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A COMPARATIVE STUDY BETWEEN GREEN TEA (*CAMELLIA SINENSIS*) AND COMMON SAGE (*SALVIA OFFICINALIS*) (CHEMICAL COMPOSITION AND ACTIVE COMPOUNDS)

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ABSTRACT: The purpose of this research is to study the chemical composition and effective compounds of leaves of green tea and sage as one of the traditional medicinal plants. It was found that green tea leaves collected from the local market contain 5.68 % moisture, 2.35% ash, 14.57 % protein, 8.55 % lipids, and 2.3% crude fiber. While sage leaves contained 8.19, 2.17, 12.21, 3.33, and 2.6 % moisture, ash, protein, lipids, and crude fiber, respectively. It was also found that green tea leaves contain 0.376 % Polyphenols and 0.188 % Flavonoids, while sage leaves contain 0.262 and 0.132 % Polyphenols and Flavonoids, respectively. HPLC showed that the aqueous extract of green tea leaves contains eighteen compounds of Polyphenols, fifteen compounds of them were identified. While the aqueous extract of sage leaves contains twenty-one compounds, nineteen of them were identified.

Key words:* *Green tea – Sage – Polyphenols – Flavonoids – HPLC.

INTRODUCTION

Green tea is the most common and consumed member at the *Theaceace* taxonomic family due to its high content of antioxidants (Prasanth *et al*., 2019). Usually after harvesting, the green tea leaves are steamed to prevent the oxidation of existing polyphenols, and also the vitamins of green tea remain active (Yamamoto *et al*., 1997). Due to its high content of polyphenols (Li *et al*., 2018; Yamamoto *et al*., 1997). Green tea was always considered as a promising candidate against antiaging, neuroprotective effects owing to the epigallocatechin gallate (Afzal *et al*., 2015; Khalatbary & Khademi, 2020), in parallel with its protective effects opposite different diseases like cancer (Mukhtar & Ahmad, 2000), cardiovascular diseases (Moore *et al*., 2009; Nagao *et al*., 2007), and obesity (Huang *et al*., 2014) Tea polyphenols fall into two main groups, catechins and flavonols. The former group includes catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), and gallocatechin gallate (GCG) as major compounds. It is important to mention that EGCG, ECG, and EGC represent around 80% of the total catechins (Cleverdon *et al*., 2018). While the latter group contains myricetin, caempherol, quercetin, chlorogenic acid, coumarylquinic acid, and theogallin (Li *et al*., 2018; Yamamoto *et al*., 1997). Common sage is the dominant genus of *Lamiaceae* family. Diterpenoids, triterpenoids, alkaloids and saccharides are the major compounds at the respective plant, and are reponsible of its medical importance (Pizzale *et al*., 2002), and the components of basic biological activity are polyphenolic compounds (phenolic acids and flavonoids). Phenolic acids have a variety of structures (monomers, dimers, trimers, tetramers, and multimers), and are found in high concentrations, on the basis of which plant quality is determined (. Wang *et al*., 2019). In addition, phenolic acids mediate

the major pharmacological activities such as antioxidant, anti-ischemia-reperfusion injury, anti-thrombosis effects (Chang *et al*., 2016; Liu *et al*., 2018). Anti-cardiovascular, anti-oxidation, anti-inflammatory, and anti-tumour effects (Eghbaliferiz & Iranshahi, 2016; Hussain *et al*., 2016; Mattera *et al*., 2017).

MATERIALS AND METHODS

1. Collection of plants

A semi-dry form of leaves of green tea and Sage were purchased from the local market (Shebin El-Kom, menofia, Egypt), identified by the departments of Horticulture, Faculty of Agriculture, Menoufia University, Shebin El-Kom (2020).

2. Determination of chemical composition

2.1. Determination of the moisture content

Moisture content (MC) was determined using air-oven based on the Association of Official Analytical Chemists (AOAC) (AOAC, 2007) with taking into account the preservation of active compounds in the medicinal plants (Muller & Heindl, 2006). The percentage of MC was calculated according to the following equation:

$$\%\,MC = \frac{A_{grams} - B_{grams}}{W_{grams}} \times 100$$

Where:
A= The initial weight (before drying).
B= The constant weigh (after drying).
W= The weight of sample.

2.2. *Determination of the crude oil content*

The crude oil was determined in the studied plants using the Soxhlet extraction method by petroleum ether (AOAC, 2007) . The percentage of crude oil was calculated as follow:

$$\%\,Oil = \frac{Weight\ of\ oil_{grams}}{Weight\ of\ sample_{grams}} \times 100$$

2.3. *Determination of the total protein content*

The total nitrogen content was determined in the studied plants using the KJELDAHL method (AOAC, 2007). The total protein in the studied samples, 6.25 was used as a conversion factor for nitrogen-to-protein.

2.4. *Determination of the ash content*

A dry ashing procedure was performed to determine the total ash content (AOAC, 2007). In brief, the studied samples were incinerated for 6 hours in the furnace at 550 ° C. The total ash content was calculated as follow:

$$\%\,Ash = \frac{Weight\ of\ incenerated\ sample_{grams}}{Initial\ weight\ of\ the\ sample_{grams}} \times 100$$

2.5. *Determination of crude fiber content in medicinal plants*

The crude fiber content was determined according to (Busuttil-Griffin *et al*., 2015). Concisely, after washing the plant samples under the running water, the samples were cut into suitable sizes and dried 40 ° C for 24 hours to be ready for grinding. Then, 2-3 grams of grinded plant were weighed and transferred into Soxhlet apparatus to remove fats using petroleum ether. After that, the defatted sample was digested using 50 ml of 1.25% H_2SO_4, Mixture was boiled under reflux for 30 min. The hot solution was filtered under suction. The insoluble matter was washed several times with hot water until samples were acid free. Sample were transferred to a flask containing 50 ml 1.25 % NaOH. The insoluble residue was washed with hot water until base free, then dried to a constant weight at 100 ° C and cooled in a desiccator and weighted (X_1). The weight sample were incinerated in a muffle furnace at 525 ° C for two hrs.,

cooled in a desiccator and reweighted (X_2). The Crude fiber was calculated as follows:

$$\%\ Crude\ fiber\ content\ = \frac{X1 - X2}{Weight\ of\ grinded\ sample} \times 100$$

2.6. *Determination of the total phenols and Flavonoids in medicinal plants*

2.6.1. *Extraction of total phenols and Flavonoids:*

We dried the samples at 55°C for 24 hours, then powdered by a mixture grinder. After that, the total phenols and flavonoids content were extracted by ethanol via 20 cycles of Soxhlet apparatus. Then concentrated by the rotary evaporator under reduced pressure.

2.6.2. *Estimation of total phenols*

Phenolic (Folin – Ciocalteu Method) Preparation of Standard Calibration Curve: 1 ml aliquots of 50 – 500 µg / ml Ethanolic Gallic acid solution were mixed with 5 ml of Folin – Ciocalteu reagent, and 4 ml of sodium carbonate (7.5%). The absorbance was read after 30 min. at 765 nm spectrophotometrically (Gansch *et al*., 2015).

To estimation of total Phenolic in extracts, 1 ml of each extract (50 mg/100 ml) was mixed with the same reagent as performed above. The absorbance was read after 30 min. at 765 nm for determination of phenolic. Total content (%) of phenolic compound in plant different extracts was calculated as Gallic acid equivalent (GAE):

$$GAE = [(C \times V)/M] \times 100$$

where,

C= the conc. of Gallic acid established from calibration curve mg/ml.

V= Volume of extract (ml); M=the weight of dried plant extract (mg).

2.6.3. *Estimation of total flavonoids*

Aluminum chloride colorimetric method (Djeridane *et al*., 2006) was used for flavonoids determination. Each plant extracts (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride 0.1 ml of 1 m Potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solution at concentration 20 to 100 µg/ ml in methanol.

2.7. Determination of Total Carbohydrates

The final carbohydrate percentage was calculated by subtracting the sum of the percentages of the ingredients obtained from 100%.

2.8. High-Performance Liquid Chromatography (HPLC) analysis of the medicinal plants

2.8.1. Preparation of plant extracts

To prepare the plant extracts of the respective medicinal plants, 250 grams of dried plants were mixed with water (1:10), boiled for 30 min at 100 °C. Then, the extracts were centrifuged, filtered, and stored at –20 °C. Before administration of the medicinal plant extracts, the lyophilized extracts were dissolved in water at the respective doses (Mohamed & Metwally, 2009).

2.8.2. HPLC

Crude extract was prepared as explained in the next section. HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A);

5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A) and post time (5 min). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 40 °C (AOAC, 2007). (By National Research Centre- 33 El Buhouth St ،Ad Doqi, Dokki, Cairo Governorate 12622).

RESULTS AND DISCUSSION

1. Chemical composition of medicinal plants used (green tea and common sage)

Data in Table (1) indicate that green tea contains 14.57 % total protein, 8.55 crude lipids and 66.3 % total carbohydrates. While the amount of the same constituents in common sage were 12.21, 3.33 and 71.5 % respectively, our results generally are similar to the studies (Grosso *et al*., 1999; Habib *et al*., 2017; Khacheba *et al*., 2014; Rahman *et al*., 2014) except humidity. The difference in humidity levels in the studied samples may be due to the different source, as they are selected from the local market in a semi-dry form.

2. Total phenolic and flavonoids compounds of green tea and Common sage extracts

Data in Table (2) showed that total phenolics and flavonoids contents in ethanolic extracts were 376 mg/100 gm dry weight for green tea while its contents were 262 mg/100 gm dry weight for common sage.

Total flavonoids contents were 188 and 132 mg/100 gm dry weight for green tea and common sage, respectively.

Table (1): Chemical composition of the studied medicinal plants (green tea and common sage)

Chemical constituent (%)	Green tea	Common sage
Moisture	5.68	8.19
Ash	2.35	2.17
Protein	14.57	12.21
Lipids	8.55	3.33
Crude Fibers	2.3	2.6
Carbohydrates	66.3	71.5

Table (2): Total phenolics and Flavonoids of green tea and common sage (mg/100 gm dry weight)

Extracts	Total phenolics	(%)	Total Flavonoids	(%)
Green tea	376	0.376	188	0.188
Common sage	262	0.262	132	0.132

3. HPLC of phenolics in green tea

HPLC analysis of the aqueous extract for the phenolic compounds Table (3) showed the presence of eighteen compounds, fifteen compounds of them were identified, gallic acid, chlorogenic acid, catechin, methyl gallate, syringic acid, pyrocatechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, taxifolin, cinnamic acid and kaempferol, its amounts were 27.6, 10.26, 59.16, 51.36, 5.79, 0.96, 3.57, 1.86, 15.93, 1.11, 1.11, 1.68, 1.71, 0.327 and 0.33 mg/100 g dry weight, respectively. There are three compounds unknown, its concentrations were 41.16, 10.26 and 4.2 mg/100 gm dry weight with RRT 0.48, 0.7 and 0.84, respectively.

The results are in agreement with those of (Galati *et al*., 2006), who demonstrated that tea phenolic acids and catechins containing Gallic acid are most abundant in green tea. Takami *et al*., (2008) investigated that green tea catechins (GTC), polyphenols extracted from the stalks and leaves of green tea were found in the different types of tea beverages and as antioxidants additives to many foods. Green tea contains polyphenols which have recently been reported to be a potent antioxidant and beneficial in oxidative stress and inhibit the initiation of aflatoxin B_1 – induced carcinogenesis in treated mice (Chen *et al*., 2004).

Table (3): HPLC analysis of poly phenol compounds of aqueous extract of green tea leaves (mg/100 gm dry weight)

Phenolic compounds	RT	RRT	Conc. (mg / 100 gm)
Unknown	2.38	0.48	41.16
Gallic acid	3.17	0.64	27.6
Unknown	3.5	0.70	10.26
Chlorogenic acid	3.77	0.76	3.3
Unknown	4.17	0.84	4.2
Catechin	4.97	1.00	59.16
Methyl gallate	4.97	1.00	51.36
Syringic acid	5.47	1.10	5.79
Pyrocatechol	6.5	1.31	0.96
Rutin	7.0	1.41	3.57
Ellagic acid	7.90	1.59	1.86
Coumaric acid	8.12	1.63	15.93
Vanillin	8.60	1.73	1.11
Ferulic acid	9.44	1.90	1.11
Naringenin	9.85	1.98	1.68
Taxifolin	12.03	2.42	1.71
Cinnamic acid	13.31	2.68	0.327
Kaempferol	14.04	2.82	0.33

4. HPLC analysis of phenolics in common sage

Data in Table (4) presented the HPLC analysis of sage water extract, showed the presence of twenty one compounds, nineteen of them were identified which were varied in its amounts, it was observed that 1,8-cinol, gallic acid, chlorogenic acid, methyl gallate, catechin, caffeic acid, syringic acid, pyrocatechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, ursolic acid, rosmarinic acid, taxifolin, cinnamic acid and kaempferol, its amounts were 21.12, 7.20, 9.93, 1.5, 2.58, 6.51, 12.78, 0.60, 0.123, 0.666, 49.02, 47.61, 17.43, 2.91, 17.43, 47.76, 1.41, 0.285 and 1.05 mg/100 g dry weight, respectively. There are two compounds unknown its concentrations were 15.51 and 6.59 mg/100 gm dry weight with RRT 0.48 and 2.13, respectively.

Table (4): HPLC analysis of poly phenol compounds of aqueous extract of common Sage leaves (mg/100 gm dry weight)

Compound	RT	RRT	Conc. (mg/100 gm)
Unknown	2.38	0.48	15.51
1,8-cinol	2.53	0.51	24.12
Gallic acid	3.17	0.64	7.20
Chlorogenic acid	3.77	0.76	9.93
Methyl gallate	4.17	0.84	1.5
Catechin	4.97	1.00	2.58
Coffeic acid	5.40	1.09	6.51
Syringic acid	5.67	1.14	12.78
Pyrocatechol	6.5	1.31	0.60
Rutin	6.88	1.38	0.123
Ellagic acid	7.9	1.59	0.666
Coumaric acid	8.12	1.63	49.02
Vanillin	8.6	1.73	47.61
Ferulic acid	9.44	1.90	17.43
Naringenin	9.85	1.98	2.91
ursolic acid	10.08	2.03	17.43
Rosmarinic acid	10.34	2.08	47.76
Unknown	10.57	2.13	6.59
Taxifolin	12.03	2.42	1.41
Cinnamic acid	13.31	2.68	0.285
Kaempferol	14.04	2.82	1.05

These data agree with those of (Hohmann *et al*., 1999; Wang *et al*., 2003), they found that common sage (*Salvia officinalis L.*) is most popular herbal remedy to treat common health as well as their antioxidative properties. (Ahl *et al*., 2015) found that the predominant medicinally valuate metabolites of sage are monoterpenes (e 9., α and β-thujone, 1,8-cinol, camphor), diterpenes (e.9-carnosic acid), triterpenes (oleanolic and ursolic acids) and phenolic compounds like rosmarinic acid. (Martins *et al*., 2014 & Cuceu *et al*., 2015) found that sage contains many biologically active compounds that can be divided into monoterpenes, diterpenes and phenolic components. They add that highly abundant phenolic components can be divided into two groups: phenolic acids (Caffeic, vanillic, ferulic and rosmarinic acid) and flavonoids (luteolin, apigenin and quercetin). (Huang and zhang 1992) reported that Sage contain several antioxidants such as water-soluble compounds, salvianolic acid A, salvianolic acid B and rosmarinic acid. (Hohmann *et al*., 2001) found that Sage also contain several phenolic glycosides, that prevent peroxidative damage, inhibit lipid peroxidation and free radicals' generations *in vivo, in vitro* and induce endogenous antioxidant deference systems.

Conclusion

In comparison between green tea and sage leaves in terms of active compounds, it was found that the aqueous extract of green tea contained 18 phenolic compounds (Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Syringic acid, Pyrocatechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Taxifolin, Cinnamic acid and Kaempferol), the proportion of catechin was the highest. While the aqueous extract of sage contained 21 phenolic compounds (1,8-cinol, Gallic acid, Chlorogenic acid, Methyl gallate, Catechin, Caffeic acid, Syringic acid, Pyrocatechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, ursolic acid, Rosmarinic acid, Taxifolin, Cinnamic acid and Kaempferol), the percent of coumaric acid was the highest, and the aqueous summary of green tea showed a higher percentage of phenolic compounds compared to with aqueous sage extract.

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دراسة مقارنة بين الشاى الأخضر والمريمية (التركيب الكيميائى والمركبات الفعالة)

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الملخص العربى

الغرض من هذا البحث هو دراسة التركيب الكيميائي والمركبات الفعالة لأوراق الشاي الأخضر والمريمية كأحد النباتات الطبية التقليدية. وجد أن أوراق الشاي الأخضر المجمعة من السوق المحلي تحتوي على 5,68٪ رطوبة ، 2,35٪ رماد ، 14,57٪ بروتين ، 8,55٪ ليبيدات ، 2,3٪ ألياف خام. بينما احتوت أوراق المريمية على 8,19 و 2,17 و 12,21 و 3,33 و 2,6٪ رطوبة ورماد وبروتين وليبيدات وألياف خام على التوالي. كما وجد أن أوراق الشاي الأخضر تحتوي على 0,376٪ بوليفينولات و 0,188٪ فلافونات بينما أوراق المريمية تحتوي على 0,262 و 0,132٪ بوليفينولات وفلافونات على التوالي. أظهر التحليل الكروماتوجرافى (HPLC) أن المستخلص المائي لأوراق الشاي الأخضر يحتوي على ثمانية عشر مركبًا من البوليفيمولات، تم التعرف على خمسة عشر مركبًا منها. بينما يحتوي المستخلص المائي لأوراق المريمية على واحد وعشرين مركبًا تم التعرف على تسعة عشر منها.

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