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Effect of *Asparagus racemosus* (Shatavari) and *Withania somnifera* (Ashwagandha) extracts on oxidative stability of ghee, in relation to added synthetic antioxidant

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Abstract
The present investigation entitled, "Efficacy of herb extracts on oxidative stability of ghee" was undertaken in laboratory of Department of Animal Science and Dairy Science, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, (M.S.) during period 2016-2018. The antioxidant activities of Shatavari (*Asparagus racemosus*) and Ashwagandha (*Withania somnifera*) extracts were evaluated and compared with BHA using β-carotene bleaching assay, DPPH. While total phenolic content of *Shatavari* extract (T_2-0.50%, 24.99) was significantly higher. Higher antioxidant activity (T_1-0.50, 67.73%) was noticed in *Ashwagandha*. Free radical-scavenging potential of *Shatavari* extract was higher (62.85%). Peroxide value (0.32) of *Ashwagandha* extract was lower. Non-significant difference for conjugated dienes was observed among the treatments. Significant rise in TBA value (T_0, 0.63) was noticed in control ghee sample. Antioxidant activity of the herbs decreased in the order Ashwagandha > Shatavari.

Keywords: Ghee (Butteroil), Shatavari (*Asparagus racemosus*), Ashwagandha (*Withania somnifera*) antioxidant activity, Radical-scavenging activity, Phenolic content

Introduction
Ghee is the most widely used milk product in the Indian sub-continent and is considered as the supreme cooking and frying medium. Ghee is important part of human diet. It is the most important ingredient in food and it is rich source of dietary energy and contains high calorific value. It is complex lipids of glycerides, free fatty acids, phospholipids, sterols, sterol esters, fat soluble vitamins, carbonyls, hydrocarbons and carotenoids (cow ghee). It is vehicle for the fat soluble vitamins. According to Hazra and Parmar (2014) [7] ghee has been considered immensely superior to other fats mainly because of the presence of characteristic short chain fatty acids, carrier of four fat soluble vitamins *viz*., A, D, E, K and essential fatty acids such as linolenic acid and arachidonic acid.

In recent decades, there has been great interest in screening essential oils and various plant extracts for natural antioxidants. In order to prolong the storage of foods, several synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are used currently, but these substances are in appropriate for chronic human consumption, as recent publications have mentioned their toxic properties for human health and the environment (Itio *et al.*, 1986) [8]. Hence, the development of alternative antioxidants of natural origin has attracted considerable attention and is thought to be a desirable development (Jia *et al*., 2007) [10].

Asparagus racemosus (Shatavari) and *Withania somnifera* (Ashwagandha) belonging to family Asparagaceae and Solanaceae, respectively have an esteemed place in Ayurveda. The active components of *Pueraria tuberosa* are puerarin, daidzein, genistein and daidzin (Pandey *et al.*, 2007) [13]; *Asparagus racemosus* are steroidal glycosides, saponins, polyphenols, flavonoids, galactose and vitamins (Thomson, 2002) [22] and *Withania somnifera* are steroidal lactones (withanolides), sitoindosides and steroidal alkaloids. All these herbs have been reported to possess several therapeutic properties. They are also known to possess antioxidant activity *in vivo* condition (Verma *et al.*, 2009) [23]. *Asparagus racemosus* is reported to have immunostimulant, antihepatotoxic and antioxytocic activities (Goyal *et al.*, 2003) [6], and antioxidant and anti-diarrheal activities in laboratory animals (Bhatnagar *et al.*, 2005) [3].

Withania somnifera (Ashwagandha) is one of the major herbal components of geriatric tonics, this plant is also claimed to have potent aphrodisiac, rejuvenative and life prolonging properties (Sharma, 1997) [19].

Shatawari (*Asparagus racemosus*)

Asparagus racemosus (family Liliaceae), is commonly called Shatavari, Satawar or Satmuli in Hindi; Satavari in Sanskrit. It is considered both a general tonic and a female reproductive tonic. The major active constituents are steroidal saponins.

The role of *Asparagus racemosus* as an immunoadjuvant in traditional therapy is well documented and therefore it can be applied to evade the toxic side effects of synthetic chemotherapeutic drugs without compromising on its anti-tumour activity. Interestingly, in Ayurvedic medicine, AIDS is thought to be a disease of decreased "ojas", defined as the essential energy of the body. The alcoholic extract of the root was found to possess *in vitro* antibacterial activity against *Staphylcoccus aureus* and *Escherichia coli*. Increase both the weight of mammary tissue and the milk yield. This effect was attributed to the action of released corticosteroids or an increase in prolactin.

Ashwagandha (*Withania somnifera*)

Withania somnifera, also known as Ashwagandha, Indian ginseng and winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. Ashwagandha in Sanskrit means "horse's smell", probably originating from the odour of its root which resembles that of sweaty horse (Puri, 2003) [17]. The species name *somnifera* means "sleep-bearing" in Latin, indicating it was considered a sedative, but it has been also used for sexual vitality and as an adaptogen.

Ashwagandha roots contain alkaloids, starch, reducing sugar, glycosides, dulcitol, withaniol acid and a neutral compound. Wide variation (0.13-0.31%) is observed in alkaloid content. Majumdar (1955) [12] isolated 8 amorphous bases such as withanine, somniferine, somniferinine, somnine, withananine, withananinine, pseudowithanine and withasomnine. *Withania* roots are astringent, bitter, acrid, somniferous, thermogenic, stimulant, aphrodisiac, diuretic and tonic. Leaf is antibiotic, antitumorous, antihepatotoxic and antiinflammatory. Seed is milk coagulating, hypnotic and diuretic.

Materials and Methods

Cream - The fresh clean cream was collected from Research Cum Development Project on Cattle, Department of Animal Husbandry and Dairy Science, M.P.K.V., Rahuri, Dist. Ahmednagar. The cream was collected immediately after morning milking.

Ashwagandha (*Withania somnifera*) and Shatavari (*Asparagus racemosus*)

Ashwagandha and Shatavari Extract as a source of natural herb were purchased from All India Coordinated Project on Medicinal and Aromatic Plants and Beetle Vine Research, MPKV, Rahuri, Dist. Ahmednagar.

Synthetic antioxidant

Synthetic antioxidant required as antioxidant agent was purchased from M/s. Dodal Enterprises, Shrihari Plaza, New Gulmandi Road, Aurangabad. 431001.

Addition of antioxidants

The ethanolic extract of Ashwagandha (*Withania somnifera*) and Shatavari (*Asparagus racemosus*) added at the rate of 0.5 per cent into the freshly prepared ghee, while synthetic antioxidant such as BHA was added at the rate of 0.02 per cent into the ghee.

Oxidative stability

The ghee samples viz. control ghee, ghee incorporated with herb extracts [*Ashwagandha* (*Withania somnifera*) and *Shatavari* (*Asparagus racemosus*)] and ghee incorporated with synthetic antioxidants (BHA) were stored at 37^0C for 4 months (ambient conditions). The ghee samples were then withdrawn at intervals of 1 month analyzed for Peroxide value, Thiobarbituric acid (TBA) value and Conjugated dienes.

Total phenolic content

Total phenolic content of herb extracts were analyzed by Folin ciocalteu method (Kahkonen *et al*., 1999).

Standard curve preparation

400µl of 10-100 µg/ml concentration of gallic acid solution was taken in place of sample for standard curve preparation.

β-carotene–linoleic acid model system

The antioxidant activity of herb extracts and synthetic antioxidants was determined according to the procedure of Marco (1969), with minor modification (used ethanol instead of methanol for sample preparation).

Radical-scavenging activity of ghee samples by DPPH model system

The radical-scavenging activity of herb extracts and synthetic antioxidants was determined according to the procedure of (Blois, 1958), with minor modification (Used ethanol instead of methanol for sample preparation).

Peroxide value

The peroxide value of ghee will be determined by Lea's method. Peroxide value of ghee samples were determined by the method as described in IS: 3508 (1966).

Conjugated dienes

Conjugated dienes will be determined as per the method of AOAC [1995].

Thiobarbituric acid (TBA) value

TBA value of ghee samples were determined by the method of Patton and Kurtz (1951).

Statistical Analysis

The data generated during the course of this investigation was analyzed with the help of Completely Randomized Design (CRD) for pre-experimental trials and final experimental trials (Panse and Sukhatme, 1985).

Results and Discussion

Oxidative Stability of Herb Extracts

The stored ghee samples were analyzed at regular intervals of 1, 2, 3 and 4 months for peroxide value, conjugated dienes and TBA. The results obtained after the addition of herb extracts (ethanolic) to ghee were compared with those

obtained with BHA, the most widely used synthetic antioxidantin the food industry.

Total phenolic content of herbs extract and bha by folin-ciocalteu method

Polyphenolic compounds are generally found in both edible and inedible plants, and they have been reported to have several biological effects with antioxidant property. Total phenolic content of *Ashwagandha*, *Shatavari*, *Ashwagandha + Shatavari* and synthetic antioxidant (BHA) extracts was determined by Folin - Ciocalteu method employing a standard curve of gallic acid (ranging from 10-100 mg/ml). The results were expressed as mg gallic acid equivalents (GAE) per gm. of herb.

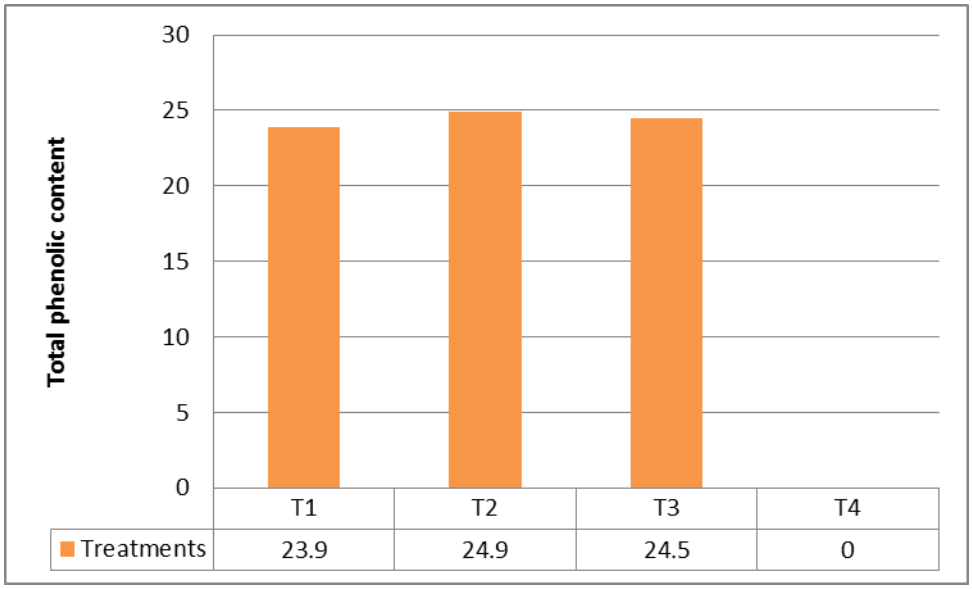


Fig 1: Total phenolic content of herbs extract and BHA

From the Figure-1, the level of total phenolic in extract of *Ashwagandha* was found to be 23.94 and extract of *Shatavari* was found to be 24.99 and combination of thereof was 24.53 mg gallic acid equivalent/g extract. The means of total phenolic content of *Shatavari* extract was found to be significantly higher ($P < 0.05$) than combination thereof and *Ashwagandh*a extracts.

Similar conclusion drown by Pawar *et al.* (2014) that phenolic content was highest in the ethanolic extract of *vidarikand* followed by ethanolic extract of *Shatavari*, aqueous extract of *vidarikand*, ethanolic extract of *Ashwagandha,* aqueous extract of *Ashwagandha* and aqueous extract of *Shatavari.*

The results obtained are in agreement to the work done by Rifat *et al.*, 2011. Siddhuraju and Becker (2003) [21] reported ethanol as an effective solvent to extract phenolic compounds.

Antioxidant activity of herb extracts and BHA by β-carotene- lenoleic system

The antioxidant activity of herb extracts, were evaluated at 200 ppm concentration using the β – carotene – linoleic acid coupled oxidation model system and the results are presented in Figure 3.

Antioxidant activity of herbs extract and BHA by β-carotene-lenoleic system at 200ppm O.D. at 470 nm

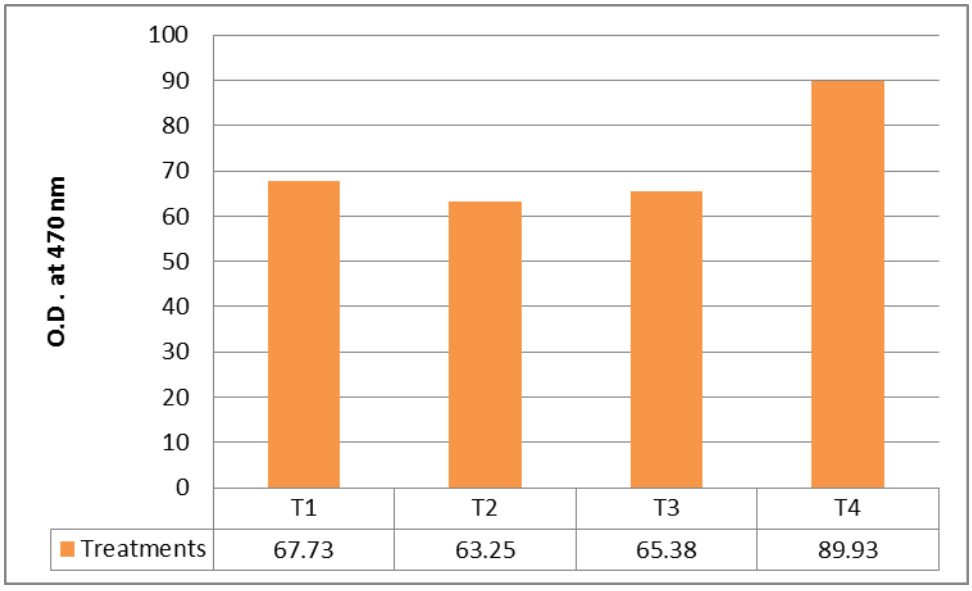


Fig 2: Antioxidant activities of herbs extract and BHA

It is evident that the extract of *Ashwagandha* showed higher antioxidant activity (67.73%) then combination thereof (65.38%) and *Shatavari* (63.25%) when incorporated at concentration of 200 ppm.

As β -carotene molecules lose their double bonds by oxidation; the compound loses its chromophore and characteristic orange colour, which is monitored spectrophotometrically. In the present study, it was observed that, the presence of ethanolic extracts of *Ashwagandha* and *Shatavari* hindered β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system. But the extent of hindrance was more for ethanolic extract of *Ashwagandha* as compared to *Shatavari* counterpart.

This difference may be due to difference in total phenolic content of the extracts. Kruawan and Kangsadalampai (2006) reported that the herbal extracts with high antioxidant activity exhibited relatively high total phenolic contents.

Radical scavenging activity of herb extracts and synthetic anti-oxidant (BY DPPH percent inhibition)

The radical-scavenging activity of herb extracts was evaluated at 200 ppm using DPPH system and the results are presented in and Figure 3.

Radical scavenging activity of herbs extract and synthetic anti-oxidant by DPPH (% inhibition)

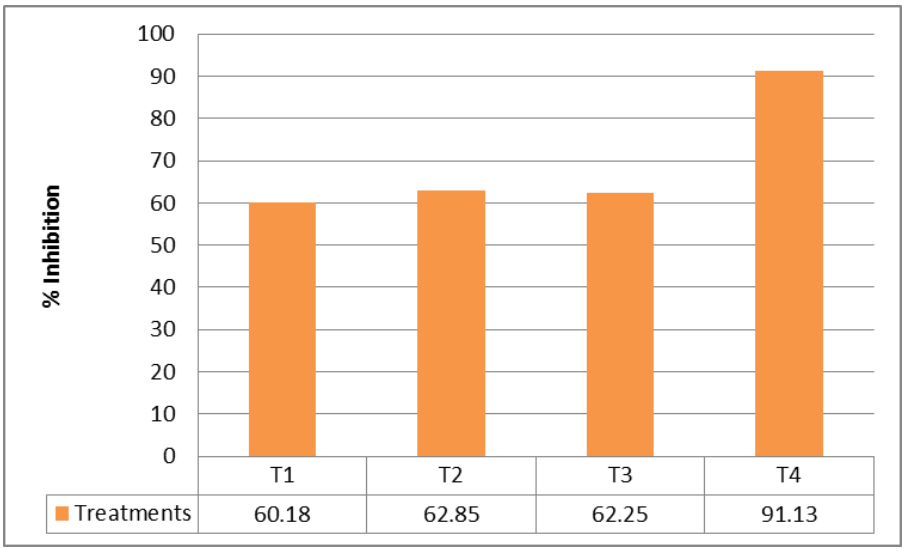


Fig 3: Radical scavenging of herbs extract and BHA

Radical-scavenging activity of extracts of *Ashwagandha*, *Shatavatri* and combination thereof was found to be 60.18 per cent, 62.85 percent and 62.25 per cent, respectively as compared to synthetic antioxidant (BHA) 91.13 per cent. These values indicated that the free radical-scavenging potential of *Shatavari* extract was significantly ($P < 0.05$) higher than the of *Ashwagandha* extract. This difference may be due to difference in total phenolic content of the extracts.

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and single oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans *et al*., 1995) [18]. They exhibited antioxidant activity by inactivation lipid free radicals or preventing decomposition of hydro peroxides into free radicals (Pokorny *et al*., 2001; Pitchaon *et al*., 2007) [16, 15].

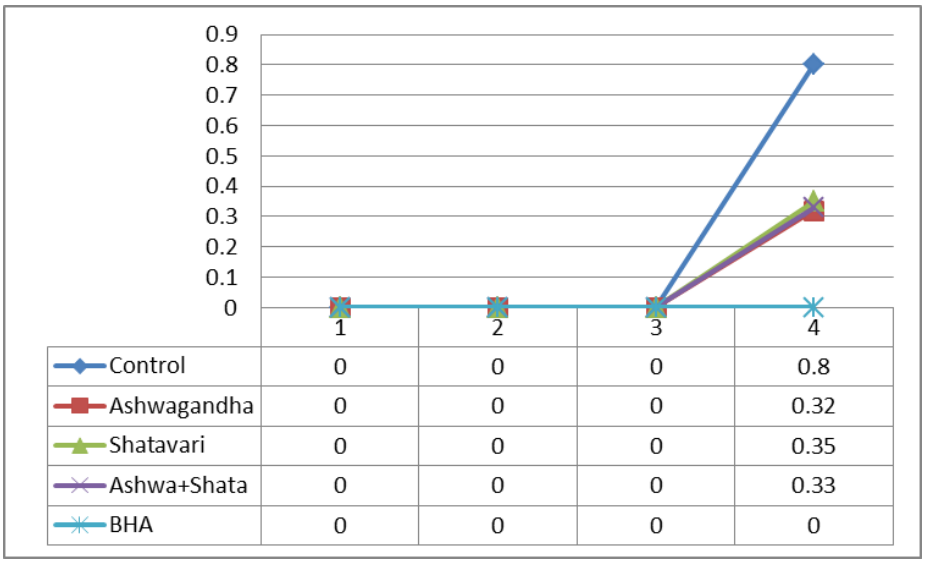
Similar conclusions were drawn by Asha *et al*. (2015) [1], ghee incorporated with orange peel extract showed maximum potential to quench the DPPH radicals than ghee incorporated with BHA and control throughout storage period. Presence of antioxidant compounds in ghee incorporated with orange peel extract and BHA exhibited stronger radical scavenging activity.

Storage study at 37±1 ^{0}C for four months

1. Peroxide value

Effect of addition of herb extracts and synthetic antioxidants on development of peroxides in ghee stored at 37±1^{0}C is observed nil up to the end of 3 rd month.

Peroxide value of ghee prepared with herbs extract and BHA stored at 37±1 ^{0}C for 1,2,3 and 4 months (Millimoles of oxygen per gram of fat)



	1	2	3	4
Control	0	0	0	0.8
Ashwagandha	0	0	0	0.32
Shatavari	0	0	0	0.35
Ashwa+Shata	0	0	0	0.33
BHA	0	0	0	0

Fig 4: Peroxide value of ghee samples stored at 37±1 ^{0}C for 1, 2, 3 and 4 months

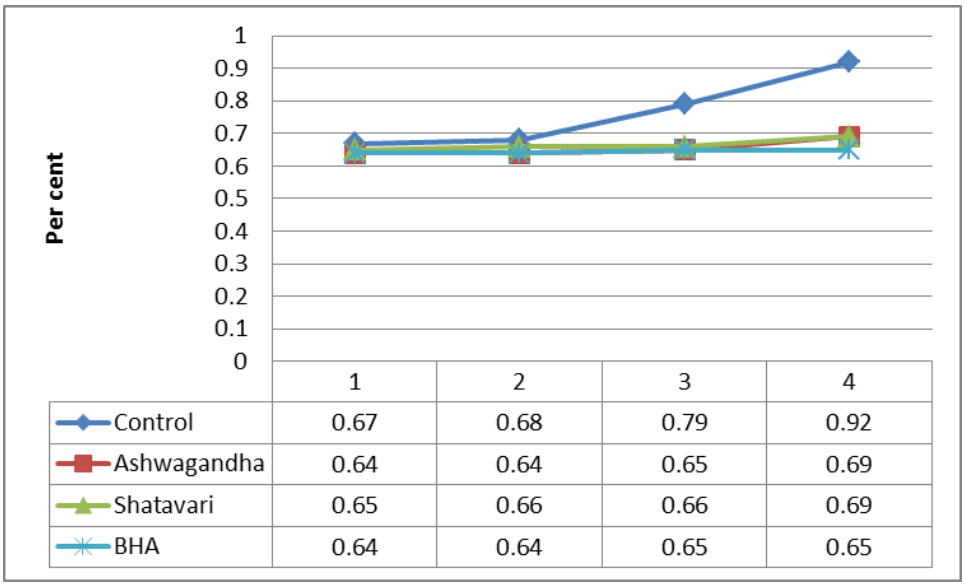
The effect of herb extract incorporation on development of peroxides during storage at 37±1 ^{0}C is presented in Figure 4. The control ghee and the ghee incorporated with *Ashwagandha*, *Shatavari* and combination thereof showed zero peroxide value at the end of 1st, 2nd and 3rd months of storage at 37 ^{0}C. At the end of 4th month of storage significant difference (P < 0.05) was observed between peroxide values of control ghee (0.80) and ghee incorporated with herb extracts. However, extracts of *Ashwagandha*, *Shatavari* and combination thereof showed peroxide value 0.32, 0.35 and 0.33.BHA (synthetic antioxidant) showed zero peroxide value at the end of 4th month of storage. This suggested that ghee containing extract of *Ashwagandha* is more effective in retarding peroxide development.

These results were in agreement with the results of Asha *et. al.* (2015) [1]. The ghee incorporated with orange peel extract was more effective than BHA treated ghee in retarding the primary oxidation. The variation in peroxides development in BHA and orange peel extract treated ghee could be due to the presence of antioxidant compounds.

2. Conjugated dienes

Effect of addition of herb extracts and synthetic antioxidants on development of conjugated dienes in ghee stored at 37±1 ^{0}C.

Overall conjugated dienes of stored ghee samples at 37±1 ^{0}C for 1, 2, 3 and 4 months (Per cent)



	1	2	3	4
Control	0.67	0.68	0.79	0.92
Ashwagandha	0.64	0.64	0.65	0.69
Shatavari	0.65	0.66	0.66	0.69
BHA	0.64	0.64	0.65	0.65

Fig 5: Conjugated dienes of stored ghee samples at 37±1^{0}C for 1, 2, 3 and 4 months

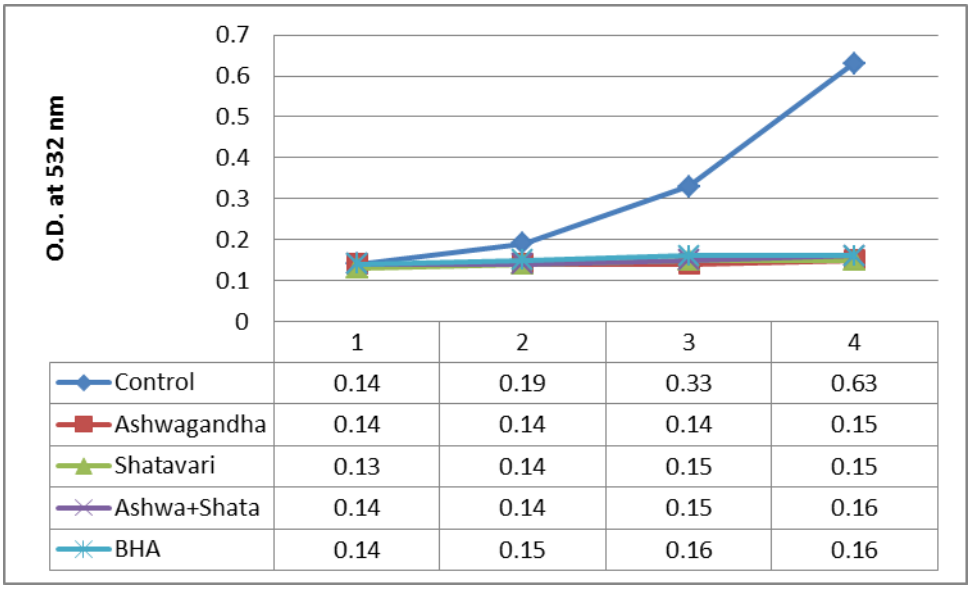
The effect of herb extract incorporation development of conjugated dienes during storage at 37±1^{0}C is presented in Figure 5. On analyzing the ghee samples for conjugated dienes at the end of 1st and 2nd months of storage, it was observed that control ghee and the ghee incorporated with herb extracts showed almost similar percentage of conjugated dienes. On analyzing the ghee samples at the end of 3rd and 4th months of storage, it was observed that the conjugated dienes (0.79 and 0.92 %) of control ghee differed significantly (P <0.05) from that of the ghee incorporated with *Ashwagandha* (0.65 and 0.69 %), *Shatavari* (0.66 and 0.69 %) and combination thereof (0.66 and 0.69 %) extracts. The conjugated dienes of *Ashwagandha* ghee shows lower per cent because of better antioxidant activity. However, ghee incorporated with extract of *Ashwagandha* (*Withania somnifera*) and *Shatavari* (*Asparagus racemosus)* and

combination thereof showed non-significant difference in conjugated dienes content (0.69%) at the end of 4th month.

3. Thiobarbituric acid (TBA) value

Thiobarbituric acid test measures the secondary products of lipid oxidation; it involves reacting thioabarbituric acid with malondialdehyde produced by lipid hydro peroxide decomposition to form a red chromophore with peak absorbance at 532 nm. This coloured complex results in the condensation of 2 moles of TBA and 1 mole of malondialdehyde, under the joint effect of the medium temperature and pH. TBA is defined as the quantity of malondialdehyde (in mg) present in 1 kg of sample. This method has, however, been the focus of much criticism. The first is that malondialdehyde only forms from fatty acid chains containing at least three double bonds, like linolenic acid, to the exclusion of linoleic and oleic acid peroxide decomposition products (Dahle *et al*., 1962) [5]. Secondly, TBA is not specific to malondialdehyde because it can react with other aldehydes, browning reaction products, protein and sugar degradation products, amino acids and nucleic acids (Janero, 1969) [9].

Thiobarbituric Acid (TBA) value of ghee prepared with herbs extract and BHA stored at 37±1 ^{0}C for 1, 2, 3 and 4 months (OD at 532nm)



	1	2	3	4
Control	0.14	0.19	0.33	0.63
Ashwagandha	0.14	0.14	0.14	0.15
Shatavari	0.13	0.14	0.15	0.15
Ashwa+Shata	0.14	0.14	0.15	0.16
BHA	0.14	0.15	0.16	0.16

Fig 6: Thiobarbituric acid value of ghee samples stored at 37±1^{0}C for 1, 2, 3 and 4 months

The effect of herb extracts and synthetic antioxidants on development of TBA value in ghee was assessed and the results are presented in Figure 6**,** control ghee and ghee incorporated with herb extracts and synthetic antioxidants showed almost similar TBA value at the end of 1st months of storage at 37^{0}C. On analyzing the samples at the end of 2nd month there is increase in TBA value of control ghee sample (0.14 to .019) and 3rd and 4th months of storage at 37^{0}C, it was observed that the TBA value of control ghee differed (0.33,0.63) significantly ($P < 0.05$) from that of the ghee incorporated with herb extracts and synthetic antioxidants (*Ashwagandha*, *Shatavari*, combination thereof and BHA is 0.15, 0.15, 0.16 and 0.16 respectively). However, control ghee sample showed a significant rise in TBA value (0.63) as compared with the *Ashwagandha* extract (0.15), *Shatavari* extracts (0.15) and combination thereof (0.16) and BHA incorporated ghee (0.16).

These results were in agreement with the results of Aditya *et.al.* (2018) [2] who reported significant difference ($p<0.01$) between all the intervals of alcoholic and aqueous extract treatment samples for 60 days of storage. The change in TBA value control, alcoholic and aqueous extract treatment samples were 0.01 to 0.52, 0.01 to 0.51 and 0.01 to 0.50 respectively. Alcoholic and aqueous extracts were reduced the development of TBA that of control sample.

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