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Qualitative and Quantitative Determination of Secondary Metabolites and Antioxidant Potential of Leaf of Brahmi, Green Tea and Bulb of Onion Extracts

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ABSTRACT

Utilization of herbs for medicinal purpose started in the early history of mankind several thousand years ago.In the last few years, there has been an exponential growth in the field of herbal medicine and gaining popularity both in developing and developed countries because of their natural origin and less side effects. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates and inhibit other oxidation reactions by thereby reducing stress responsible for many degenerative disorders. The aim of the present study was to determine qualitative and quantitative phytochemical and *in vitro* antioxidant activities of leaf of Green tea, Brahmi and bulb of Onion collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic content was carried out by Folins Ciocalteau reagent method. The *in vitro* antioxidant activity of methanolic extract of the leaf and bulb was assessed against DPPH assay method using standard protocols. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total phenolic content of leaf of Green tea, Brahmi and bulb of Onion was 195.26 ± 12.64, 136.92 ± 21.73 and 112.23 ± 11.36 mg/100mg respectively. The activities of methanolic leaves and bulbs extract against DPPH assay method were concentration dependent with IC 50 values of ascorbic acid and extracts 20.05±1.86 and 55.98±2.56, 104.45±3.13, 144.37±5.45µg/ml respectively. These studies provided information for correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: Green Tea, Brahmi, Onion, Phytochemical constituents, Antioxidant activity

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INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country[1]. Herbal medicine is still the mainstay of about 75%-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in photochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines[2].The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, resins, fatty acids, gums which are capable of producing definite physiological action on body[3]. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques[4]. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available[5]. Reactive oxygen species (ROS) are highly reactive molecules which may be both important mediators of some physiological functions and also potential prooxidants. Imbalance between ROS generation and antioxidant capacity induces a condition known as oxidative stress which may play a major role in the initiation and

progression of numerous pathologies including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes mellitus, hypertension and ischemia/reperfusion injury[6]. The potential damage caused by an excess of ROS is controlled by a series of antioxidant defence mechanisms and among them, a key protective role is played by the antioxidant enzymes gluthatione (GSH) peroxidase, superoxide dismutase (SOD) and GSH reductase[7]. Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage[8]. These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation[9,10].

Centella asiatica (L.) Urban (*Syn*. *Centella coriacea* Nannfd., *Hydrocotyle asiatica* L., *Hydrocotyle lunata* Lam., and *Trisanthus cochinchinensis* Lour.) is a tropical medicinal plant from Umbeliferae family native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar[11]. *C. asiatica*, commonly known as "Gotu kola, Asiatic pennywort, Indian pennywort, Indian water navelwort, wild violet, and tiger herb" in English, is a tropical plant, which has been also cultivated successfully due to its medical importance in some countries including Turkey, and it has a long history of utilization in ayurvedic and Chinese traditional medicines since centuries[12]. The leaves, which are edible, are in yellowish-green color, thin, alternate with long petioles, and quite characteristic reniform, orbicular, or oblong-elliptic shapes with seven veins[13]. Various chemical constituents are reported in *Centella asiatica* like asiaticoside, madecassoide, madecassicacid, asiatic acid, glucose, rhamnose, terpenoids, sitosterol, stigmasterol, fatty oils consist of glycerides of palmitic acid, stearic acid, linoleic acid, linolenic acid vitamins like ascorbic acid. It also contains calcium, iron, and phosphate[14, 15]. In addition to neuroprotective effect of *C. asiatica*, it has been reported to own a wide range of biological activities desired for human health such as wound healing, anti-inflammatory, antipsoriatic, antiulcer, hepatoprotective, anticonvulsant, sedative, immunostimulant, cardioprotective, antidiabetic, cytotoxic and antitumor, antiviral, antibacterial, insecticidal, antifungal, antioxidant, and for lepra and venous deficiency treatments[16]. Onion (*Allium cepa* L.), one of the most frequently consumed vegetables, is known to have many health benefits because of its flavonoids contents. Onion extracts can function as potent cardiovascular and anticancer agents that exert hypocholesterolemic, thrombolytic, and antioxidant effects[17-19]. Flavonols, which are primarily detected as glycosides of quercetin and kaempferol in onions, have been identified as major contributors to the antioxidative activities and health benefits[20,21]. Orange colored onion peel including several phenolic compounds, such as quercetin, gallic acid, ferulic acid, and kaempferol[22]. The major flavonoid in the onion peel is quercetin, which is present in one of its conjugated forms, as quercetin 4'-O-β- glycopyranoside, quercetin 3, 4'-O-β-diglycopyranoside, and quercetin 3, 7, 4'-O-β-triglycopyranoside[23]. The onion ranked the highest in quercetin content among 28 vegetables and 9 fruits[24], and its flavonoid compounds are mostly present in the onion peels at levels significantly higher than in the edible portion of the onion[25]. Green tea is made from leaves of *camellia sinensis* that have undergone minimal oxidation during processing. Green tea originates from china[26]. Over the last few decades green tea has been subjected to many scientific and medical studies to determine the extent of its long purported health benefits, with some evidence suggesting that regular tea drinkers may have a lower risk of developing heart disease[27] and certain types of cancer [28]. Although green tea does not raise the metabolic rate enough to produce immediate weight loss, green tea extract containing polyphenols and caffeine has been shown to reduce thermo genesis and stimulate fat oxidation, boosting the metabolic rate without increasing the heart rate[29]. Flavanoid are a group of phytochemicals present in most plant products that are responsible for health effects such as anti oxidative and anti carcinogenic functions[30]. Polyphenols found in tea are mostly flavanoid[31]. The polyphenols are large group of plant chemicals that include the catechins, are thought to be responsible for health benefits that have traditionally been attributed to tea, especially green tea[32]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and in vitro antioxidant activity of leaf of Green tea, Brahmi and bulb of Onion.

MATERIALS AND METHODS

Plant material

Plant materials *Allium cepa* (fresh bulb) and *Centella asiatica* (dried leaves) were collected from local market of District Haridwar and leaves of *Camellia sinensis* was collected from tea garden of Kokrajhar District Assam. The sample was identified by Emeritus scientist Dr. Sunita Garg, CSIR-NISCAIR New Delhli. A herbarium of plants was submitted to Raw Material Herbarium and Museum Delhi (RHMD). The specimen voucher no. of *Camellia sinensis* is NISCAIN/RHMD/consult/2017/3032-59, for *Centella asiatica* and *Allium cepa* 3023-50-1 and 50-2 respectively. The plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction by hot continuous percolation process

100 gm of each dried material were exhaustively extracted with methanol using hot continuous percolation for 24 hrs. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[33].

Qualitative analysis of phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in methanolic extracts of Green Tea, Brahmi and Onion. The above phytoconstituents were tested as per the standard method[34].

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in

plant extracts. For total phenolic content. Methanolic extracts of Green Tea, Brahmi and Onion plant material of subjected to estimate the presence of TPC by standard procedure.

Total phenolic content estimation

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Concentration of (10-50µg/ml) of gallic acid was prepared in methanol. Concentration of 100 µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10 fold dilute folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and it was then Incubated at room temperature for 30 mins with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was calculated by the standard regression curve of Gallic acid and the results were expressed as gallic acid equivalent (mg/g) [35].

Antioxidant activity

DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.,* 2006[36] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

RESULTS AND DISCUSSIONS

Phytochemical analysis of methanolic extracts of leaf of Green Tea, Brahmi and bulb of Onion showed the presence of carbohydrate, flavonoids, phenolics, tannin, saponins and result shown in Table 1.

Table 1 Result of phytochemical screening of green tea, brahmi and onion

Phytochemical compounds	Methanolic extract		
	Onion	Green Tea	Brahmi
Tannins	+ve	+ve	+ve
Saponins	+ve	-	-
Flavonoids	+ve	+ve	+ve
Glycosides	+ve	-	+ve
Terpenes	-	-	-
Alkaloids	+ve	+ve	+ve
Steroid	+ve	+ve	-
Phenols	-	+ve	+ve
Gallic acid.	-	+ve	-
Carbohydrate	+ve	+ve	+ve
Terpenoids	-	+ve	-
Anthraquinonne	-	-	+ve
Amino acids	+ve	-	+ve
Proteins	+ve	-	-

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of methanolic extract of leaf of Green tea, Brahmi and bulb of Onion showed the content values of 0.214 and 0.174 respectively Table 2 and Figure 1.

Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 25 µg/ml to 300µg/ml. A dose dependent activity with respect to concentration was observed Table 3.

Table 2 Total phenolic content of extracts

S. No.	Methanolic Extract	Total Phenolic Content
1.	Green Tea	195.26 ± 12.64
2.	Brahmi	136.92 ± 21.73
3.	Onion	112.23 ± 11.36

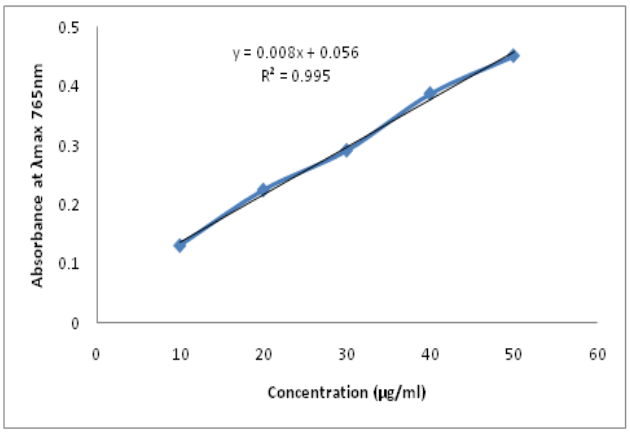

Fig 1 Graph of estimation of total phenolic content

Table 3 DPPH assay of ascorbic acid, methanolic extract

S. No.	Concentration (µg/ml)	Ascorbic Acid	Green Tea Extract	Brahmi Extract	Onion Extract
1.	25	61.85±2.13	58.36±0.41	42.11±2.59	35.46±1.26
2.	50	76.87±3.18	64.16±0.33	48.92±3.76	42.89±2.56
3.	100	78.95±2.72	71.94±0.56	57.44±2.44	45.52±3.15
4.	150	83.62±3.64	75.98±0.45	66.89±3.25	51.64±1.89
5.	200	89.35±1.47	81.23±0.35	75.13±2.25	63.95±2.67
6.	250	92.75±2.65	82.78±0.64	79.15±4.58	69.68±2.08
7.	300	93.12±2.11	86.25±2.54	81.62±1.32	75.12±2.45
8.	**IC 50**	20.05±1.86	55.98±2.56	104.45±3.13	144.37±5.45

CONCLUSION

Qualitative and quantitative analysis of phenolics from leaves extract of Green tea, Brahmi and bulb of Onion was achieved first time in this work. The observed level of phytoconstituents revealed that Green Tea, Brahmi and Onion is a rich source of antioxidant compounds. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant and others activity and to explore the existence of synergism if any, among the compounds.

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