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Research Article

Phytochemical Investigations and Antioxidant Activity of Green Tea

Rana R, Madan S*, Gullaiya S, Agarwal S.S

Amity Institute of Pharmacy, Amity University, Noida

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ABSTRACT

Green Tea is one of the most ancient and popular therapeutic beverages consumed around the world. It is obtained from the plant *Camellia sinensis* by minimal oxidation during processing is "Natural secret for a healthier life. Green tea is reported to contain thousands of bioactive ingredients which are almost contributed by polyphenols which plays a key role in prevention and treatment of many diseases. The main constituent present in green tea is Epigallocatechin-3-gallate. This component is responsible for all the biological activities that it shows like anticancer, antioxidant, antidiabetic, antiobesity, antihypertensive, antiplatlet, use in cardiovascular diseases etc. The current research assists for the pharmacognostical standardization of the plant *Camellia sinensis.* The study also includes comparing various phytochemical parameters like ash value, extractive value, loss on drying, as well as preliminary phytochemical tests to detect the different constituents present in the plant. The anti-oxidant activity of the plant is also carried out by *in-vitro* DPPH method.

Keywords: *Camellia sinensis*, anti-oxidant activity, epigallocatechin-3- gallate.

INTRODUCTION

Tea is the second most commonly consumed beverage in the world after coffee, beer, wine and carbonated soft drinks[1,2]. Tea, the drink, is an infusion of variously processed leaves and flowers of one of the varieties of an evergreen shrub botanically called *Camellia sinensis*. Depending on the manufacturing process, teas are classified into three major types: 'non-fermented' green tea (produced by drying and steaming the fresh leaves to inactivate the polyphenol0 oxidase and thus, non oxidation occurs); 'semi-fermented' oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying); and 'fermented' black tea which undergo a post-harvest fermentation stage before drying and steaming. Black tea is one of a varieties of preparations made from *Camellia sinensis.* Green tea is made from unfermented leaves and contains the highest concentration of powerful antioxidants. The processes of obtaining the various types of tea are different.

Green tea has number of pharmacological activities such as anticancer, lipid lowering, neuromuscular blocking action, immunomodulatory effect, antiviral, antibacterial[3], antispasmodic, antioxidant[4]. A large number of phytoconstituents like alkaloids (caffeine, theobromine), proteins, enzymes, carbohydrates, lipids, polyphenols, catechins (epicatechins, epicatechins 3-gallate, epigallocatechin and epigallocatechin-3-gallate), carbohydrates, tannins, vitamins and minerals have been reported to be present in this plant[5].

Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases (www.nlm.nih.gov/medlineplus/antioxidants). Another study shows that green tea is also helpful in reducing stroke, myocardial infarction and coronary heart diseases[6]. One study found green tea polyphenols to be more potent antioxidants than Vitamin C, Vitamin E, rosemary extract, and even cur cumin in some systems. Curcumin, a potent antioxidant and chemo preventive agent, has recently been found to be capable of inducing apoptosis in human hepatoma and leukemia cells by way of an elusive mechanism, but green tea is proved to be more effective than curcumin[7].

Green tea lowers blood pressure and helps prevent hypertension. Tea increases body's production of nitric oxide, which dilates arteries and thereby reduces blood pressure. Among persons consuming tea regularly for at least one year, the risk of developing high blood pressure was 46% lower among those who drank half cup to two and a half cups per day and 65% less among those consuming more than two and a half cups per day[8].

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. (St Louis, MO, USA), Merck (Darmstadt, Germany) and Qualigens (Mumbai, India). Reference standard ascorbic acid 99% (sigma Aldrich), Spectral and absorbance measurements were carried out on a Shimadzu UV-Vis 1601 spectrophotometer by using 1.0 cm quartz cells.

*Author for Correspondence

Table 1: Result of total ash, acid insoluble ash, water soluble ash (% w/w)

Parameter	
Total ash	7.5
Acid insoluble ash	1.20
Water soluble ash	2.0

Table 2: Result of loss on drying (% w/w)

Plant name	Loss on drying
C. sinensis	5

Table 3: Result of hot soxhlation

Type of extract	% w/w of Extractive value
Methanol	30
Ethanol	25
Water	32

Table 4: Result of DPPH radical scavenging activity of aqueous extract of *C. sinensis*

S. No.	Extract	IC_{50} (µg/ml)
1.	aqueous extract	68.11
2.	ascorbic acid	26.38

Collection of plant material

Dried leaves of *C. sinensis were* procured from Sood brothers, Lahori gate, Old Delhi. The identification of the dried leaves of *C. sinensis* were verified by Dr. (Mrs) Sunita Garg, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi and a voucher specimen number NISCAIR/RHMD/Consult/2013/2347-127 for the leaves were obtained.

Physico-chemical analysis

The percentage physico-chemical values, *viz*, total ash, acid insoluble ash, water soluble ash, loss on drying and extractive values after successive extractions in various solvents such as aqueous, methanol and ethanol were determined according to the official methods prescribed[9] and the WHO guidelines on quality control methods for medicinal plant materials[10].

Ash values

Ash content of crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration. Total ash is the residue remaining after incineration. Acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. Water soluble ash is the part of total ash, which is soluble in hot water.

Methodology

Total ash

2 gm of the powdered drug was accurately weighed in a tared silica crucible. The powdered drug was spread as a fine layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450ºC until free from carbon. The crucible was cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated with reference to air dried drug[6].

Acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 min. The insoluble ash was collected on an ashless filter paper by filtration and it was washed with hot water. The insoluble ash was transferred into tarred silica crucible, ignited, cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to air dried drug.

Water soluble ash

The ash obtained as described in the determination of total ash was boiled for 5 min with 25 ml of water. The insoluble matter was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a tared silica crucible and ignited at a temperature not exceeding 450ºC. The procedure was repeated until a constant weight was observed. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug[6].

Loss on drying

An excess of water in medicinal plant material will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water[11].

Methodology

About 10 gm of the drug was placed after accurately weighing it in a tared evaporating dish. After placing the above said amount of the drug in the tared evaporating dish, it was dried at 105º C for 5 hours and weighed. Drying and weighing were continued at one hour interval until difference between two successive weighing corresponded to not more than 0.25%. The loss in weight on drying was then calculated.

Extraction of the dried leaves

The ***C. sinensis*** leaves were dried in an oven to remove any moisture content and were coarsely powdered. The powdered material was defatted with petroleum ether (40-60ºC). Further extraction were made by using Soxhlet apparatus in different solvents like ethanol, methanol.

Aqueous extract

About 50 gm of ***C. sinensis*** air dried crushed drug was defatted by extracting the drug in a soxhlet apparatus for about 6 hours with petroleum-ether (40-60ºC) as the solvent. After 6 hours extract was decant off and the marc were air dried for the removal of any petroleum ether left. The marc left is further subjected to Soxhlet by using distilled water for 6-8hrs. After completion of extraction solvent was evaporated and extract was concentrated using rota vapour assembly, cooled, transferred into a petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the aqueous extract was kept in desiccator to

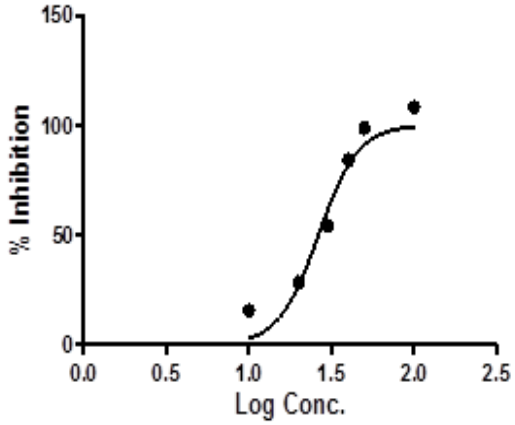

Figure 1: Percent inhibition graph for ascorbic acid

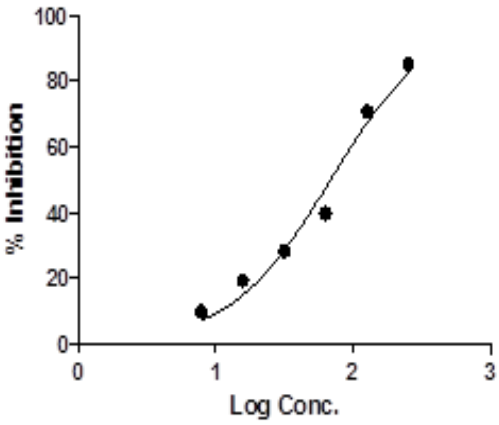

Figure 2: Percent inhibition graph for aqueous extract of *C.sinensis*

remove the excessive moisture and was used for further studies.

Methanolic extract

About 50 gm of ***C.sinensis*** air dried crushed drug was defatted by extracting the drug in a soxhlet apparatus for about 6 hours with petroleum-ether (40-60ºC) as the solvent. After 6 hours extract was decant off and the marc were air dried for the removal of any pet.ether left. The marc left is further subjected to Soxhlet by using methanol as solvent for 6-8hrs.

After completion of extraction solvent was evaporated and extract concentrated on rota vapour assembly, cooled, transferred into a petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the methanolic extract was kept in desiccator to remove the excessive moisture and was used for further studies.

Ethanolic extract

About 50 gm of ***C.sinensis*** air dried crushed drug was defatted by extracting the drug in a soxhlet apparatus for about 6 hours with petroleum-ether (40-60ºC) as the solvent. After 6 hours extract was decant off and the marc were air dried for the removal of any pet.ether left. The marc left is further subjected to soxhlet by using ethanol as solvent for 6-8hrs. After completion of extraction solvent was evaporated and extract concentrated on rota vapour assembly, cooled, transferred into a petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the ethanolic extract was kept in desiccators to remove the excessive moisture and was used for further studies.

Preliminary Phytochemical Screening

The phytochemical tests are carried out for methanolic extract of *C. sinensis* to detect the presence of various classes of phytoconstituents.

DPPH free radical scavenging method

The free radical scavenging capacity of aqueous extract of *C. sinensis* was determined using established DPPH method[12,13]. The aqueous extracts of *C. sinensis* was mixed with 95% methanol to prepare the stock solution (1.0 mg ml^{-1}). The 1.0 ml of DPPH solution (0.10 mM) prepared in 95% methanol was taken in test tubes then 1.0 ml of each sample was added followed by serial dilutions (7.8-250 µg ml^{-1}) to every test tube. Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration (1.0 mg ml^{-1}) followed by serial dilutions (10-100 µg ml^{-1}). The absorbance was measured after 10 min at 515 nm. The control sample was prepared containing the same volume without any extract and reference ascorbic acid. Methanol (95%) was used as blank. The percentage scavenging activity of the *C. sinensis* against DPPH free radical was measured using the following equation:

$$\% \text{ Inhibition} = [(A0-A1) / A0] \times 100$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance of the extract or standard.

RESULT AND DISSCUSSION

Results of Physicochemical analysis

Physico-chemical parameters i.e. ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 1) of the powdered *C.sinensis* leaves revealed a high concentration of total ash of 7.5% w/w. Loss on drying of the powdered *C.sinensis* leaves revealed the presence of 5% w/w of moisture in a drug (Table 2). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The results of methanol, ethanol and water soluble extractive values are shown in Table 3.

Preliminary phytochemical screening of the leaves of *C.sinensis* showed the presence of tannins, alkaloids, saponin, terpenoids, cardiac glycosides and flavanoids.

The results of antioxidant activity of standards ascorbic acid and green tea aqueous extract showed IC_{50} values of 26.38 and 68.11 µg/ml respectively (Table 4 & Fig 1 & 2). This free- radical scavenging activity can be attributed to the high amounts of flavonoids and phenolics present in green tea extract of *C.sinensis*.

In conclusion, the extracts from the leaves of *C.sinensis* possessed significant antioxidant activity. The present

work was taken up with a view to lay down standards, which could be useful to detect the authenticity of this medicinally useful plant

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