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Effect of Vidarikand (Extracts) on Oxidative Stability of Ghee: A Comparative Study

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Abstract

Antioxidant activities of vidarikand (Pueraria tuberosa) extracts were evaluated and compared with BHA, TBHQ, rosemary and green tea using a β-carotene bleaching assay, a 2, 2-diphenyl-β-picrylhydrazyl (DPPH) assay and the Rancimat method. Phenolic content and antioxidant activity (β-carotene–linoleic acid model system and DPPH assay) of ethanolic extract of vidarikand was more compared to its aqueous extract. Ethanolic extract of the vidarikand was more effective in preventing the development of the peroxide value and conjugated diene value in ghee compared to its aqueous extract. Vidarikand ethanolic extract showed the higher induction period as compared to its aqueous extract in the Rancimat.

Keywords: *antioxidant activity, ghee (butter oil), vidarikand (Pueraria tuberosa), phenolic content, radical-scavenging activity, rancimat, antioxidant activity*

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INTRODUCTION

Ghee considered as the Indian name for clarified butterfat, is usually prepared from cow milk or buffalo milk or combination thereof and has a pleasing and appetizing aroma. About 30–35% of the milk produced in India (112 million tons in 2009-10) is converted into ghee [1]. Ghee is the most widely used milk product in the Indian sub-continent and is considered as the supreme cooking and frying medium. It has a considerably longer shelf-life as compared to other indigenous dairy products. Ghee undergoes oxidative degradation during storage, resulting in alteration of major quality parameters such as color, flavor, aroma and nutritive value affecting suitability for consumption. The primary autoxidation products are hydroperoxides, which have no taste and flavor, while their degradation products, called secondary oxidation products have detectable taste and flavor [2]. Development of rancidity reduces the shelf-life of the product, which ultimately affects consumer acceptability [3].

In recent decades, there has been a great interest in screening essential oils and various plant extracts for natural antioxidants because of their good antioxidant properties. In order to prolong the storage of foods, several synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) are used currently, but these substances may be inappropriate for chronic human consumption, as recent publications have mentioned their possible toxic properties for human health and the environment [4, 5]. Hence, the development of alternative antioxidants of natural origin has attracted considerable attention and is generally thought to be a desirable development [6]. Many herbs and spices are known to exhibit antioxidant activity in food lipids [7]. The majority of published work in the area of natural antioxidants has focused on tocopherols [8]. Rosemary extract which is also available commercially exhibits potent antioxidant activity [9]. Green tea extracts also exhibit potent antioxidant activity in vegetable oil and animal fat [10]. Spices and herbs, in addition to contributing taste and aroma to foods, also contain a variety of bioactive substances which are of considerable use from the standpoint of food science and technology. However, addition of herbal extracts in dairy products is a newly emerged area [11]. Merai *et al*. [12] reported that water-insoluble fraction of Tulsi (*Ocimum sanctum* L.) leaves

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possess good antioxygenic properties and phenolic substances present in Tulsi leaves were the main factors in extending the oxidative stability of ghee (butterfat).

Puerariatuberosa has an esteemed place in Ayurveda and is very widely used in treating various diseases including diabetes, cancer, etc. [13]. It restores and balances body functions that assist in achieving the maximum potential. *Pueraria tuberosa* which belongs to Fabaceae family, one of the important and potential medical plants in traditional and folklore system, is also commonly known as vidarikand and Indian Kudzu. Theisoflavonoids of *Pueraria tuberosa* such as puerarin, daidzein, genistein and daidzin have been reported to possess several therapeutic properties. It acts as an antioxidant under *in vivo* condition [14]. Though a large number of plants worldwide show strong antioxidant activities [15, 16], the antioxidant properties of vidarikand extract have not been elucidated before. This study reports the beneficial effects of vidarikand *(Pueraria tuberosa)* extract in ghee (butter oil) during accelerated oxidation in order to understand its potential use as an antioxidant in the food industries.

MATERIALS AND METHODS

Preparation of Ghee

The aqueous extracts of vidarikand and green tea were added to the cream at the rate of 1% (w/w) and ghee was prepared by direct creamery method. Ethanolic extracts of vidarikand, rosemary as well as synthetic antioxidant (BHA and TBHQ) were added directly to the freshly prepared ghee at the rate of 0.5, 0.5, 0.02 and 0.02% (w/w), respectively. Ghee without any added antioxidant or herb extract served as control. Ghee samples stored in hot air oven at 80 ± 1 C were analyzed at regular intervals of 0, 3, 6, 9, 12, 15, 18 and 21 days for peroxide value, conjugateddienes and radical-scavenging activity by DPPH assay. Accelerated stability test (Rancimat) was also used to determine the induction period (IP) or oxidative stability index (OSI) of freshly prepared ghee samples.

The results obtained after the addition of vidarikand extracts (aqueous and ethanolic) to ghee were compared with those obtained with different reference products: BHA and TBHQ, the most widely used synthetic antioxidants employed in the food industry and two commercially available extracts of natural origin with high antioxidant activity: rosemary and green tea extract.

Chemicals and Reagents

β-carotene, linoleic acid, 2,2-diphenyl-1-picrylhydrazylhydrate free radical (DPPH), Folin-Ciocalteu's reagent and Tween-40 were obtained from Sigma Chemical Co., USA. Organic solvents, namely, chloroform and gallic acid (Lobachemie Pvt. Ltd., Mumbai, India) and ethyl acetate (RFCL Ltd., New Delhi, India) were used. Sodium carbonate was obtained from Qualigens Fine Chemicals, Mumbai, India.

Herb Extracts

The aqueous and ethanolic extract of vidarikand was obtained from National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh, India. The ethanolic extract of rosemary and aqueous extract of green tea were obtained from Synthite Industry Ltd., Kerala, India.

Synthetic Antioxidants

The synthetic antioxidants such as butylatedhydroxyanisol (BHA) and *tert*-butylhydroquinone (TBHQ) were obtained from Sigma Chemicals, USA.

Addition of Antioxidants

As vidarikand and green tea were available as aqueous extracts, these were initially added to cream @ 1.0% and then ghee was made. Alcoholic extracts of vidarikand and rosemary were added directly to ghee @ 0.5%. Synthetic antioxidants, BHA and TBHQ, were also added directly to ghee @ 0.02%.

Total Phenolic Content

Total phenolic content of herb extracts was analyzed by Folin Ciocalteu method [17].

β-carotene-linoleic Acid Model System

The antioxidant activity of herb extracts and synthetic antioxidants was determined according to the procedure of Marco [18], with the following modification (ethanol used as a solvent instead of methanol for sample preparation).

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Radical-scavenging Activity by DPPH Model System

The radical-scavenging activity of herb extracts and synthetic antioxidants was determined according to the procedure of Blois [19], with minor modification (used ethanol instead of methanol for sample preparation).

Radical-scavenging Activity of Ghee Samples by DPPH Assay

The capacity of antioxidants to quench DPPH radicals in ghee was determined before and after accelerated oxidation tests [20]. Ethyl acetate was used as a better solvent for hydrophobic compounds.

Peroxide Value

Peroxide values of ghee samples were determined by the method as described in IS: 3508 [21].

Conjugated Dienes

Conjugated dienes were determined as per the method of AOAC [22].

Accelerated Test – Rancimat 743

The resistance to auto-oxidation was measured using Rancimat 743 (Metrohm, Herisau, Switzerland) instrument at 120 °C with airflow rate of 20l/h. The oxidative stability was expressed as induction period (h) or oxidative stability index (h).

Statistical Analysis

Results are presented as means ± standard error from three replicates of each experiment. A *P*-value < 0.05 was used to denote significant differences among mean values determined by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Total Phenolic Content

The amount of total phenolics, measured by Folin-Ciocalteu method, varied widely in herb materials and results are presented in Table 1. The calculation of total phenolic content of ethanolic and aqueous extracts of vidarikand was carried out using the standard curve of gallic acid and presented as mg gallic acid equivalents (GAE) per gram. The ethanolic extract contained the highest amount of phenolic compounds (44.8 ± 0.14 mgGAE/gm) while the lowest amount was present in aqueous extract (24.95 ± 0.18 mgGAE/gm) of vidarikand. Both the herb extracts had significantly ($P < 0.05$) different content of phenolics.

Table 1: *Total Phenolic Content of Vidarikand Extracts (Ethanolic and Aqueous).*

Herb extracts	Gallic acid equivalents (mg/gm)
Vidarikand ethanolic	44.8 ± 0.14^{a}
Vidarikand aqueous	24.95 ± 0.18^{b}

Data are presented as means of three determinations ± SEM (n = 3). Means with different superscripts letters are significantly different (P < 0.05).

Significant differences between the results of aqueous and ethanolic extract of vidarikand was likely due to difference in the solubility of antioxidative compounds in the water and ethanol and/or due to the difference in the temperatures used in their preparation. The amount of polyphenols in the extract is also dependent on the extraction method. Philip *et al*. [23] also showed that ethanolic extract of *Andrographis paniculata* leaves had a higher content of total phenolic compounds (75.86 ± 0.82 mg of GAE/g) than its aqueous extract. Ethanol is reported as an effective solvent to extract phenolic compounds [24].

β-carotene-linoleic Acid Model System

The antioxidant activity of vidarikand (aqueous and ethanolic), rosemary and green tea extract, as well as butylated hydroxyl anisole (BHA) and *tert*-Butylhydroquinone (TBHQ), were evaluated at 200 ppm using the β-carotene-linoleic acid coupled oxidation model system and the results are presented in Figure 1 and Table 2. The antioxidant activity exhibited by ethanol (86.05 ± 0.13%) was greater than the aqueous (84.44 ± 0.18%) extracts of vidarikand at 200 ppm. The antioxidant potential of synthetic antioxidant TBHQ and BHA (90.02 ± 0.11% and

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89.97 ± 0.16% respectively) was almost equal. The synthetic antioxidant TBHQ showed the maximum activity of 90.02 ± 0.11%, whereas the aqueous extract of vidarikand showed least activity of 84.44 ± 0.18% when incorporated at the concentration of 200 ppm. The inhibition percentage of rosemary and green tea extract (88.57 ± 0.20% and 88.11 ± 0.25% respectively) was found to be almost equal and higher than the vidarikand extracts (aqueous 84.44 ± 0.18% and ethanolic 86.05 ± 0.13%). Significant differences between the results were likely due to genotopic and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested, time of taking samples and determination methods [25, 26].

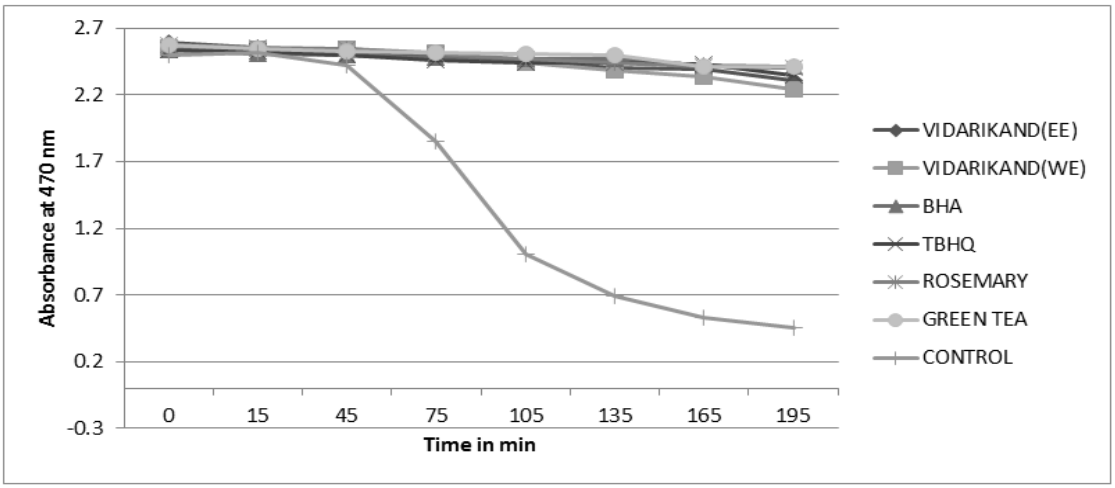

Fig. 1: *Antioxidant Activity of Herb Extracts and Synthetic Antioxidants. Vidarikand (EE): Vidarikand ethanolic, Vidarikand (WE): Vidarikand aqueous*

Table 2: *Antioxidant Activity (%) of Herb Extracts and Synthetics Antioxidants.*

Particulars	**Antioxidant activities at 200 ppm Concentration**
TBHQ	90.02 ± 0.11[a]
BHA	89.97 ± 16[a]
Rosemary	88.57 ± 0.20[a]
Green tea	88.11 ± 0.25[a]
Vidarikand ethanolic	86.05 ± 0.13[b]
Vidarikand aqueous	84.44 ± 0.18[b]

Data are presented as means of three determinations ± SEM (n = 3). Means with different superscripts letters are significantly different (P < 0.05).

Based on the result, antioxidant activity exhibited by the ethanolic extract of vidarikand was greater (86.05 ± 0.13%) than its aqueous (84.44 ± 0.18%) extract. This difference between antioxidant activity of ethanolic and aqueous extracts of vidarikand might be attributed to the higher temperature used in the preparation of the latter causing damage to some compounds with antioxidant activity and/or different solubility of antioxidant compounds in water and ethanol.

Radical-scavenging Activity by DPPH Model System

The radical-scavenging activity of vidarikand (aqueous and ethanolic), rosemary and green

tea extract, as well as butylatedhydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ), were evaluated at 200 ppm in the DPPH system and the results are presented in Table 3. It is evident from the table that radical-scavenging potential of ethanolic extract was significantly higher (72.89 ± 0.34%) than the aqueous (51.17 ± 0.44%) extract of vidarikand, but significantly lower than all other additives used, namely, TBHQ, BHA, rosemary and green tea extract with the percent inhibition of 96.14 ± 0.11%, 91.21 ± 0.23%, 88.69 ± 0.10% and 87.88 ± 0.20% respectively at 200 ppm concentration.

Table 3: *Radical-scavenging Activity of Herb Extracts and Synthetic Antioxidants.*

Particulars	% Inhibition
BHA	91.21 ± 0.23^{a}
TBHQ	96.14 ± 0.11^{b}
Rosemary	88.69 ± 0.20^{ac}
Green tea	87.88 ± 0.16^{ac}
Vidarikand ethanolic	72.89 ± 0.34^{d}
Vidarikand aqueous	51.17 ± 0.44^{e}

Data are presented as means of three determinations ± SEM (n = 3). Means with different superscripts letters are significantly different (P < 0.05).

The radical-scavenging potential of green tea and rosemary extracts was found to be almost equal and significantly higher than the vidarikand extracts but lower than the synthetic antioxidants. Aqueous extracts of vidarikand showed the weakest radical-scavenging potential among all the compounds studied.
Thus, we can conclude that results obtained were consistent with the results obtained from β-carotene-linoleic acid model system.

Several studies have shown that there is a difference between antioxidant capacity of ethanolic and aqueous extract of herbs. For example, Philip *et al*. [23] reported that the ethanolic extract of *A. paniculata* leaves had a higher stable DPPH free radical scavenging activity (86.87%) and higher content of total phenolic compounds (75.86 ± 0.82 mg of GAE/g) than aqueous extract.

Only a combination of different methods of antioxidant activity evaluation can give more reliable results as a single method alone may not be reliable. Several studies [24–27] found a good correlation between total content of phenolic compounds and the antioxidant activity in different plants. Other studies however contradicted the above statement. For example, Nsimba *et al*. [28] showed that the antioxidant activity of *Chenopodiumquinoa* and *Amaranthus spp.* seeds, determined using three different assays (carotene bleaching, FRAP and DPPH), poorly correlated with total content of phenolic compounds. There were also no significant relationships between the antioxidant activities (determined using three different methods namely FRAP, DPPH and carotene bleaching assays) and total contents of phenolic compounds for fifteen genotypes of selected Turkey *Zizyphus jujube Mill. Fruits* [29]. Based on the results obtained in the present study, there is a positive linear correlation between total content of phenolics, DPPH free radical scavenging activities and β-carotene-linoleic acid model system of vidarikand extract (aqueous and ethanolic). This correlation confirms that phenolic compounds play an important role as free radical scavengers.

Accelerated Oxidation Studies

Peroxide Value

Peroxide value (PV) represents primary reaction products of lipid oxidation, which can

be measured by their ability to liberate iodine from potassium iodide. It is considered to represent the quantity of active oxygen (mg) contained in 1 g of lipid. The results obtained after addition of vidarikand (aqueous and ethanolic), rosemary and green tea extracts and synthetic antioxidants, namely, BHA and TBHQ, to ghee on the peroxide development are presented in Figure 2.

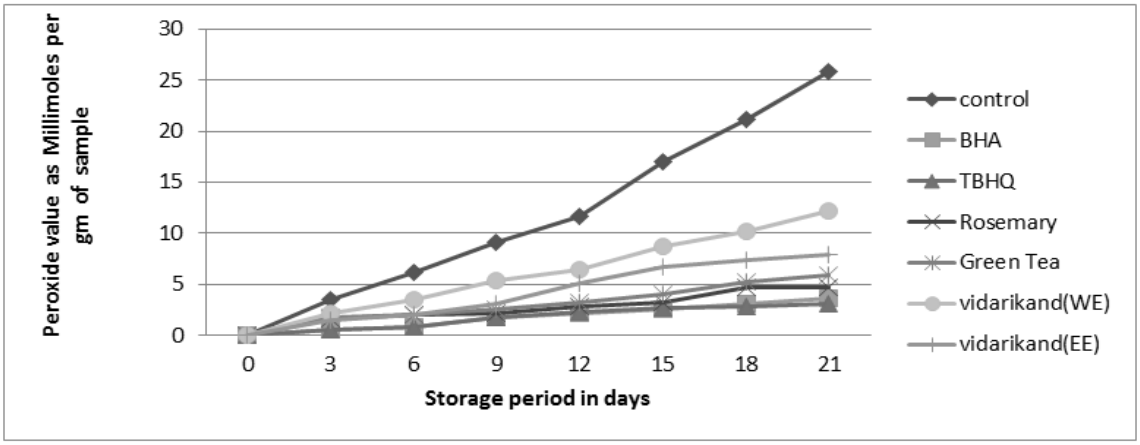

Fig. 2: *Peroxide Value of Ghee Samples Stored at 80 ± 1 °C as Milli Moles of Oxygen per kg of Fat. Vidarikand (EE): Vidarikand ethanolic, Vidarikand (WE): Vidarikand aqueous*

It was observed that the vidarikand extracts (aqueous and ethanolic) significantly ($P < 0.05$) lowered the peroxide value throughout 21 days of storage at 80 ± 1 °C as compared to the control. However, ghee incorporated with aqueous extract of vidarikand showed a significant ($P < 0.05$) rise in peroxide value as compared with its ethanolic extract. This indicated that ghee containing ethanolic extract was more effective than the ghee containing aqueous extract of vidarikand in retarding peroxide development. The peroxide value of ghee containing both the extracts of vidarikand was significantly ($P > 0.05$) higher than the synthetic (BHA and TBHQ) and natural antioxidants (rosemary and green tea extract) throughout 21 days of storage at 80 ± 1 °C as shown in Figure 3.

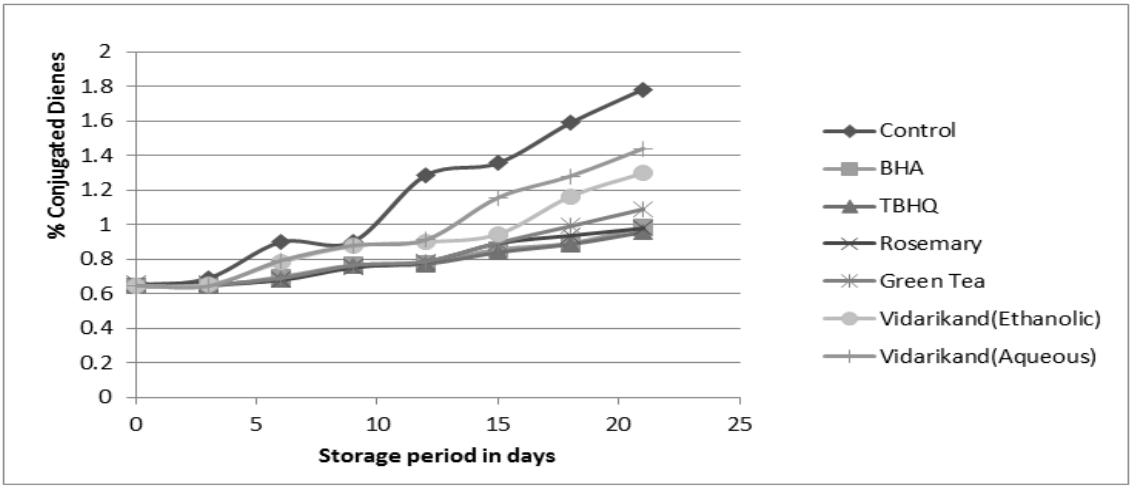

Fig. 3: *Conjugated Dienes (%) Content of Ghee Samples Stored at 80 ± 1 °C. Vidarikand(EE): Vidarikand ethanolic Vidarikand(WE): Vidarikand aqueous*

Zia-ur-Rehman *et al.* [30] have reported that addition of ginger extract to sunflower oil greatly inhibited the rise in peroxides under accelerated conditions. In southern Indian villages, people use the fresh leaves of *Moringaoleifera* during preparation of cow

and buffalo ghee to increase its shelf life [31]. Merai *et al.* [12] reported that addition of 0.6% of silica gel charcoal treated fraction of Tulsi leaves powder to ghee was more effective than the BHA at 0.02% until the peroxide value of 5 meq of peroxide oxygen was reached. The green tea extract exhibited antioxidant activity as two folds of sage extract and four folds of caraway extract as indicated by peroxide value during the storage periods of sunflower oil [32].

Conjugated Dienes

According to Silva *et al.* [33], the polyunsaturated fatty acid oxidation occurs with the formation of hydroperoxides and the double bond displacement followed by the formation of consequent conjugated dienes (CD). Over 90% of hydroperoxides formed by lipoperoxidation have a conjugated dienic system resulting from stabilization of the radical state by double bond rearrangement. These relatively stable compounds absorb in the UV range (235 nm) forming a shoulder on the main absorption peak of nonconjugated double bonds (200–210 nm), so they can be measured in the UV spectrum by absorption spectrophotometry [34].

Figure 3 shows that vidarikand extracts (aqueous and ethanolic) were significantly ($P < 0.05$) capable of lowering conjugated dienes formation throughout 21 days of storage at 80 ± 1 °C as compared to the control, but significantly ($P < 0.05$) less effective in retarding conjugated dienes formation as compared to other natural (rosemary and green tea extracts) and synthetic (BHA and TBHQ) antioxidants. The ethanolic extract of vidarikand significantly ($P < 0.05$) lowered conjugated dienes formation as compared to the aqueous extract of same herb.

In a research carried out by Frankel *et al* [35] to assess the antioxidant activity of rosemary in several oil types stored at 60 °C, for 20 days, rosemary extract containing 44 mg/kg carnosic acid and 6 mg/kg carnosol, added to soybean oil at a concentration of 1000 mg/kg, inhibited diene formation when compared to the control. Siddiq *et al.* [36] reported that the addition of methanolic and acetone extracts of *Moringaoleifera* to sunflower oil was found to show least contents of conjugated dienes as compared with control.

Evaluation of Radical Scavenging Activity (RSA) of Ghee Samples towards DPPH Radicals

The ghee incorporated with herb extracts, synthetic antioxidants and control ghee were evaluated for the potential to quench DPPH radicals before oxidation and after completing the accelerated tests and the results are presented in Table 4. It is evident from Table 4 that on zero day (before oxidation), the radical scavenging activity of ghee incorporated with ethanolic and aqueous extracts of vidarikand, rosemary and green tea extract were found to be 62.00 ± 0.45, 58.74 ± 0.57, 90.08 ± 0.26 and 80.77 ± 0.33%, respectively whereas for control ghee it was 20.67 ± 0.31%. However, the radical scavenging activity of ghee containing BHA and TBHQ was found to be 82.95 ± 0.43 and 96.87 ± 0.23%.

Table 4: *Radical-scavenging Activity of Herb Extracts and Synthetic Antioxidants Incorporated Ghee Samples.*

Samples	Storage period in days	
	0	21
Control	20.67 ± 0.31^{a}	5.47 ± 0.29^{a}
BHA	82.95 ± 0.43^{b}	74.86 ± 0.51^{b}
TBHQ	96.87 ± 0.23^{c}	92.30 ± 0.16^{c}
Rosemary	96.08 ± 0.26^{c}	92.05 ± 0.28^{c}
Green Tea	80.77 ± 0.33^{d}	75.90 ± 0.40^{d}
Vidarikand ethanolic	62.00 ± 0.45^{e}	43.95 ± 0.38^{e}
Vidarikand aqueous	58.74 ± 0.57^{f}	37.87 ± 0.15^{f}

Data are presented as means of three determinations ± SEM (n = 3). Means with different superscript letters are significantly different (P < 0.05).

This indicates that the ghee samples with added vidarikand extract (ethanolic and aqueous) have significantly higher antioxidant potential than control. Ghee incorporated with

ethanolic extract of vidarikand showed stronger activity in quenching DPPH radicals in system both before and after oxidation than that shown by the aqueous extract of the herb. This could be due to presence of higher amount of total phenolic compound in ethanolic extract of vidarikand. Furthermore, the radical scavenging activity of ghee added with vidarikand extract (ethanolic and aqueous) showed a significantly lower value than those with rosemary and green tea extract. Nogala-Kaluka *et al*. [37] reported that rosemary extract was a more effective antioxidant than tocopherols in rapeseed oil tri-acylglycerol (TAG). Before and after the accelerated storage of the samples (i.e., on zero day and 21st day) at 80 ± 1 °C, the ability to quench free DPPH radicals was in the following order:
TBHQ = rosemary > BHA > green tea > vidari kand (ethanolic) > vidarikand (aqueous) > cont rol.

It can be concluded that rosemary and TBHQ had the strongest activity in quenching DPPH radicals in system before and after oxidation followed by green tea extract. In the same system, vidarikand (aqueous) extract had the lowest antioxidant activity and a very low capacity to quench radicals both before and after oxidation.

Evaluation of Antioxidative Potential of Ghee Samples by Using Rancimat 743

The effect of herb extracts, viz., vidarikand (ethanolic and aqueous), rosemary (ethanolic), green tea (aqueous) and synthetic antioxidants (BHA and TBHQ) incorporation on oxidative stability of ghee was evaluated by Rancimat equipment and results are presented in Table 5. The induction time was used as a major indicator of antioxidative potential of antioxidant used. The induction period (IP) is also known as oxidative stability index (OSI) measured as the time required to reach an endpoint of oxidation corresponding to either a level of detectable rancidity or a sudden change in the rate of oxidation [38, 39]. Measurements of IP under standard conditions were used as an index of antioxidant effectiveness.

Table 5: *Oxidative Stability of Ghee Samples Incorporated with Natural and Synthetic Antioxidants.*

Sample	Induction period
Control	10.45 ± 1.01^{A}
Vidarikand (A@1%)	14.67 ± 1.09^{B}
Vidarikand (E@0.5%)	17.89 ± 1.01^{C}
BHA (0.02%)	20.59 ± 0.92^{D}
TBHQ (0.02%)	33.87 ± 2.14^{E}
Green tea (A@1%)	31.27 ± 1.37^{F}
Rosemary (E@0.5%)	ND
Rosemary (E@0.25%)	45.92 ± 1.14^{G}

ND not detected. A Aqueous E Ethanolic. Data are presented as means of three determinations ± SEM (n = 3).Different superscripts in column (A, B) means significant difference between groups (p < 0.05).

The ethanolic extract of vidarikand (IP 16.83 ± 0.17 h) was found to be more effective in stabilizing ghee against oxidative deterioration as compared to its aqueous extract (IP 13.53 ± 0.14 h). However, induction periods of both the extract of vidarikand-added ghee was found to be significantly higher ($P < 0.05$) than that of control ghee (IP 10.33 ± 0.17 h). Nahak and Sahu [40] reported that phenolic compounds

are considered to be the most important antioxidative components of herb and other plant materials, and a good correlation exists between the concentrations of plant phenolics and the total antioxidant capacities.

It is evident from Table 5 that both the extracts of vidarikand (ethanolic and aqueous)-added ghee were found to have significantly lower ($P < 0.05$) antioxidative potential than that shown by rosemary (IP 45.47 ± 0.43) and green tea (IP 31.55 ± 0.48)-added ghee. Synthetic antioxidants, viz., BHA (IP 20.14 ± 0.28) and TBHQ (IP 33.87 ± 1.16)-added ghee also showed significantly higher ($P < 0.05$) antioxidative effectiveness as compared to vidarikand (ethanolic and aqueos) extracts-added ghee. The induction period of the ghee incorporated with 0.5% rosemary extract was not obtained even after 72 h. Therefore, rosemary extract at the concentration of 0.25% was used. The IP was found to be 45.92 ± 1.14 h.

The most effective antioxidant in stabilizing ghee by the Rancimat test was found to be rosemary extract (IP 45.47 ± 0.43 h). In fresh rosemary leaves, carnosic acid is the major phenolic diterpene. During the extraction of rosemary extracts carnosic acid was partially converted either into carnosol or into other diterpenes, which could degrade further to produce other phenolic diterpenes with -and -lactone structure [41, 42] or compounds of unknown structure [43]. These compounds were more lipophilic than carnosic acid and act as antioxidants [43, 44], but their antioxidant activities were lower than those of carnosic acid and methyl carnosate [9]. Antioxidative activity of these degradation products was relatively high at temperatures similar to frying temperature [7, 45]. When compared to other antioxidants, rosemary diterpenes were reported to have higher antioxidant activity than many commonly used phenolic antioxidants.

It can be inferred from Table 5 that the aqueous extract of green tea-added ghee exhibited significantly higher ($P < 0.05$) antioxidant activity as compared to vidarikand extract (aqueous IP 14.670 ± 1.09 h and ethanolic IP 17.89 ± 1.01 h)-added ghee. This may be due to the reason that the green tea extract contains phenolic antioxidants (flavonoids) such as catechins of different structure and antioxidant activity which are polar and their glycosides are very polar [7].

The antioxidative effectiveness of BHA (IP 20.14 ± 0.28 h)-added ghee was found to be significantly lower ($P < 0.05$) than the TBHQ (IP 33.87 ± 1.16 h)-added ghee. Higher antioxidant activity of TBHQ might be due to presence of more number of hydroxyl groups than that contained by BHA. However, both the synthetic antioxidants showed significantly lower antioxidant potential in ghee when compared to rosemary extract. This could be explained by higher volatility of synthetic antioxidants at higher temperature [38]. In contrast, the antioxidative activity of degradation products of carnosic acids present in rosemary is relatively high at temperature similar to frying temperature [39]. The antioxidant activity of herb extracts and synthetic antioxidants in ghee as measured by Rancimat was in the following order: rosemary > TBHQ > green tea > BHA > vidari kand (ethanolic) > vidarikand (aqueous).

CONCLUSIONS

A positive correlation between antioxidant potential, free radical-scavenging capacity and total phenolic content was found in the aqueous as well as ethanolic extracts of vidarikand (*Puerariatuberosa*). Total phenolic content, antioxidant activity as determined by the β-carotene linoleic acid model assay and radical scavenging activity as determined by the DPPH assay were more for the ethanolic extract of vidarikand as compared to the aqueous extracts of the vidarikand. Ghee incorporated with the vidarikand ethanolic extract showed better radical scavenging activity as compared to the ghee incorporated with the aqueous extract of the same. Aqueous and ethanolic extracts of vidarikand were found to be capable of retarding oxidative degradation in ghee but were less effective than natural (rosemary and green tea) and synthetic (BHA and TBHQ) antioxidants. Vidarikand ethanolic extract showed the higher induction period as compared to its aqueous extract in the Rancimat. Hence, vidarikand could be used as a natural antioxidant to preserve the food system apart from providing other beneficial benefits.

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