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In-vitro antioxidant properties of *Ocimum tenuiflorum*, *Curcuma longa* and *Camellia sinensis*

Tuhin Miah[1], Jabin Sultana[1]*, Md. Forhad Uddin[2], Shah Jalal[3], Shuvo Singha[4], Tuli Dey[4], Sonnet Poddar[5]

[1]Department of Physiology, Biochemistry & Pharmacology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong – 4225, Bangladesh
[2]Department of Microbiology, University of Chittagong, Chittagong, Bangladesh
[3]Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong – 4225, Bangladesh
[4]Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong – 4225, Bangladesh
[5]Department of Anatomy and Histology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong – 4225, Bangladesh

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***Corresponding Author**

Jabin Sultana
✉ jabinsultana1010@gmail.com

ABSTRACT

In this study, the fractionated ethanolic extracts of dried leaves of *Ocimum tenuiflorum* (Tulsi), *Camellia sinensis* (Tea) and dried stem of *Curcuma longa* (Halud) were evaluated for the antioxidant activity or free radical scavenging activity. This was achieved by screening the leaves and its extracts for estimating free radical scavenging properties using ascorbic acid as standard antioxidant. Total phenolic content was estimated in leaves extracts showing more antiradical activity in tea leaves (72.22%) is higher in average than in Tulsi (44.10%) and Halud (62.15%) where 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was assayed. Quantification of ascorbic acid showed 95.83% - 100% antiradical activities which were assumed as a standard for antioxidant properties in the present study. Upon further fractionation, the highest average levels of DPPH radical scavenging activities was found in the Tea leaves and the lowest level was found in the Tulsi leaves. All three plants Tulsi, Halud and Tea leaves would exert several beneficial effects by virtue of their antioxidant activity and could be hardnosed as drug formulation.

INTRODUCTION

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants protect the human body from free radicals and ROS (Reactive Oxygen Species) effects (Yan and Asmah 2010; Miliauskas et al., 2007). They retard the progress of many chronic diseases as well as lipid peroxidation. They have been widely used as food additives to provide protection against oxidative degradation of foods (Gulcin et al., 2005). The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counter act the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer (Kinnula and Carpo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000), mild cognitive impairment (Guidi et al., 2006), Parkinson's disease (Bolton et al., 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis, aging (Hyun et al., 2006) and atherosclerosis (Upston et al., 2003). All aerobic organisms have antioxidant defense systems to offset harmful effects caused by free radicals. In the case of failure of the antioxidant defense system, antioxidants need to be supplemented from outside sources. An organism's metabolism fights against oxidative effects with its own antioxidant defense systems. Elimination and neutralization of reactive oxygen

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species is handled by both enzymatic and non-enzymatic antioxidant mechanisms. In practice, total antioxidant status (TAS) represents all of these compounds. Antioxidants can be found naturally in foods (Kedare and Singh, 2011). A majority of antioxidants naturally present in foods occur in phenolic structures and especially in flavonoid structures. In addition, antioxidants are added to nutrients to prevent deterioration intheir taste, smell, and color. Butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and propyl gallate (PG) can be included in this group, which are known as synthetic antioxidants (Koksal and Gulcin, 2008).Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.). Generally in vitro antioxidant tests using free radical traps are relatively straight forward to perform. A rapid, simple and inexpensive method to measure antioxidant capacity of any substances the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Chanda and Dave, 2009; Badarinath et al., 2010) So far, very limited research was found with a view to explore the antioxidant activities of mostly available plant ingredients used commonly for some remedies to meet benefit both human and domesticated animals in Bangladesh. Here, in this study, attempts have been taken to include in vitro method to analyze the antioxidant activity of three plants *Ocimum tenuiflorum* (Tulsi), *Curcuma longa* (Halud) and *Camellia sinensis* (Tea leaves) in compare to ascorbic acid.

MATERIAL AND METHODS

Sample, area and duration

Leaves and stem sample from 3 plants - *Ocimumtenuiflorum* (Tulsi), *Curcuma longa* (Halud) and *Camellia sinensis* (Tea leaves) were selected to perform the study. Five hundred grams (500 gm) of each sample were obtained from the plants at March 20, 2017 from Saghata Upazilla under Gaibandha district in Rangpur Division. Undesirable leaves were removed and samples were dried. The leaves were taken in a polybag and air tighten to ensure presence of no moisture within container and transported to laboratory of Department of Physiology, Biochemistry & Pharmacology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong – 4225, Bangladesh.

Preparation of extracts

Dried samples (at room temperature; 25±2ºC) were powdered using mortar and pestle. Fifty grams (15gm) powder of *Ocimum tenuiflorum*, 20gm of powdered *Curcuma longa* were placed in amber color bottles and added ethanol 10 times more than samples and then kept these in dark place. After filtering, sample's solution was extracted by using rotary evaporator at 170 rpm for 4 – 5 hours at 50°C water temperature. About 20 gm powder of *Camellia sinensis* was mixed with 200 ml distilled water and boiled for 2 hours at 80ºC using electric heater and filtered. The extraction was then made moisture free using rotary evaporator at 170 rpm for 4 – 5 hours at 80ºC.

In-vitro antioxidant activity

Molecule 1, 1-diphenyl-2-picrylhydrazyl (1, 1-diphenyl-2-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole. Delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. Phenolic compounds and polyphenols are the most abundant structures in plants. Determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts.

Preparation of standard solution and test sample

Required quantity of Ascorbic acid was dissolved in ethanol to prepare the concentration of 5mg/5 ml or 1mg/ml or 1µg/µl. Stock solutions (plant extracts) of samples were prepared by dissolving 5mg of dried ethanolic extract in 5ml of ethanol to give concentration of 1mg/ml. 4.3 mg of DPPH was dissolved in 3.3 ml of ethanol. It was protected from light by covering the test tube with aluminum foil.

Absorbance was taken at 516 nm in UV-visible spectrophotometer after 30 minutes using ethanol as a blank. 150µl DPPH solution was added to 3ml of ethanol and absorbance was taken immediately at 516nm for control reading. 100µl, 200µl, 400µl and 800µl of Ascorbic acids were dissolved separately in each 3ml of ethanol test tube and then added 150µl of DPPH solution in each test tube and incubated for 30 minutes. Absorbance was taken at 516 nm in UV-spectrophotometer after incubation. 100µl, 200µl, 400µl and 800µl of test samples were dissolved separately in each 3ml of ethanol test tube and added 150µl of DPPH solution in each tube and incubated for 30 minutes. Absorbance was measured at 516 nm. The percent reduction and IC_{50} were calculated as follows. The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\% \text{ Antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Each experiment was carried out in triplicate and results are expressed as mean percent antiradical activity.

RESULT AND DISCUSSION

Percentage of antiradical activity of Ascorbic acid, Tulsi, Halud and Tea leave are shown in (Table 1)

Table 1
Concentration and percentage of antiradical activity of Ascorbic acid, Tulsi, Halud and Tea leave.

Variables	Concentration (1µg/µl)	% antiradical activity	
Ascorbic acid	100µl	95.83	97.92
	200µl	97.22	
	400µl	98.61	
	800µl	100	
Tulsi	100µl	29.16	44.10
	200µl	41.67	
	400µl	44.44	
	800µl	61.11	
Halud	100µl	13.89	62.15
	200µl	72.22	
	400µl	80.55	
	800µl	81.94	
Tea leaves	100µl	61.11	72.22
	200µl	73.61	
	400µl	76.39	
	800µl	77.78	

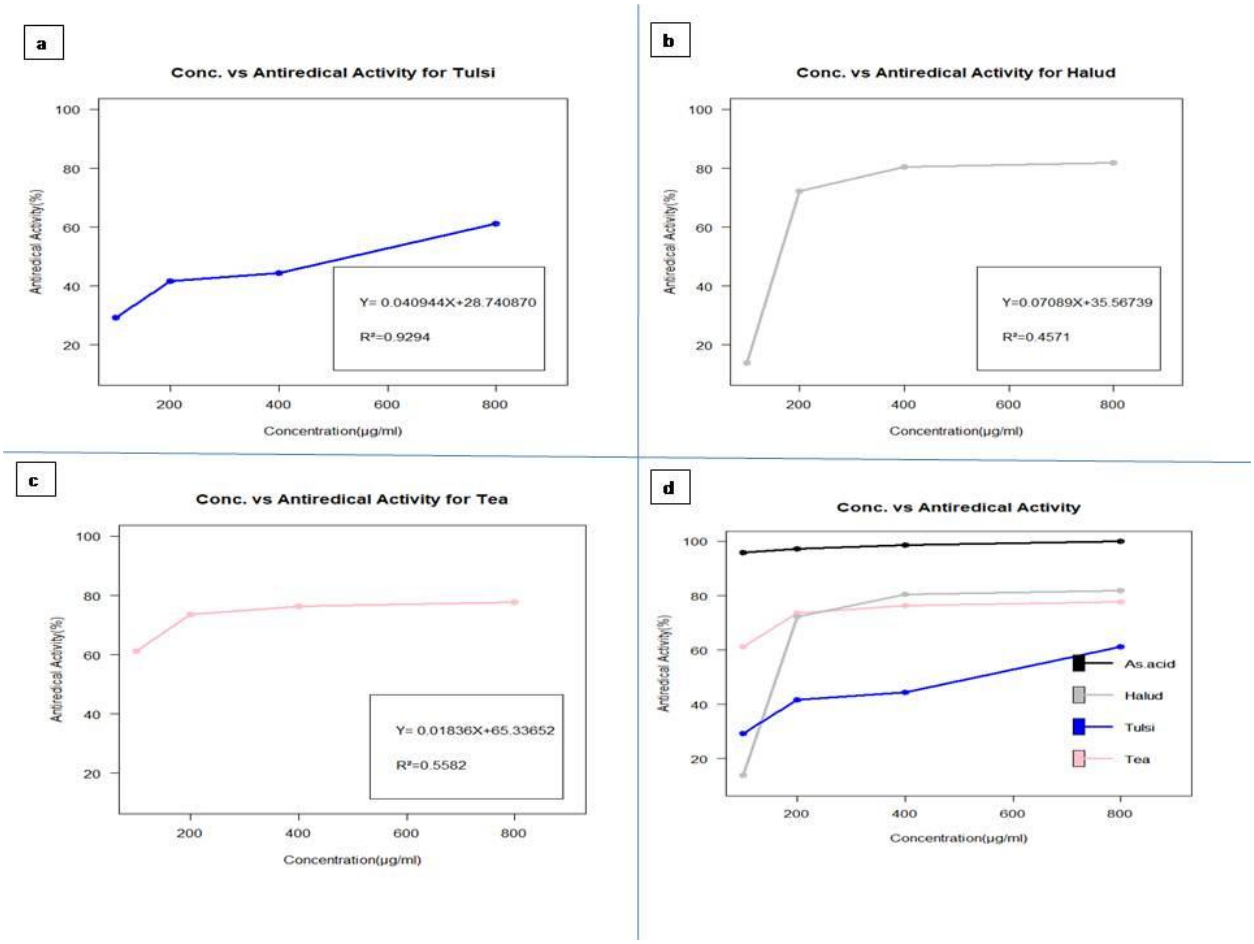

Figure 1
a. Concentration VS Antiradical activity for Tulsi, b. Concentration VS Antiradical activity for Halud, c. Concentration VS Antiradical activity for Tea leaves and d. Concentration VS Antiradical activity for comparison

Ascorbic acid is supposed to have 100% antioxidant property. So, the antioxidant properties of the extracts used in the study may be estimated in comparison with the value of Ascorbic Acid.

The highest and lowest % Antiradical activity of Ascorbic acid is 100% and 95.83% in respect of the value of the concentration at 800µl and 100µl. The highest and lowest % Antiradical activity of Tulsi were 61.11% and 29.16% in respect of the value of the concentration at 800µl and 100µl. The highest and lowest % Antiradical activity of Halud is 81.94% and 13.89% in respect of the value of the concentration at 800µl and 100µl. The highest and lowest % Antiradical activity of Tea is 77.78% and 61.11% in respect of the value of the concentration at 800µl and 100µl. Increase scavenging of DPPH radicals in dose dependent manner due to the scavenging ability of these ethanolic extracts were illustrated in (Figure 1).

Ocimum tenuiflorum showed high inhibition of DPPH activity of 61.11% and 29.16% at 800µl and 100µl concentration of leaf extract which differs according to Balaji et al., (2011) found *Ocimum tenuiflorum* (Tulsi) extract was the most potent scavenger (81.1%) while *O. americanum, O .minimum, O. citriodorum, O. kilimandscharicum, O. grandiflorum, O. lamiifolium and O. selloi* had significantly lower scavenger activity as 77.4%, 70.1%, 60.6% 56.2%, 51.3%, 46.2% and 42.4% respectively. It also differs from the study of Goretti et al., (2008).

Curcuma longa (Halud) also showed marked inhibition of DPPH activity shows that 81.94% and 13.89% in respect of the value of the concentration at 800µl and 100µl in stem extract which also shows some dissimilarity with an article reported by Tanvir et al., (2017) that the ethanolic extract of *Curcuma longa* (Halud) contained the highest concentrations of polyphenols (16.07%), flavonoids (9.66%) and ascorbic acid (0.09 mg/100 g) resulted in high yields (17.39%). Our findings strongly suggest that the Halud (Halud) varieties from Bangladesh are promising sources of natural antioxidants by their considerable DPPH free radical-scavenging activities (Hasan and Mahmud, 2014; Tanvir et al., 2017).

Camellia sinensis (Tea leaves) was identified in the study as highest average inhibition of DPPH activity that was reflected from 77.78% and 61.11% in respect of the value of the concentration at 800µl and 100µl with the average value of 72.22% which was dissimilar with the study of (Izzreen and Fadzelly, 2013; Chan et al., 2007). The cause of the variation may be due to ethanolic extracts were found with the highest frequency for antioxidant study in the present study but in the referral research works some other methods were used to draw a conclusion of antiradical activities of the plant extracts. DPPH is the most easy, simple and reasonably costly method to estimate the antiradical activities but it doesn't ensure maximum precision and the samples were not maximum to conclude the result as fruitful for further advanced research works relation to the present study.

CONCLUSION

The present study of *Ocimum tenuiflorum*, *Curcuma longa* and *Camellia sinensis* might be useful to supplement information in regarding to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs. The DPPH free radical scavenging activity of ethanolic extract was determined which show significant in-vitro antioxidant activity in a higher dose than standard antioxidant. Further extensive studies are recommended with maximum sample of extracts as molecular identification of antiradical activity analysis to make the research fruitful.

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