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# Phenolic Content and Antioxidant Properties of Herbal Sandesh

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**A B S T R A C T**

Natural sources of antioxidant viz. basil (*Ocimum sanctum*), coriander (*Coriandrum sativum*), pudina (*Mentha arvensis*) have been used to fortify sandesh, in the form of herbal juice @ 4%, 5%, 5% (w/v basis of chhana) level individually into sandesh, a heat-acid coagulated product which is analogous to cottage cheese. Incorporation of herbs at this level significantly ($p<0.01$) affect the total polyphenol content and antioxidant level of sandesh. Herbal Sandesh (Pudina) showed the highest antioxidant level as compared to other laboratory made Herbal Sandesh. Considering the findings it can be concluded that pudina (mint) might constitute a rich and novel source of natural antioxidants and may be suggested as a new potential source of natural antioxidant and could be used as food additive.

## Introduction

The term phytochemical is used to refer chemical compounds that occur naturally in plants (phyto means plant in Greek), chemicals that may have biological significance but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals with the potential to affect diseases such as cancer, stroke, or metabolic syndrome. Plants need amino acids, sugars, organic acids, etc., for primary metabolism or their development. In addition, all higher plants produce one or several representatives, called as secondary metabolites, which are not essential for a plant for its metabolism (Wink, 2003).

Phyto constituents are the natural bioactive compounds found in plants. These phyto constituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions (Koche *et al.,* 2010). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids,

tannins, and many others (Nonita *et al.*, 2010).

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases (Shahidi, 1997). It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996).

Consumers now prefer food products that are natural, non-thermally processed and have an acceptable shelf life with assured quality and safety. This has necessitated using natural substances from various sources as natural preservatives which have capacity to inhibit microbial growth, ability to inactivate microorganisms in food and promote the growth of desirable microorganism without adversely affecting most of their nutritional and organoleptic properties.

These products have attained more demand in the markets as they along with the common nutritional attributes claim many health benefits, like anti-cholesteromic, antistress, antirheumatic, anthelmintic, anti-inflamatory, anticarcinogenic, antibacterial properties etc.

Thus many herbs and spices that have been implicated by research to impart such attributes become ideal for use (Dharmani and Palit, 2006).

In the present study Sandesh, a chhana-based (heat-acid coagulated product) popular sweet delicacy of the eastern part of India, especially West Bengal is prepared with fortification of different types of herbs juice (Tulsi juice @ 4% or Pudina juice @ 5% or Coriander juice @ 5%).

Methanolic extracts of tulsi, pudina, coriander and their corresponding fortified Sandesh were investigated for their phenolic content and antioxidant properties.

## Materials and Methods

### Raw material

Authentic pooled samples of raw cow milk was procured from a herd situated near the Mohanpur campus of the West Bengal University of Animal and Fishery Sciences, West Bengal and was standardized at Solids not fat (SNF) to fat ratio of 1 : 8. Fine crystalline cane sugar, Tulsi, Pudina and Coriander herbs were purchased from the local market. Other chemicals used were of Analytical Grade.

### Preparation of herbs juice

Herbs (Tulsi, Pudina and Coriander) were washed thoroughly; roots were removed by using a sharp knife and air dried at $40^0$C for 5 minutes (min) in tray drier.

After total air drying herbs were ground. The juice was obtained by pressing the grinded herbs through a muslin cloth. The juices thus obtained were kept in three separate sterile containers and placed in refrigerator at $7^0$C till use.

### Preparation of chhana

The method outlined by De and Ray (1954) was followed with suitable modification given by Bhattacharya *et al.*, 1971. The standardized cow milk was heated up to 75$^o$C. The freshly prepared coagulant (1% citric acid solution) was heated to 75$^o$C and then added slowly in a thin continuous stream with continuous gentle agitation till clear whey separated out.

Stirring was then stopped and the coagulum was allowed to remain in whey for about 5

min. It was then drained through a hang with muslin cloth (10 min) and then used for preparation of herbal sandesh.

## Preparation of Herbal Sandesh

The chhana (milk casein) was kneaded for 30 min. Then it was properly mixed with previously grounded sugar (at 30% level of chhana) and divided into three equal lots. One lot of total chhana was taken in a karahi and cooked for 15 min in low flame with continuous stirring and scrapping until a thick consistency was obtained.

The second lot of chhana was then added and re-cooked for 15 min. After that the last lot of chhana was added and cooking for 5 min. until the final pat formation stage appeared. After that herbs juice (for herbal sandesh preparation) was added (Tulsi juice @ 4% or Pudina juice @ 5% or Coriander juice @ 5%). Then the mixture was moulded in different desired shapes, packed and stored at $7\pm1^{0}$C (in Free Radical Scavenging Activity refrigerator).

Free radical scavenging activity was determined by ABTS method (Re *et al.*, 1999; Hern´andez-Ledesma *et al.*, 2005). The results were expressed as trolox equivalent antioxidant capacity (TEAC) values *i.e.* mmol of Trolox equivalence/ 100 gm.

## DPPH radical scavenging activity

Radical scavenging activity against stable 2, 2 diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams *et al.*, 1995.

## Total phenolics estimation

The total phenolics of all extract were measured at 765nm by Folin Ciocalteau reagent (McDonald *et al.*, 2001) and expressed in terms of gallic acid equivalent (mg/g of dry mass),which is a common reference compound.

## Statistical analysis

The data were statistically analyzed using statistical tool ANOVA described by Snedecor and Cochran (1968).

## Results and Discussion

There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants. The extracts of *Ocimum sanctum, Mentha arvensis and Coriandrum sativum* exerted good antioxidant activity.

## Total phenolic compounds present in herbs and their corresponding herbal sandesh

The total phenolic contents in methanolic extracts obtained from tulsi, pudina and coriander leaves extract are shown in figure 2 (a) and table 1. Standard curve for gallic acid is shown in figure 3. The highest contents (28.33 mg GAE/100gm) were observed in extract of pudina (*Mentha arvensis*) followed by tulsi (*Ocimum sanctum*) (21.75 mg GAE/100gm) and coriander (*Coriandrum sativum*) (19.46mg GAE/100gm). The herbal sandesh prepared by the above mentioned herbs also exerted good phenolic content with the highest value observed in Herbal Sandesh (5% pudina extract) 0.835 mg GAE/100gm followed by 0.812, 0.799 and 0.582 mg GAE/100gm for Herbal Sandesh (4% tulsi extract), Herbal Sandesh (5% coriander extract) and control sandesh respectively. All the values differ significantly at $p<0.01$. Triantaphyllou *et al.*, (2001) reported that the extracts of Mentha species contained bound

phenolic acids and flavonoids. The major phenolic acids reported in water-soluble *Mentha spicata* extract are eriocitrin, luteolin glucoside, rosmarinic acid and caffeic acid (Dorman *et al.,* 2003). Phenolic compounds present in these extracts are reported to have beneficial effects on chronic diseases such as coronary heart disease (Forester and Waterhouse, 2009). These health effects are reported to be due to antiradical and antioxidant properties of phenolics in plants and plant derivatives (Lurton, 2003).

## Free radical scavenging activity of herbs and their corresponding herbal sandesh by DPPH method

The antiradical activities of herbal extracts and their corresponding herbal sandesh were assessed using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay. The results of the assay for antioxidant activity are shown in table 1 and figure 2(b).

The highest free radical scavenging activity (72.2%) was observed for the extract of pudina leaves followed by that of tulsi and coriander leaves extracts (67.4% and 26.41% respectively). The antioxidant activity of pudina leaves was found to be 7.12% and 173% higher than tulsi and coriander leaves.

In case of different types of herbal sandesh the assay for antioxidant activity are shown in table 1 which revealed that herbal sandesh (5% Pudina extract) showed the highest free radical scavenging activity (1.85%).

**Table.1** Total polyphenol content and antioxidant level of different types of herbs and herbal Sandesh

| **Herbs** | **Total phenolic content (mg GAE/gm) (avg±sd)** | **% DPPH scavenging (Extract concentration 50µg/ml) (avg±sd)** | **TEAC (mmol of Trolox/ 100 g) (avg±sd)** |
|---|---|---|---|
| Pudina (Mint) | 28.33±0.78 | 72.2 ± 0.50 | 31.26± 0.16 |
| Tulsi (Basil) | 21.75±0.65 | 67.4±0.35 | 25.58± 0.14 |
| Coriander | 19.46±0.32 | 26.41± 0.68 | 7.82± 0.04 |
| | | | |
| Level of Significance | * | * | * |
| | | | |
| **Herbal Sandesh** | **Total phenolic content (mg GAE/gm) (avg±sd)** | **% DPPH scavenging (Extract concentration 50µg/ml) (avg±sd)** | **TEAC (mmol of Trolox/ 100 g) (avg±sd)** |
| Herbal Sandesh (5% Pudina extract) | 0.835±0.027 | 1.85± 0.042 | 2.99± 0.03 |
| Herbal Sandesh (4% Tulsi extract) | 0.812±0.026 | 1.36± 0.025 | 2.84± 0.011 |
| Herbal Sandesh (5% Coriander extract) | 0.799±0.023 | 0.92± 0.014 | 1.61± 0.025 |
| Control Sandesh | 0.582±0.011 | 0.34± 0.012 | 1.24± 0.014 |
| | | | |
| Level of Significance | * | * | * |
| | | | |

(avg ± sd)= Average ± standard deviation, n=3; * Significant at p<0.01

**Fig.1 (a-c)** Total polyphenol content and antioxidant level of different types of herbs

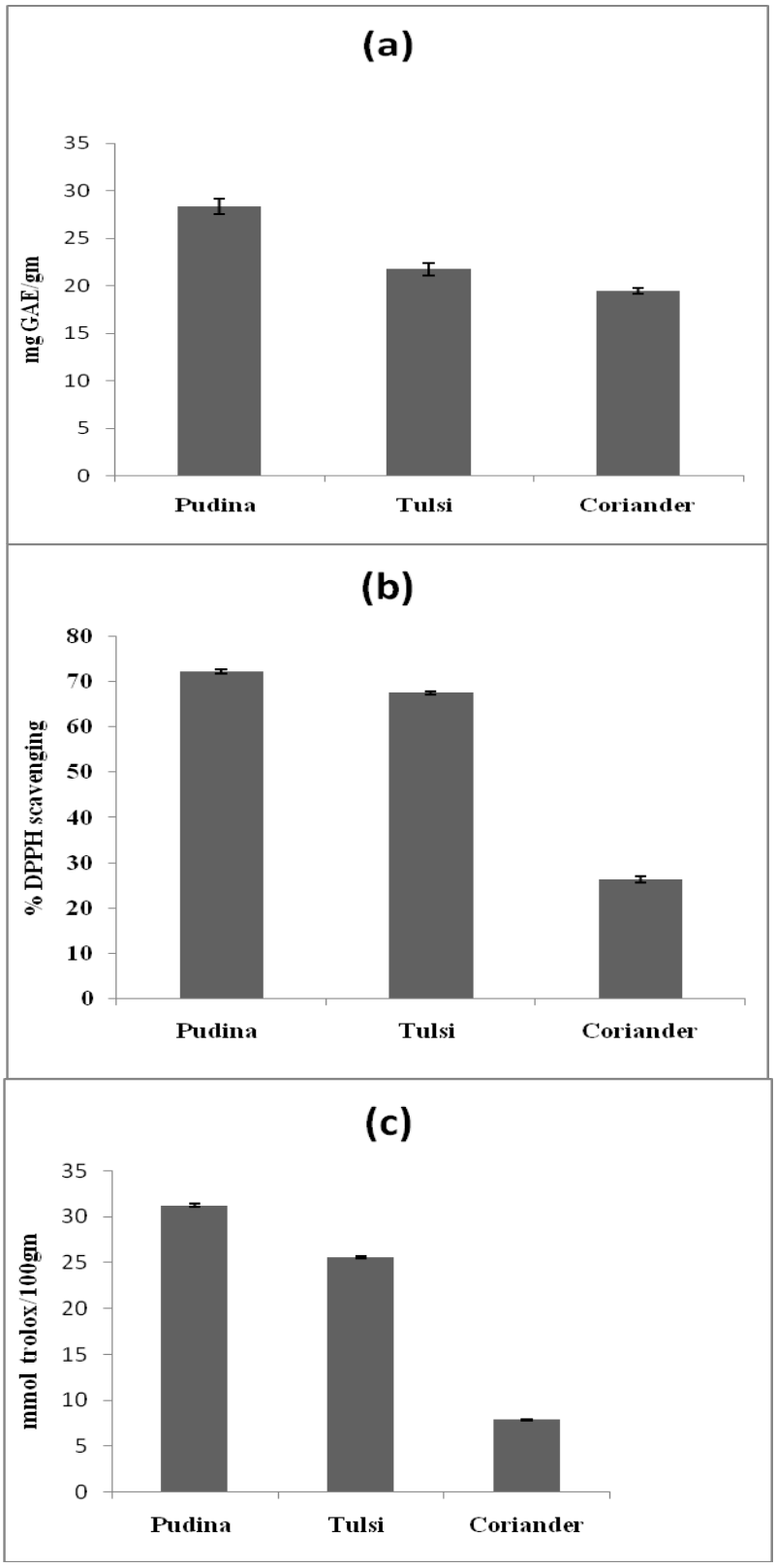


**Fig.2 (d-f)** Total polyphenol content and antioxidant level of different types of herbal Sandesh

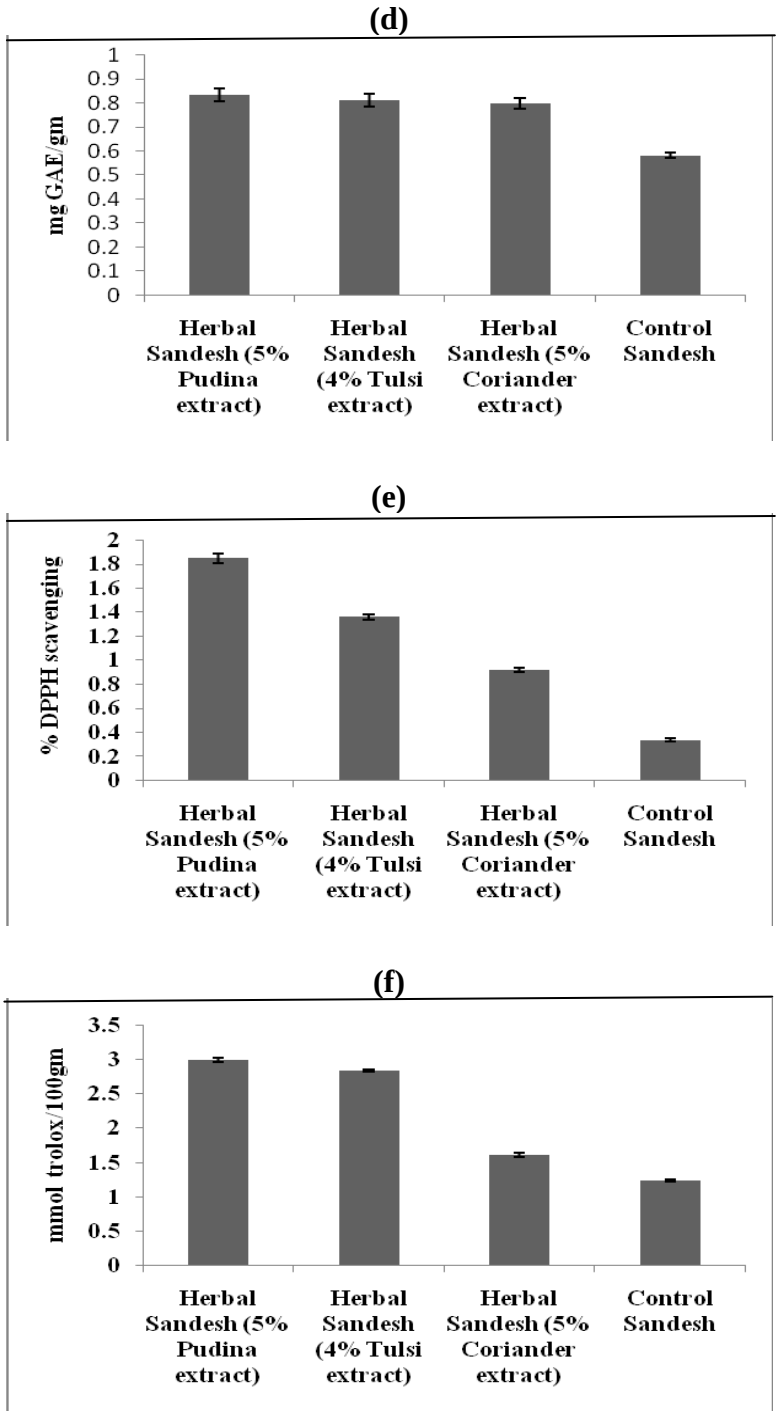
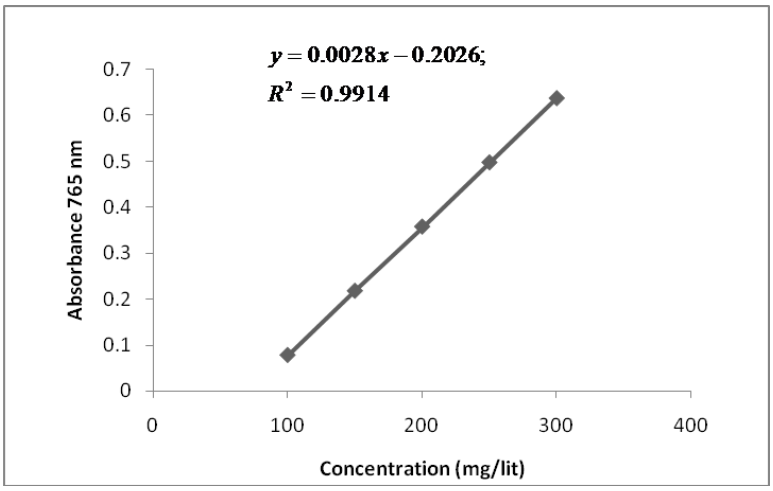


**Fig.3** Standard curve for gallic acid




The antioxidant activity of Herbal Sandesh (5% pudina extract) was found to be 36%, 101% and 444% higher than Herbal Sandesh (4% tulsi extract), Herbal Sandesh (5% coriander extract) and control sandesh. All the values differ significantly at $p<0.01$.

Albano and Miguel (2011) found that the total phenols and antioxidant activities, especially DPPH scavenging activities of herbs from *Lamiaceae* were higher than those from *Apiaceae*. They also observed that the extracts of these herbs showed good anti-inflammatory activities such that they were able to inhibit 5-lipoxygenase. Similar differences in total phenols among these two families were also documented by Hinneburg *et al.,* (2006). There was a correlation in the antioxidant activities and total phenolics, more evident in case of herbal leaves' extract. Such correlations have also been observed in other studies (Ghafoor and Choi, 2009; Lim *et al.,* 2010).

## Studies on the total antioxidant level of herbs and their corresponding herbal sandesh samples by ABTS method

Table 1 and figure 1(c) showed the total antioxidant capacity of herbs and their corresponding herbal sandesh samples based on the capture of the $ABTS^{\bullet+}$ radical, and higher values of mmol Trolox/100 g also correspond to higher antioxidant capacities. Table 1 showed the antioxidant capacity of the different types of herbs extracts as measured using the ABTS method. The test gave a value of 31.26 mmol Trolox/100 g for the pudina extract, a result significantly higher ($p<0.01$) than the values found for tulsi and coriander extracts (25.58 and 7.82 mmol Trolox/100 g respectively).

From table 1 it has been observed that Herbal Sandesh (5% pudina extract) had the highest antioxidant level (2.99 mmol of Trolox/ 100 g) as compared to other laboratory made herbal sandesh samples.

The antioxidant level (2.84 mmol of Trolox/ 100 g) of Herbal Sandesh (4% tulsi extract) was quite close to Herbal Sandesh (5% pudina extract) whereas Herbal Sandesh (5% coriander) showed lower antioxidant content (1.61 mmol of Trolox/ 100 g) than other herbal sandesh. The lowest antioxidant content was observed for control sandesh

(1.24 mmol of trolox/ 100 g). All the values differ significantly at $p<0.01$.

The effect of addition of natural antioxidant in sandesh was also observed by Bandyopadhyay *et al.,* 2007. They found that addition of beet or mint alone in sandesh showed lower antioxidant level than the addition of ginger alone. However, combination of beet with ginger showed highest antioxidant level among the natural sources. It was also observed by them that among the four forms of herbs such as paste, tray-dried powder, freeze-dried powder and solvent extracted form, addition of solvent extracted form in sandesh showed highest antioxidant level than any other form.

In conclusion, the work provided a better understanding of desired antioxidant properties imparted by the herbs to the Herbal Sandesh. All the herbs that were analyzed have high antioxidant contents. Although herbs contribute little weight on the dinner plate, they may still be important contributors to our antioxidant intake, especially in dietary cultures where herbs are used regularly.

The Herbal Sandesh were prepared by standard procedure with incorporation of tulsi extract (4%), coriander extract (5%) and pudina extract (5%) extract. Antioxidant activity is measured in terms of ABTS and DPPH radical scavenging method and the best sandesh sample incorporated with 5% pudina extract showed exceptional result as compared to other samples. A good correlation between the antioxidant activities determined by DPPH and ABTS methods versus total phenol content (Folin Method) was observed for all the samples in this study. It has been suggested that the antioxidant capacity of herbs and their corresponding herbal sandesh samples are strongly correlated to the type of phenolic compounds present in them. This points out the predominant role of phenols in antioxidant activity. The evidence from this study suggests that herbs additives in Herbal Sandesh increases the acceptability of sandesh.

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