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Analysis of antioxidant activity of herbal yoghurt prepared from different milk

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Abstract
In the present study different levels of (*Zingiber officinale)* and *(beta vulgaris)* was used to produce the herbal yoghurt of cow, buffalo and goat milks. The aim of the study was to find the antioxidant activities of the herbal yoghurt samples. The samples were analysed by using 2,2 diphenyl-1 picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP). The result indicated that highest antioxidant activities with DPPH & FRAP methods were found in 2% level of ginger rhizome goat milk yoghurt and 2% level of beet root extracts of goat milk yoghurt followed by 2% level of ginger extract in cow milk yoghurt the lowest antioxidant activity were found in buffalo milk herbal yoghurt. The yoghurt was prepared by this method is subjected to sensory properties of herbal yoghurt. Thus it was concluded that yoghurt prepared by this method have high antioxidant properties which are beneficial for human health.

Keywords*:* Antioxidant activity, DPPH, FRAP, antidiabetic, antimicrobial

1. Introduction
Yoghurt is among the most popular fermented milk products consumed all over the world because of its excellent sensory properties, as well as high nutritive and therapeutic values. It is offered in a variety of types concerning fat and total solids content, the form of the body (drinking, set-style, frozen, concentrated), with or without additives, probiotic microflora and in different flavours [Grochulska 2008]. Yoghurts may also be fortified with bioactive components like: caffeine, guarana, green tea extract, coenzyme Q10, ginseng, aloe vera, cranberry, fibre [Stankiewicz 2009]. The most popular flavourings for yoghurts are of fruit types. Among non-traditional additives also vegetable powders, pulps and natural extracts obtained from row vegetable vegetables have been used in the production of fermented milk products [Tamime and Robinson 1999]. Vegetables present a valuable source of nutrients and are also low in calories. They are rich in dietary fibre, minerals as well as many bioactive compounds, such as antioxidants, e.g. carotenoids, ascorbic acid, tocopherols, phenolic substances [Czapski 2001]. Biological antioxidants are substances which in low concentration are able to delay or prevent the oxidative damage of various bio molecules connected with various diseases including cancer, liver disease, Alzheimer's disease, aging, arthritis, inflammation, diabetes, Parkinson's disease, atherosclerosis and AIDS [Moon and Shibamoto 2009]. Increased fruit and vegetable consumption is an effective strategy to increase antioxidant intake and may help to prevent developing chronic diseases, especially cancer and cardiovascular disease [Song *et al.* 2010]. Supplementation of yoghurt with selected vegetables will provide additional health properties, especially concerning antioxidant properties and will result in the development of novel functional dairy products.
The incorporation of beetroot juice into goat milk yogurt can enhance nutritional value of the yogurt in addition to the health benefits. [Damunupola *et al.* 2014].
The aim of the present study was to produce yoghurts with the addition of different levels of extracts of ginger rhizome & beet root (0.5%, 1%, 1.5%, 2%) preparations and to determine the effect of selected herbs on the antioxidant capacity and organoleptic acceptance of the obtained fermented milk products.

2. Material and Method
2.1 Yoghurt preparation
Fresh cow, buffalo, and goat milk were used for preparation of yoghurt. Milk composition was adjusted to achieve the desired fat and solids content (4%fat and 10% snf). Dry milk was added to increase the amount of whey protein to provide a desirable texture.

The milk mixture was pasteurized at 185 °F (85 °C) for 30 minutes or at 203 °F (95 °C) for 10 minutes. The milk was cooled to 108 °F (42 °C) to bring the yogurt to the ideal growth temperature for the starter culture. The starter cultures (*L. bulgaricus, S. thermophillus*) were mixed into the cooled milk. Ginger rhizome and beet root extracts in different concentrations of 0.5%, 1 %, 1.5%, 2% were added and stirred The milks were hold at 108 °F (42 °C) until a pH 4.5 is reached. This allows the fermentation to progress to form a soft gel and the characteristic flavour of yogurt. This process can take several hours. The yogurt was cooled to 7 °C to stop the fermentation process and stored at ambient temperature.

2.2 Preparation Of extracts

The ground herbs were suspended in distilled water (dH2O) in the ratio 1:10. The mixture was left to incubate overnight in a water bath (70 °C) followed by centrifugation (15 minutes, 2000 rpm at 4 °C). The supernatant (water herbal extract) was used in the experiments.

2.3 Antioxidant activity

DPPH Assay

Antioxidant activity of herbal yoghurt was determined using stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described by Brand-Williams *et al*. (1995) [3]. Antioxidant activity analyzed by Diphenylpicrylhydrazyl (DPPH) radical scavenging activity was determined method is based on the ability of the antioxidant to scavenge the DPPH cation radical. The hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple colored MeOH solution of DPPH. This spectrophotometric assay uses stable radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) as a reagent. Briefly, 0.1 ml of sample extract or standard was added to 5 ml of DPPH reagent (0.00039 gm in 1 liter methanol) and vortexed vigorously. The reaction tubes were incubated in dark for 30 min, at room temperature and the discolouration of DPPH was measured against a reagent blank at 517 nm. Percentage inhibition of the discolouration of DPPH by the sample was expressed as gallic acid equivalents.

2.4 Calculation

All values obtained are acquired from UV spectrophotometer/colorimeter for assays.

 % Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

2.5 FRAP

The Ferric reducing antioxidant power (FRAP) test was conducted according to the method Described by Benzie andStrain (1996) [2].

2.6 Procedure

Micro liters of sample and 100 µl of standard were taken in different two tubes. 3 ml of FRAP reagent was added. Absorbance at 593 nm was measured at 0 minutes after vortexed. Samples were then placed at 37 ºC in water bath and absorption was again measured after 4 minutes. Ascorbic acid standards (100 µM-1000 µM) were to be processed in the same way. The content of above tubes was mixed well. OD of the Standard and Test were measured at Zero minute and again after four minutes at 593 nm.

3. Result

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. These free radicals are the major points in lipid peroxidation. The antioxidants may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions. Several synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially available but are quite unsafe and their toxicity is a problem of concern. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive which are capable of absorb and neutralize free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Recently focus has been concentrated on identification of plants with antioxidant ability that may be used for human consumption (Raushan Kumar, *et al*,. 2013). The herbal yoghurt prepared by different concentration of ginger rhizome and beet root extracts 0.5%,1%, 1.5%, and 2% respectively. The maximum % antioxidant activity of herbal yoghurt was found in 2% level of herb ginger rhizome extracts in goat milk yoghurt (68.869±0.02) followed by 1.5% of ginger extract in goat milk yoghurt (54.089 ± buffalo milk yoghurt (56.112±0.05), 1.5% of ginger rhizome extract in buffalo milk yoghurt(56.04±0,013), 2% of beet root extract in goat milk yoghurt (46.707±0.304), 2% of beet root extract in buffalo milk yoghurt (46.495±0.016), 2 % of ginger extract in cow milk yogurt (42.404±0.031), 2% of beet root extracts in cow milk yoghurt (40.104±0.101).The minimum concentration (i.e.0 .5% and 1%) of natural herb extracts of ginger and beet root based yoghurt was found minimum antioxidant power.

Ferric reducing antioxidant power analyzed of different level herbs powder used in herbal ice cream gives high value which was confirmed by method used for the FRAP assay. The maximum Ferric reducing antioxidant power of herbal yoghurt were found maximum in 2% ginger rhizome extract in goat milk (1.0876 ±0.023), followed by 1.5% of ginger extract in goat milk (1.036±0.04), 2% ginger rhizome extract in cow milk yoghurt (1.0354±0.023), 2% beet root extract of goat milk yoghurt. The minimum concentration (i.e. 0.5% and 1%) of natural herbal extract based yoghurt was found minimum antioxidant power.

Table 1: Table for %DPPH radical scavenging activity and ferric reducing antioxidant power of different levels of beet root extracts

S.N	Treatment combinations	Level of herbs	% antiradical activity	FRAP (µM)
1	$T_0/B_0/G_O$		0.00	0.00
2	T_cBr_1	0.5%	16.60±0.02	0.519±0.03
3	T_cBr_2	1%	30.46±0.016	0.622±0.015
4	T_cBr_3	1.5%	36.8±0.018	0.880±0.045
5	T_cBr_4	2%	40.10±0.015	0.889±0.053
6	T_bBr_1	0.5%	20.620±0.113	0.390±0.022
7	T_bBr_2	1%	35.42±0.023	0.467±0.065
8	T_bBr_3	1.5%	37.01±0.013	0.570±0.03
9	T_bBr_4	2%	46.49±0.016	0.587±0.012
10	T_gBr_1	0.5%	30.51±0.042	0.77±0.05
11	T_gBr_2	1%	62.08±0.014	0.847±0.05
12	T_gBr_3	1.5%	32.203±0.04	0.897±0.033
13	T_gBr_4	1.5%	46.702±0.034	0.940±0.03

Table 2: Table for %DPPH radical scavenging activity and ferric reducing antioxidant power of different level of ginger rhizome

S.N	Treatment combinations	Level of herbs	% antiradical activity	FRAP (µM)
1	$T_0/B_0/G_O$		0.00	0.00
2	T_cG_1	0.5%	25.51±0.03	0.674±0.13
3	T_cG_2	1%	31.39±0.01	0.803±0.083
4	T_cG_3	1.5%	35.64±0.011	0.900±0.102
5	T_cG_4	2%	42.40±0.031	1.03±0.001
6	T_bG_1	0.5%	14.80±0.03	0.416±0.031
7	T_bG_2	1%	35.42±0.05	0.519±0.023
8	T_bG_3	1.5%	56.04±0.013	0.65±0.08
13	T_bG_4	2%	56.112±0.04	0.689±0.011
18	T_gG_1	0.5%	29.00±0.015	0.77±0.02
19	T_gG_2	1%	31.82±0.017	0.93±0.013
20	T_gG_3	1.5%	54.08±0.06	1.03±0.04
21	T_gG_4	2%	63.429±0.014	1.087±0.023

Tc - Cow milk
Tb - Buffalo milk
Tg - Goat ilk
G- Ginger extract
Br- Beet root extract

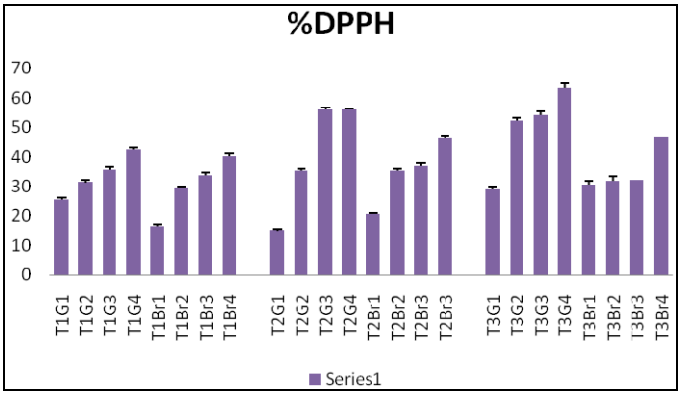

Fig 1: DPPH radical scavenging activity of the different percentage of water extracts of *Zingiber officinale and Beta vulgaris* in cow, buffalo & goat milk yoghurt

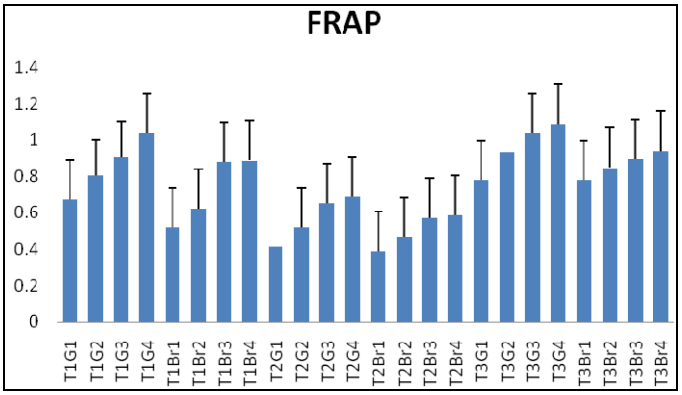

Fig 2: Ferric reducing antioxidant power of the different percentage of water extracts of *Zingiber officinale and Beta vul*garis in cow, buffalo & goat milk yoghurt

4. Conclusion

The antioxidant activity of herbal yoghurt prepared by different level of selected medicinal herbs can be determined accurately, conveniently, and rapidly using DPPH testing. The results of the present study revealed that the inclusion of herbs extracts in the yoghurt significantly altered the antioxidant properties of the yoghrt samples. Among the cow, buffalo and goat milk yoghurt, maximum antioxidant activity were found in 2% levels of ginger and beet root in goat milk yoghurt followed by cow and buffalo milk herbal yoghurt. And among the two herbs ginger rhizome extract shows high antioxidant activities than beet root extract. Hence it may be recommended that the above herbs ginger and beet root could be added at 2 % in the preparation of herbal yoghurt.

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