts of the world as a sweetener and grown commercially in Central America, Korea, Brazil, Thailand, and China used the leaves of this small, herbaceous, semi-bushy, perennial shrub to sweeten their bitter drinks due to its leaves contain a natural complex mixture of eight sweet diterpene glycosides, including isosteviol, sativoside, rebaudioside (A, B, C, D, E, F), steviolbioside and glucoside A [15,16]. Stevia is recently cultivated on a large scale by entrepreneurs in Ethiopia for herbal production due to its potential uses in treatment against various chronic and non-chronic diseases like diabetes, cardiovascular disease, cancer, renal disease, obesity, inflammatory bowel disease, and dental caries [16,17]. Stevia is also grown like other vegetables in the kitchen garden [15,17]. Therefore, exploring the potential of phytochemicals content in the herbal tea formulated from dried Moringa and stevia, due to their health benefits.

Materials and Methods

Sample Collection and Preparation

Fresh moringa and stevia leaves were obtained from Arba Minch and Wondo Genet Agriculture research centers (Ethiopia), respectively. The samples were packed in polyethylene (plastic) bags and transported to Wondo Genet Natural Product Research Laboratory.

The fresh leaves of uniform shape, color, and size were selected and subjected to shade drying at ambient temperature for about one week according to Killedar, et al. [3]. The leaves were spread thinly on paper-lined wooden trays and protected from direct sunlight to prevent the loss of volatile aroma compounds or even photo-oxidation.

The dried samples were separately milled using an electric Blender (Model BLG401, Zhejiang YiLi Tool Co., Ltd., China). The milled samples were sieved using 2 mm (Verder 008SAW2.00) sieve size to separate the milled leaves. From the sieved samples, formulations were prepared and kept in an air-tight container and stored at room temperature until further analysis.

Treatments and Experimental Design

The dried moringa and stevia leaves were mixed in varying proportions to obtain 7 different formulations (Table 1) based on the preliminary test. The chemicals, antioxidant value, and sensory acceptability of their tea were analyzed. The experimental design was a completely randomized design (CRD).

Herbal tea formulation	Moringa leaves %	Stevia leaves (%)
TF1	100	0
TF2	95	5
TF3	90	10
TF4	85	15
TF5	80	20
TF6	75	25
TF7	70	30
TF8	65	35

Table 1: Formulations of herbal tea in the experimental design.

Preparation of Herbal Tea

The herbal tea was prepared according to the method developed by Horžić, et al. [18] formulated samples 2 g were infused in 200 mL boiling water for 5min at 97°C to mimic normal tea preparation till it becomes an appealing fragrance. Herbal tea was prepared from all formulated herbal teas and controls. The formulated herbal teas were unsweetened using sugar and considered for the analysis in this experiment.

Preparation of Extracts from Herbal Tea

The extract was prepared according to Mingarro, et al. [19] & Koh, et al. [20]. The decoction was boiled for 5 min at

97°C as usual for the normal tea prepared. The decoction was filtered through a double-layered muslin cloth to get rid of the large particles and filtered through a filter paper (Whatman® Grade 1 Qualitative Filter Paper - Sigma-Aldrich). The filtered product was then allowed to concentrate at 45°C for three consecutive days by evaporating excess water and obtain the dried. The extract was weighed and its respective percentage yield was recorded. The crude extract 1 g was dissolved in 50 mL of respective solvent (methanol 99.85 % grade) to make a stock solution of 20 mg/mL. The prepared stock solution was kept at 4°C in a refrigerator, to serve as the working solution for all the phytochemicals and antioxidant tests.

Phytochemicals

Total Phenolic Content (TPC): The total phenolic content of the herbs and their formulated herbal teas were estimated according to the method used by Shan, et al. [21] using gallic acid as a standard. Folin-Ciocalteu reagent (diluted ten times) (1 mL) was added to 0.1mL of the extract (1 mg/ mL). The mixture was left for 5 min and 1 mL (7.5% w/w) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with a UV-visible double beam spectrophotometer (Spectronic 20, UK) after incubation for 90 min at room temperature. The TPC was estimated from gallic acid calibration curve ($y = 0.0073x -0.0462$, $R^2 = 0.973$) and results were expressed as milligram gallic acid equivalent/gram of dried extract (mgGAE/g).

Total Flavonoid Content (TFC): Total flavonoid content (TFC) of the herbs and their formulated herbal teas were determined according to Ayoola, et al. [22]. The extracts (1mg/ mL) were diluted with 1.25 mL distilled water and 0.75 µL 5% $NaNO_2$ was added to the mixture. After 6 min, 150 µL 10% $AlCl_3$ was added and after another 5min, 1mL Na OH was added to the mixture. Immediately the absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 510 nm. All the calculations were done using the standard equation Catechin obtained from standard calibration curves ($y=0.0014x+0.0192$, $R^2=0.97(p<0.001)$). Results were expressed as milligrams of catechin equivalents per gram of dry extract (mgCE/g).

Condensed Tannins Content (CTC): The condensed tannins content of the herbs was assayed as described by Chew, et al. [23]. The undiluted crude extract 0.5 mL was first mixed with 3 mL of vanillin reagent (4%, w/v, in absolute methanol), and followed by the addition of 1.5 mL of concentrated HCl (37%). The mixture was stored in a dark at room temperature for 15 min. Blank was prepared by replacing 0.5 mL of the undiluted crude extract with 0.5 mL of deionized water. The absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 500 nm. Catechin was used for calibration of the standard curve ($y=0.0042x+0.0331$, $R^2=0.995(p<0.001)$, and the results were expressed as mg catechin equivalent per 100 g dry weight sample (mg CE/100 g).

Statistical Analysis

All data were analyzed using one-way ANOVA with traits as an independent variable. The means were separated using Tukey's HSD test at $p<0.05$. Principal Component Analysis (PCA) for all numerical results was performed using XLSTAT version 2016.03.30882 (Addinsoft, New York).

Results and Discussions

Phytochemical Contents of the Formulated Herbal Tea

The phytochemicals studied in this research were total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content (CTC) of the dried moringa and stevia leaves and their formulated herbal tea infusions.

Total Phenolic Content (TPC)

The total phenolic contents (TPC) of all the herbal tea formulations showed considerable variations from 1.9 - 3.1 (mg GAE*/g) (Table 4.1). Among the herbal tea formulations, only TF6, TF7, and TF8 had a higher ($p<0.05$) level of TPC compared to TF1 (100% moringa /control). This indicated that herbal teas made with a higher level of stevia (within this study limit: 25-35%) improved the TPC of moringa-stevia infusions as a result of the high TPC in the dried stevia leaves. However, TF2, and TF3 had lower ($p < 0.05$) TPC content compared 100% moringa (TF1), while TF4 and TF5 were comparable ($p > 0.05$) to the control (TF1). The increase in the phenolic contents with the increasing proportion of stevia makes the new stevia-based herbal tea an excellent source of natural antioxidants and hence this can be considered as a healthy functional tea.

Total Flavonoid Content

All the formulated herbal teas had considerable variation in their TFC and ranged from 6.3 – 20 mg CE/ g) (Table 2). The TF5, TF6, TF7, and TF8 herbal tea formulations had higher ($p<0.05$) TFC compared to the control TF1 (100% moringa), but without significant differences with TF7 and TF8. The herbal tea samples with a higher level of stevia TF7 and TF6 resulted in a higher ($p<0.05$) level of TFC. This is indicative of the herbal teas made with the high level of stevia (within this study limit: 20-35%) improved the TFC of moringa-stevia infusions as a result of the high TFC in the dried stevia leaves. However, other formulations TF2, TF3

and TF4, had lower ($p<0.05$) TFC compared to reference sample TF1 (100% moringa). Generally, the higher levels of flavonoids in the stevia-based herbal tea formulations make the new product healthier as a source of natural antioxidants that might help the consumers to have resistance to oxidative stresses and inflammations.

Herbal tea	TPC (mg GAE*/g)	TFC (mg CE/g)	CTC (mg CE/g)
TF1	1.9 ± 0.01^{h}	6.3 ± 0.5^{g}	1.6 ± 0.2^{f}
TF2	2.4 ± 0.1^{g}	10.4 ± 0.3^{f}	$1.7\pm0.\ 1^{f}$
TF3	2.5 ± 0.1^{fg}	11.3 ± 0.4^{ef}	2.2 ± 0.1^{e}
TF4	2.7 ± 0.2^{de}	14.2 ± 0.1^{d}	2.5 ± 0.1^{d}
TF5	2.8 ± 0.1^{cd}	18.6 ± 0.2^{c}	3.7 ± 02^{b}
TF6	3.0 ± 0.1^{bc}	20.0 ± 0.1^{b}	3.8 ± 0.1^{b}
TF7	3.1 ± 0.1^{b}	20.0 ± 0.1^{b}	4.4 ± 0.1^{a}
TF8	2.6 ± 0.1^{ef}	11.9 ± 0.2^{e}	3.3 ± 0.1^{c}

Table 2: Total phenolic contents (TPC), total flavonoid contents (TFC) and condensed tannin contents of herbal tea formulated from moringa and stevia.
Values are mean ± SD (n=2). Means with a different letter *same column* of superscripts are significantly different at $p<0.05$.

Condensed Tannin Content (CTC)

The CTC of all the formulated herbal tea ranged from 1.6 – 4.4 mg CE/ g and variations were considerable (Table 2). The formulated herbal tea, TF6, TF7 and TF8 herbal tea formulations had higher ($p<0.05$) CTC compared to the control TF1 (100% moringa) without significant differences compared to TF5 and TF6. These herbal tea samples had a high level of stevia (25-35%) (TF6, TF7 and TF8 and this is indicative of the samples resulted in higher level of CTC. This can be attributed to the high CTC level in the dried stevia leaves (Table 2).

Total phenolic content expressed as gallic acid equivalent per g of the dried extract; 2: Total flavonoid content expressed as cateichen equivalent per g of the dried extract;

Where **TF1** (100% moringa), TF2 (95% moringa and 5% stevia), TF3 (90% moringa and 10% stevia), TF4 (85% moringa and 15% stevia), TF5 (80% moringa and 20% stevia), TF5 (75% moringa and 25% stevia), TF6 (70% moringa and 30% stevia), and TF7 (65% moringa and 35% stevia).

Generally the increments and decrements of phytochemicals result between the blending of Moringa and Stevia is due to the effect of synergism because of the presence of diversified molecules in the plants, the synergic activity of the extracts is due to the cumulative effects produced by interactions between the compounds present in the herbs ,interaction between them resulted in protecting the active substances from decomposition; thus, the overall effects increased the anti-oxidant efficiency [24,25]. This is exemplified by the reduction in platelet aggregation observed for the total mixture as opposed to that given by individual compounds, because of their activity as platelet-activating factor antagonists [26].

Conclusions and Recommendations

This study to investigate the phytochemical (total phenolic, total flavonoid, and condensed tannin) content of herbal teas formulated from dried moringa (*Moringa Stenopetala*) and stevia (stevia rebaudiana bertoni) leaves. The result of herbal tea brewed from 25-35% of stevia had the highest total phenolic, total flavonoid, and condensed tannin content than the herbal tea brewed from 100% moringa herbal tea infusion. Using this formulated herbal has advantage to the consumer rather than using moringa alone is evident from this work. However, there is a need to carry out further herbal tea composition profile to explore the potential chemicals present in the formulated herbal tea and commercialization and promotion of herbal tea including this product.

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Conflicts of Interest

There is no confilect of interst exist concerning the work.

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