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Research Article:

Characterization and Quantification of Active Components of Yerba Mate Extract

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Abstract

Background: Yerba Mate is commonly used as a popular tea due to its delicious taste and bright color after preparation. It contains active ingredients that might have therapeutic benefits. This study aimed to identify and quantify active ingredients present in Yerba Mate extract (YME). **Methods:** To do so, YME was prepared by wet digestion and the extract was dried at room temperature. Vitamin E and C were determined using the UV-visible spectrophotometer, minerals were determined by atomic absorption and organic polyphenols were determined using "high-performance liquid chromatography". **Results:** The contents disclosed the presence of vitamin E (9.8 mg/gm), vitamin C (12.5 mg/gm), phosphate (145.9 µg/gm), iron (80.9 µg/gm), calcium (80.6 µg/gm), magnesium (66.3 µg/gm), and zinc (41.9 µg/gm). Additionally, gallic acid (98.8 µg/gm), ferulic acid (60.5 µg/gm), caffeic acid (55.9 µg/gm), apigenin (30.5 µg/gm), chlorogenic acid (24.8 µg/gm), and kaempferol (12.9 µg/gm). **Conclusion:** the concentration and active ingredient present in YME enable its use in some diseases including diabetes mellitus, hyperlipidemia, and bone diseases due to their antioxidant, lipid-lowering and bone-strengthening effects.

1. Introduction

A herbal product *Ilex paraguariensis* represents about 450 species flourishing in the tropical regions of South America and Asia. It belongs to the Aquifoliaceae family which are physically leaves of a tree called Mate folium and is usually consumed as a tea after being dried and roasted domestically known as Yerba Mate. Ilex trees originated in South America: in northern Argentina, southern Brazil, Uruguay, and Paraguay (1–3). *I. paraguariensis* planted 8-15 metres as a subtropical, dioecious, evergreen tree (4). Yerba Mate (YM) is a Spanish word, where it have been used as a tea by people (4,5). Yerba mate tea, a traditional beverage steeped in cultural significance, plays a vital role in the daily lives and social customs of Argentina, Brazil, and Paraguay. These countries are not only the primary producers of this distinctive tea but also its chief consumers, with most of their production being utilized domestically rather than exported (1).

Being used as tea is a popular beverage traditionally used by people; however, being rich in phenolic compounds, the concentrated extract was used as a pharmaceutical product, cosmetics, and food additive (6). The main therapeutic actions of YM include the reduction of serum glucose, lipid profile, and oxidative stress, offering use in obesity, diabetes, and metabolic syndrome (7). The YM leaves are harnessed for the production of cold beverages and energy drinks, moreover, they're used as a preservative for food products due to their antioxidant action (8). This study sought to quantify the contents of the YM extracts which has been purchased from local traditional market in order to investigate the quality used by the consumers in the locality.

2. Materials and Methods

Yerba Mate is a soft drink prepared from the roasted and toasted leaves and twigs of *Ilex paraguariensis* (Argentina)

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related to Aquifoliaceae with a pale green colour (**Figure 1**). To prepare the YME, 50 grams of YM is measured and combined with 300 ml of distilled water. This mixture is then heated to 55°C for 7hr. After heating, the mixture is covered and allowed to rest overnight. The following day, the solution is filtered using filter paper to separate the liquid from the solid residue. The resulting liquid extract is air-dried, converting it into a powder form. The exact weight of the powder was dissolved for the subsequent step of quantification (7,8).

Figure 1. Dried rough-grinded Yerba Mate.

Determination of vitamin E content: Dry leaves (0.5g) plants were submerged in ethanol (20 ml, 30 min, 85˚C). Following that, the solution was left to cool down and filtered in a separating funnel. In the next step, heptane (10ml) was mixed with the solution stirred for 2min and left to disperse into layers. The total tocopherols were estimated by "UV- VIS 545nm" versus the standard solution of tocopherols in ethanol (0.5 ml) (9).

Estimation of ascorbic acid: The powder (10g) was transferred to a flask mixed with 50ml acetic acid solution, shaken, and 4-5 drops of bromine water were added continuously until the solution became coloured. To start with, the surplus bromine in the solution was removed via the addition of 3-5 drops of thiourea solution forming a clear solution. Following this step, a sufficient amount of 2, 4-Dinitrophenyl hydrazine solution to the tested samples and serial dilution of ascorbic acid to extrapolate the calibration curve and quantify the ascorbic acid in the plant extracts via measurement of standard and samples absorbance using a UV-visible spectrophotometer (Shimadzu, 1600–Japan) (10,11).

Determination of minerals: Minerals detected by wet maceration of dry powder based on the method described in APHA (2017). To start with, the plant powder (3g) is mixed with a concentrated perchloric acid solution (3ml) in a beaker and placed on gentle heating (65°C) to facilitate digestion. The beaker remained on heating until dry. Once cooled down, concentrated nitric acid solution (3ml) was added and heating restarted to complete digestion until we reached a light clear colour. The fluid was then evaporated until the sample was dry. Next, 5ml hydrochloric acid (1:1 diluted) was added and samples were exposed to heating (65°C) to ascertain the dissolution of any lees. After 10 minutes of heating, distilled water (50ml) was added. The formed solution became ready for analysis using the atomic absorption device of the type (Shimadzu AA 7000)(12).

The organic polyphenolic compounds were estimated using high-performance liquid chromatography (HPLC, Sykamn, Germany) assembled with a C18-ODS column (250 × 4.6 mm, 5 µm). The samples (100 µL) of YME were injected into the HPLC column. The experiment was conducted using a mobile phase at a rate of 1 mL/min. The mobile phase was composed of solvent A (95% acetonitrile+0.01% Triflouroacetic acid) and solvent B (5% acetonitrile+0.01% Triflouroacetic acid). The gradient program was as follows: 10% A from 0–5 min; 25% A from 5-7 min; 40% A from 7–13 min; then returning to initial conditions. The detection of phenolic compounds was carried out with a UV-visible detector at 278 nm(2).

3. Results

After injecting the extract into the high-performance liquid chromatography, the resulting peaks were compared to the standard of caffeic acid, gallic acid, apigenin, kaempferol, chlorogenic acid, and ferulic acid which represent the component of YME (**Figure 2** and **Table 1**), The result showed that the main organic polyphenol components were gallic acid and the lowest was with kaempferol. Phosphate also was the main mineral in the extract, and vitamins E and C were nearly similar. Moreover, organic polyphenols were characterized by HPLC chromatogram (**Table 2**).

Table 1. Components extracted from YME using the HPLC technique

Components	Conc.
Vit C (mg/gm)	12.5
Vit E (mg/gm)	9.8
P (µg/gm)	145.9
Fe (µg/gm)	80.9
Ca (µg/gm)	80.6
Mg (µg/gm)	66.3
Zn (µg/gm)	41.9
Gallic acid (µg/gm)	98.8
Ferulic acid (µg/gm)	60.5
Caffeic acid (µg/gm)	55.9
Apigenin (µg/gm)	30.5
Chlorogenic acid (µg/gm)	24.8
Kaempferol (µg/gm)	12.9

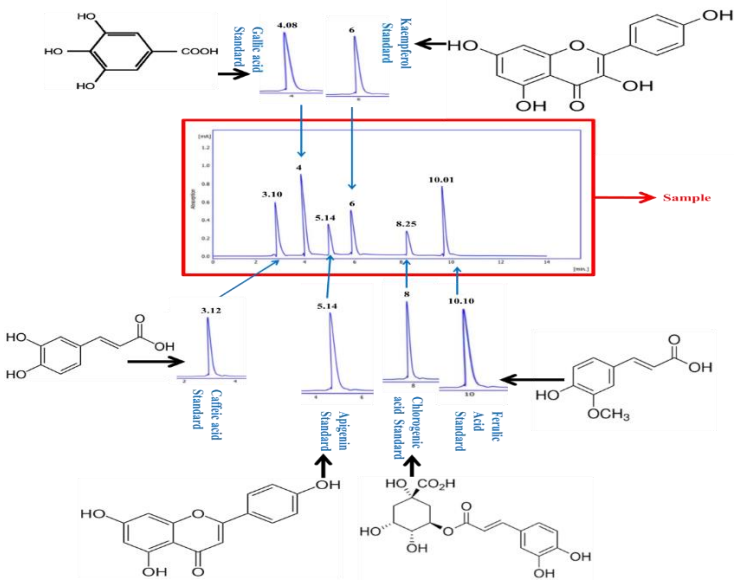

Figure 2. The HPLC chromatogram of the YME sample compared to standard peaks.

Table 2. HPLC analysis results in characteristics for isolated compounds

Compounds	Retention time (Min.)	Area (mAU.s)	Height (mAU.s)	Area (%)	Height (%)	W05 (Min)	M.wt.(g/mol)
Caffeic acid	3.1	10236	590.14	20	20	0.2	180.16
Gallic acid	4	12564.08	820.35	25	25	0.25	170.12
Apigenin	5.14	5254.19	350.64	13	13	0.15	270.0528
Kaempferol	6	9652.31	430.55	15	15	0.18	286.23
Chlorogenic acid	8.25	4154.89	260.14	8	8	0.08	354.31
Ferulic acid	10.01	13225.65	750.11	19	19	0.2	194.18
Total		56078.14	3201.39	100	100		

4. Discussion

The presence of various species of the plant yerba has raised the importance of their quantification in different countries which necessitates their content analysis and quantification of individual components to assess their potential therapeutic value compared to already available sources. The present study confirmed the presence of minerals, polyphenols, vitamin E and vitamin C in YME.

The presence of these components has given yerba its therapeutic value for the treatment of inflammation, oxidative stress, and bone strengthening.

The YME have shown a good content of vitamin C and vitamin E hence providing interesting therapeutic antioxidant value. Similarly, a previous study confirmed that YME contains vitamins E and C alongside other vitamins (13). The presence of these vitamins and other phenolic compounds has gained YME its antioxidant effects and thereby their application in the therapy of certain diseases where oxidative stress is the mainstay. For example, YME was found to be applied as an antioxidant to decrease lipid peroxidation and lipid profile in hyperlipidemic patients on statin therapy(14). Moreover, the antioxidant profile of YME contributes to the reduction of oxidation of glycated protein and thereby could be effectively used to reduce advanced glycation end product (AGE)(15)

The YME has shown potential therapeutic mineral contents represented by its content of calcium, magnesium, zinc, phosphate, and iron. In congruent with the present findings, Camotti Bastos et al. (2014), have found that 1 gram of yerba mate dry powder contains a relatively reasonable amount of several minerals, such as Cu, Mn, K, P, Mg, Fe, Zn, Ca, and Na when tested by UV-VIS I spectrophotometer and atomic absorption spectrophotometry with flame. However, the amount could be different across different geographical regions (16). Lorini et al. (2021) also reported an estimation of relatively large quantities of several minerals including copper, zinc, iron, and manganese, but not calcium and were shown that the quantities present are sufficient and meet the recommended daily requirements and effectively could meet the demand of minerals for bone healing (17)

In addition to the detected antioxidant vitamin and presence of minerals, there have been detected organic compounds represented by caffeic acid, gallic acid, apigenin, kaempferol, chlorogenic acid, and ferulic acid, which potentially provides the YME therapeutic value.

The presence of a reasonable amount of caffeic acid in YME provides the extract with potential therapeutic antioxidant

effects and reduces the AGE formation (15), moreover, it stimulates CNS to the same extent as coffee drinks with no incidence of nervousness (18). Nonetheless, caffeine together with other polyphenols carries a negative impact on bone density and encourages fractures an action which could be antagonized by a diet with good calcium content (19). This impact was also reported in an animal study with the reported mechanism of bone loss being related to osteoclastogenesis (20). Conversely, caffeic acid has been reported to preserve and increase the viability of osteoblastic cells in an in vitro cell culture model (21). This negative osteoclastogenesis could be neutralized by the presence of chlorogenic acid which prevents RANKL-induced osteoclastogenesis (22), moreover, the chlorogenic acid encourages bone cell specialization of human dental pulp stem cells through Wnt signalling (23) and halts osteoporosis in ovariectomized rats through the Shp2/phosphoinositide 3-kinase/Akt pathway (24).

Chlorogenic acid present in YME has provided this extract with therapeutic effects including antioxidant effects, reducing lipid peroxidation, and hypoglycemic effects making YME applicable for the treatment of metabolic diseases (25,26). Moreover, chlorogenic acid has been shown antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Alternaria alternata, Aspergillus niger, Candida albicans, and Fusarium oxysporum* (27). In skin products, chlorogenic acid in YME has provided penetration-enhancing properties on the skin by acting as an emulsifier (28). Apigenin is the main flavonoid in YME (29,30) with useful applications in diabetes, amnesia and Alzheimer's disease, depression and insomnia, and cancer (31)

Kaempferol, which is also found in YME, is known to engage multiple pathways involved in cancer progression. It promotes apoptosis triggered by kaempferol and alters various cellular signalling pathways, effectively targeting cancer cells while sparing normal cells. This selectivity offers an advantage over traditional chemotherapy drugs by focusing specifically on cancer cells (32). Despite the reported antimicrobial activity of kaempferol, it has shown only weak bacteriostatic effects on S. epidermidis and E. coli. As a part of phenolic compounds present in YME, it has been found that the extract also contains hydroxycinnamates, of which the most important is ferulic acid (33), which has been reported to have a selective bacteriostatic effect on pathogenic microorganisms with no impact on normal flora (34,35).

5. Conclusion

The present study quantified the active components separated from YME and the main components were antioxidant vitamins, bone protection minerals, and organic polyphenols. This enables the use of the yerba plant for the treatment of diabetes, hyperlipidemia, cardiovascular, and bone diseases. The concentration of present compound represent the standard content of YME and these compound concentration is reasonably acceptable and could be used by consumers safely with reported and recorded therapeutic activity.

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توصيف وتقدير المكونات النشطة لمستخلص شاي الجزويت

الخلاصة

المقدمة: يشيع استخدام يربا ماتي كشاي شعبي بسبب المذاق اللذيذ والألوان الزاهية بعد التحضير. يحتوي على العنصر النشط الذي قد يضفي فوائد علاجية. كان الهدف من هذه الدراسة هو تحديد وقياس المكونات النشطة الموجودة في شاي الجزويت. **الطريقة:** للقيام بذلك ، تم تحضير شاي الجزويت عن طريق الهضم الرطب وتم تجفيف المستخلص في درجة حرارة الغرفة. تم تحديد فيتامين ي وفيتامين ج بواسطة طريقة مقياس الطيف الضوئي المرئي للأشعة فوق البنفسجية ، وتم تحديد المعادن عن طريق الامتصاص الذري ، بينما تم تقدير البوليفينول العضوي بواسطة كروماتوغرافيا سائلة عالية الأداء. **النتائج:** كشفت المحتويات عن وجود فيتامين ي9.8) مجم / جم) وفيتامين ج (12.5 مجم / جم) والفوسفات (145.9 ميكروغرام / جم) والحديد (80.9 ميكروغرام / جم) والكالسيوم (80.6 ميكروغرام / جم) والمغنيسيوم (66.3 ميكروغرام / جم) والزنك (41.9 ميكروغرام / جم). بالإضافة إلى ذلك ، حمض الغال (98.8 ميكروغرام / جم) ، حمض الفيروليك (60.5 ميكروغرام / جم) ، حمض الكافيين (55.9 ميكروغرام / جم) ، أبيجينين (30.5 ميكروغرام / جم) ، حمض الكلوروجينيك (24.8 ميكروغرام / جم) ، وكيمبفيرول (12.9 ميكروغرام / جم). **الخلاصة:** التركيز والعنصر النشط الموجود في شاي الجزويت يمكنه من استخدامه في بعض حالات الأمراض بما في ذلك داء السكري وفرط شحميات الدم وأمراض العظام بسبب مضادات الأكسدة وخفض الدهون وتقوية العظام.

الكلمات المفتاحية: يربا ماتي ، إيليكس باراجوارينسيس ، بوليفينول ، تحليل كروماتوغرافي.