Natural Antioxidants - The Key to Safe and Sustainable Life

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Abstract- This review work analytically explicates the food sources, chemistry and benefits of natural antioxidants. Naturally occurring antioxidants are not only capable of abolishing the ill effects of free radical damage to food system, but their consumption also boost the body's endogenous antioxidant mechanism to combat oxidative stress. Natural ingredients in food are gaining much contemplation in both the areas of food industry and research, as these are considered safer option than synthetic additives. This review explores the possible uses of natural antioxidants as additive and components of functional food formulations. Critical analysis of natural antioxidants as additive in food is reviewed, addressing food safety and health benefit for the consumers.

Keywords: polyphenol, flavonoid, oxidative stress, food safety, nutraceutical.

I. INTRODUCTION:

An antioxidant is a molecule that inhibits the oxidation of other molecules and delays or prevents oxidative damage. Biochemical reactions occurring in biological systems continuously produce various free radicals. These are highly reactive and may further react with other bio molecules and thus initiate a chain reaction [1]. The body has several antioxidant mechanisms to protect against oxidative stress. This is achieved by either the actions of externally supplied antioxidant through foods or the endogenous ones, for example, enzymes like glutathione peroxidise, superoxide dismutase, catalase, and metabolites like uric acid, glutathione, L-arginine, etc. Vitamins, minerals and phytochemicals from food are exogenous antioxidants [2]. The antioxidants neutralise the load of free radicals, protect the cells against their deleterious effect and thus help in disease prevention.

Natural antioxidants are obtained from the biological system. Human diet contains an array of different compounds that possess antioxidant activities. The most prominent representatives of dietary antioxidants are vitamin C, tocopherols, carotenoids, flavonoids, antioxidant polysaccharides and amino acids & its compounds. In the diet, there may be synergistic effects of these various dietary compounds and they work as an orchestra where interactions between constituents bring about the effects. The need of antioxidants in food industry is not only to preserve flavour and colour and increase the shelf life of food, but also as components of nutraceutical food. Intake of antioxidant rich diet protects against deleterious degenerative diseases. In food system, naturally occurring antioxidant mechanism are often lost during processing or storage. This necessitates the addition of exogenous food additives - either natural or synthetically produced antioxidants. Butylated hydroxylanisole (BHA), Butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ) are the frequently used synthetic antioxidant in food, whereas, natural antioxidants generally added in food are tocopherols, ascorbic acid etc. However, antioxidants are effective at very low concentration, higher doses may generate toxic effects. The cytotoxicity of BHA and BHT have been found in human promeylocytic leukemia cell lines (HL-60) and squamous carcinoma cell lines [3]. This food safety issue with these synthetic antioxidants limits their use as additive. On the other hand, the higher production cost and lower efficiency of generally used natural antioxidants like ascorbic acid, tocopherol etc. have triggered the need of exploring alternative natural and probably safer sources of food antioxidants. Fruits and vegetable processing in India generates huge quantities of waste and residues. It has been reported that these wastes and by-products of fruits and vegetables like seeds, peel and pomace are abundant sources of antioxidants [4], but these have not been conventionally used as

additive. In this review the sources and potential uses of these less explored antioxidant stores is also discussed.

II. BENEFITS OF ANTIOXIDANT IN FOOD:

In biological system, oxidative stress generates excess free radical formation, which, in turn induces disease process in different organs and physiological systems. The role of antioxidants in foods is to retard or control oxidation. The process of auto-oxidation and development of rancidity in foods involves a free radical chain mechanism via initiation, propagation and termination steps. While radicals are produced in the 'initiation' step, they attack a compound by abstracting a hydrogen atom in the 'propagation' step [5]. The reactions in the propagation step make up a chain reaction until a 'termination' reaction occurs. In addition to auto-oxidation, lipid quality deterioration in foods may occur due to photo-oxidations, oxidation via a lipoxygenase-assisted process, or oxidation under thermal conditions as during frying of foods. Many of the products formed as a result of oxidation of foods, regardless of the conditions experienced, are deleterious and affect health adversely. Thus, all oxidation processes should be controlled in order to protect food lipids from deterioration & off-flavour formation and disease propagation in man. Free radical damage to cell affects many organs and physiological systems (figure 1) which may lead to chronic disease pathogenesis. Thus food antioxidants not only help to improve food quality but also have a role in disease prophylaxis.

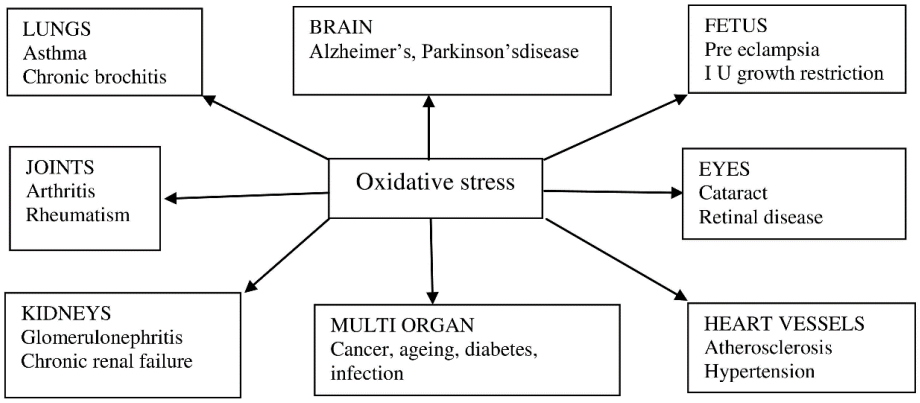

Figure 1: Adverse effects of free radicals in human body.

The presence of antioxidants in food prevents this damage caused by oxidative stress. Each antioxidant functions in biological system in its own unique way. Vitamin E plays an important role to prevent cardio vascular disease, ischemic heart disease, cataract, arthritis, cancer in colon, breast and prostate in the prevention against colon, prostate and breast cancers. Flavonoids, Vitamin C and beta carotene have been reported to delay the process of ageing and delay various chronic degenerative diseases such as cardiovascular disease, Alzheimer's disease, cancer, cataract, memory loss and act as immune-modulator, fighting against infections and inflammation [6]. The antioxidants in food not only help to prevent against the chronic diseases, their addition to food also prevent lipid oxidation or auto-oxidation of food.

Function of antioxidants in foods is based on the antioxidant activity of a particular compound, a mixture of compounds, or a natural source containing such compounds. It is generally related to their ability to scavenge free radicals, decompose them, or to quench singlet oxygen or possibly act as metal chelators or a synergists with other components present. Antioxidants from natural sources are often present in combinations involving many different compounds. Each compound may be present together with its precursor and reaction product. Thus, the mode of action of natural sources of antioxidants may be varied and could involve multiple mechanisms of action, depending on the type and source of the material used.

III. MECHANISM OF ANTIOXIDANT ACTIVITY

A. *Activity of some commonly used synthetic antioxidant additives:* BHA, BHT and TBHQ are widely used synthetic antioxidant additives in food. These are phenolic compounds. BHA and BHT are fairly stable to heat and are often used for stabilization of fats in baked and fried products. Some antioxidants, such as BHA and BHT, are used in combination with resulting synergistic effects [7]. BHA is also synergistic with propyl gallate [8]. These antioxidants act as a terminating agent that suppresses auto-oxidation. They stop the chain reaction by the following mechanism:

$RO_2 + ArOH \rightarrow ROOH + ArO\cdot$
$RO_2 + ArO\cdot \rightarrow$ Harmless non radical product
Where 'R' is alkyl or aryl group, and ArOH is BHA or BHT.

The oxidative characteristics and/or metabolites of BHA and BHT are under investigation due to their possible contribution to carcinogenicity or tumorigenicity. This food safety issue with these synthetic antioxidants limits their use as additive.

B. *Activity of some natural antioxidants*: Foods are natural store house for antioxidant compounds like polyphenols, bioactive peptides, cellular enzymes and vitamins etc. Antioxidant compounds have been found in grains, oilseeds, spices, fruits and vegetables and even in food residues or wastes. Natural antioxidants act in various ways, by binding metal ions, scavenging free radicals, decomposing peroxides or by combining these methods together causing synergism.

Table 1: Classification of natural antioxidants according to their antioxidant mechanism [9].

Antioxidant class	**Mechanism of activity**	**Examples of antioxidants**
Proper antioxidants	Inactivating lipid free radical	Phenolic compound
Hydroperoxide stabiliser	Preventing hydroperoxide decomposition into free radical	Phenolic compound
Synergist	Promoting activity of proper antioxidants	Citric acid, ascorbic acid
Metal chelator	Binding heavy metals into inactive compound	Phosphoric acid, Maillard compounds, citric acid
Singlet oxygen quencher	Transforming singlet into triplet oxygen	carotenes
Substances reducing hydroperoxides	Reducing hydroperoxide in a non-radical way	Proteins, amino acid

Apart from the above mentioned mechanisms, natural antioxidants in biological environment also act synergistically with each other with the final outcome of free radical scavenging, for instance tocopherol prevents lipid per-oxidation and itself gets oxidised; oxidised tocopherol is reduced by ascorbic acid to its antioxidant active state (figure 1). Oxidised ascorbic acid (dehydro ascorbate) is converted into harmless compounds such as 2, 3- diketogulonic acid which then decomposes to oxalate [10]. Phenolic acids and flavonoid compounds act as metal chelator and free radical scavenger [11]. These interact with body's endogenous antioxidant enzyme system and boost the overall antioxidant mechanism (figure 2).

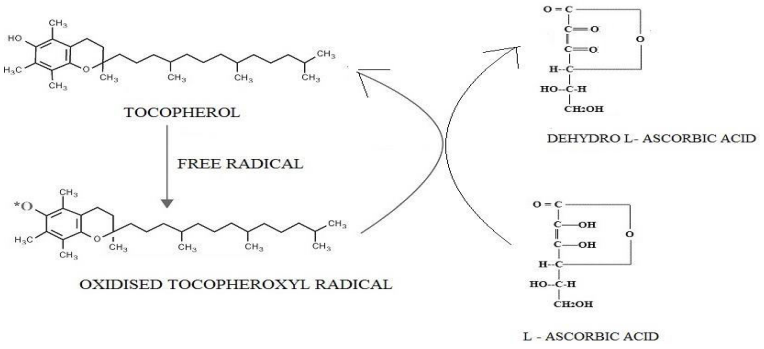

Figure 2: Antioxidant mechanism of tocopherol and ascorbic acid.

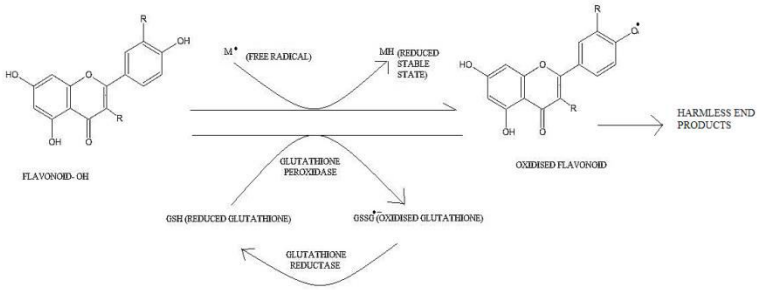

Figure 3: Interaction between flavonoid compound, glutathione peroxidise and glutathione reductase enzymes for free radical scavenging

IV. SOURCES OF NATURAL ANTIOXIDANTS:

A. *Food sources of natural antioxidants*: Plants are a rich source of naturally occurring antioxidants like phenols, phenolic acid and their derivatives for example, whole grain has found to contain ferullic acid, vanillic acid and syringic acid [12]. Fruits and vegetables contain abundant source of antioxidants. Fruits, especially berries, cherries and citrous fruits contain high levels of antioxidants. The polyphenol content in berries varies from 30-2000mg / 100 g which include anthocyanins, proanthocyanidins and flavonols. Among tropical fruits guava contains highest quantities of polyphenol (126 mg/100g) showing antioxidant activity [13].Vegetable provide rich sources of antioxidant, specially anthocyanins, flavonones, flavonols and vitamin C [14], [15]. Spices and herbs like garlic, ginger, etc. also have antioxidant potential [16]. Fruits contain strong anti-oxidant compounds [17], for example berries [18], [19], cherries [20], [21], [22], citrus [23] and in kiwi fruit [24] has been found to be rich in antioxidants. High activity anti-oxidants were found in olive oil [25], [26]. A comprehensive study on the role of phenolic compounds in the oxidative process of fruits reveals the effects of processing and storage on its antioxidative capacity. [27]Several studies have analysed the antioxidant potential of a wide variety of vegetables [28], [29], [30]. Wines contain a variety of polyphenolic compounds, the most abundant being anthocyanins [31], [32], antioxidant activity was also reported in whiskeys [33] .

Green and black tea extracts contains upto 30% phenolic compound of dry weight, which mainly include (-)epigallocatechin, (-)epicatechin 3-gallate, (-)epicatechin, (+)gallocatechin and (+)catechin and reports show that green tea extracts have greater antioxidant efficieny than BHT (ÿ)epigallocatechin, (ÿ)epicatechin 3-gallate, (ÿ)epicatechin, (+)gallocatechin and (+)catechin were also identified as good as synthetic antioxidants [34]. A number of studies deal with the antioxidant activity of extracts from herbs, medicinal plants and spices [35]. Antioxidant component in different herbs and elucidation of their antioxidant mechanisms has also been addressed [36], [37]. Other potential sources, such as canola [38] has also been explored.

Waste/Residual sources: Food residues and waste of food product, though considered as waste, are

an unconventional source of antioxidants. For example, potato peel extract, Apple pomace, Oat hulls, Lemon seeds, orange peel, Lentil seed coat (brown), Lemon peel, Grape seed extract have been found to contain polyphenolic antioxidants [39].

V. NATURAL V/S SYNHETIC ANTIOXIDANT – A COMPARATIVE APPROACH

Natural antioxidants are generally preferred by consumers and may gain legislative approval more easily than synthetic additives do. However, the fact that a substance is commonly found in a food is no guarantee that it is entirely non-toxic. Synthetic antioxidants are tested for carcinogenic or mutagenic effects, but many natural food compounds have not yet been tested. The advantages and disadvantages of synthetic and natural antioxidants are summarized.

Table 2: comparison of synthetic and natural antioxidant

Synthetic antioxidants	**Natural antioxidants**
Inexpensive	Expense depends upon source and extraction process
Widely applied	Restricted use
Medium to high antioxidant capacity	Wide range of antioxidant activity
Low water solubility	Broad range of solubility
Decreasing interest	Increasing interest
Some of them are stored in adipose tissue	Completely metabolised

VI. EXPERIMENTAL APPLICATION OF SOME NATURAL ANTIOXIDANT EXTRACT IN FOOD

The antioxidant activity of green tea extract was compared with the effects of the commonly-used antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) at 200ppm and α-tocopherol at 500ppm. Green tea extract at >200 ppm exhibited excellent antioxidant activity in both oils and its efficacy was higher than that of BHA, BHT and a-tocopherol, but less than that of TBHQ [40]. Antioxidant activity of Grape Seed Extract and Blueberry extract was observed in cooked pork patties. The addition of grape seed extract resulted in minor increases in the surface colour of raw and cooked pork. The sensory quality of cooked pork was unaffected and these exhibited potent lipid antioxidant activity in raw and cooked pork [41]. The search for cheap and abundant sources of antioxidant compounds is attracting worldwide interest. Investigations with vegetables, spice extracts and their residues as sources of antioxidant additive are being carried out. Garlic, lemon and orange fiber has also been used as antioxidant in meat [42]. Residual sources like peels of pomegranate, orange and lemon have been investigated as antioxidant additive in paneer and have found to be a better substitute for synthetic antioxidants [43].

VII. CONCLUSION

Here, it has been discussed that many food and food residue /waste are important antioxidant sources and they have promising prospective as antioxidant additive in food. In some studies, natural antioxidants have shown greater anti-oxidant efficiency than synthetic antioxidants. But before any practical application, detailed study and consideration of the possible toxicity must be done. Much research is needed in this subject in order to identify more food and residual origins of natural antioxidants; optimisation of the application method in food must be done before practical scale application.

VIII. ACKNOWLEDGEMENT

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REFERENCES:

[1] R. Amarowicz, R.B. Pegg,P. Rahimi-Moghaddam,B. Barl, J.A. Weil,"Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies",*Food Chem*, vol. 84,pp. 551–562, 2004.

[2] P.G. Pietta, "Flavonoids as antioxidants",*J Nat Prod*, vol. 63,pp.1035–1042,2000.

[3] R. Kahl, H. Kappus, "Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E",*Z Lebensm Unters Forsch.,* vol.196, no. 4,pp.329-38, 1993.

[4] N. Balasundram, K. Sundaram,S. Samman, "Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses.", *Food Chemistry,* vol. 99,pp. 191-203.2006.

[5] F. Shahidi,"Antioxidants: principles and applications", *Handbook of Antioxidants for Food Preservation*, pp.1, 2015.

[6] R.Winter, "Vitamin E: Your Protection Against Exercise Fatigue, Weakened Immunity, Heart Disease, Cancer, Aging, Diabetic Damage, Environmental Toxins",*Three Rivers Press*. 2013.

[7] K. Omura,"Antioxidant synergism between butylated hydroxyanisole and butylated hydroxytoluene",*J Amer Oil Chem Soc*, vol. 72, pp.1565–1570.1995.

[8] D.S. Rawat, G. Joshi, B.Y. Lamba, A.K. Tiwari, and P. Kumar, "The effect of binary antioxidant proportions on antioxidant synergy and oxidation stability of Jatropha and Karanja biodiesels." *Energy,* vol. 84, pp. 643-655. 2015.

[9] J. Pokorny, N.Yanishlieva, and M.H. Gordon, eds. "Antioxidants in food: practical applications",*CRC press*, 2001.

[10] V. Valpuesta, and M.A. Botella. "Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant.", *Trends in plant science* vol. 9, no.12,pp. 573-577.2004

[11] M. Carocho and ICFR Ferreira. "A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives.", *Food and Chemical Toxicology,* vol. 51,pp.15-25. 2013.

[12] H. Zielinski& H. Kozlowska, "Antioxidant Activity and Total Phenolics in Selected Cereal Grains and Their Different Morphological Fractions",*J. Agric. Food Chem.*, vol. 48, no. 6, pp.2008–2016.2000.

[13] K.R. Maatta-Riihinen, A. Kamal-Eldin, P.H. Mattila, A.M Gonzalez-Paramas and A.R. Torronen, "Distribution and contents of phenolic compounds in eighteen Scandinavian berry species", *Journal of Agricultural and Food Chemistry,*vol. *52*, pp. 4477-4486. 2004.

[14] S. Furuta,Y. Nishiba, and I. Suda, "Fluorometric assay for screening antioxidative activity of vegetables",*Journal of Food Science*, vol. 62,pp.526-528. 1997.

[15] Liu,H. Rui, "Health-promoting components of fruits and vegetables in the diet.", *Advances in Nutrition: An International Review Journal,* vol.4, no. 3, pp. 384S-392S, 2013.

[16] O.I. Aruoma, J. P. E. Spencer,D. Warren, P. Jenner, J. Butler and B. Halliwell, "Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations",*Food Chemistry*, vol.60, pp. 149-156. 1997.

[17] W Kalt., C. F Forney, A. Martin and R.L Prior,"Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits",*Journal of Agricultural and Food Chemistry*, vol. 47, pp. 4638-4644 . 1999

[18] H Wang, M.G. Nair, G.M. Strasburg, A.M. Booren & J. I., Gray, "Novel antioxidant compounds from tart cherries (Prunus cerasus)",*Journal of Natural Products*, vol. 62,pp. 86-88. 1999.

[19] H. Wang, M.G. Nair, G.M. Strasburg,Y.C. Chang,A.M. Booren and J.I. Gray,"Antioxidant polyphenols from tart cherries (Prunus cerasus)",*Journal of Agricultural and Food Chemistry*, vol. 47,pp. 840-844.1999.

[20] H. Wang, M.G. Nair, G.M. Strasburg,Y.C. Chang,A.M. Booren, J.I. Gray and D.L. DeWitt, "Antioxidant and anti-inflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries",*Journal of Natural Products*, vol. 62,pp. 294-296.1999

[21] M. M. Saleh, F. A. E.M. Hashem andK. W Glombitza, "Study of Citrus taitensis and radical scavenger activity of the flavonoids isolated",*Food Chemistry*, vol. 63,pp. 397-400. 1998.

[22] H. W. Dawes andJ. B. Keene,"Phenolic composition of kiwi-fruit juice",*Journal of Agricultural and Food Chemistry*, vol. 47, pp. 2398- 2403.1999.
[23] G. Blekas andD. Boskou, "Antioxidative activity of 3, 4-dihydroxyphenylacetic acid and rt—tocopherol on the triglyceride matrix of olive oil. Effect of acidity.",*Grasas y Aceites*, vol. 49, pp. 34-37.1998.
[24] G. Papadopoulos and D. Boskou,"Antioxidant effect of natural phenols on olive oil",*Journal of the American Oil Chemists' Society*, vol. 68, pp. 669-671.1991.
[25] K.Robards,P.D. Prenzler, G. Tucker, P. Swatsitang and W. Glover, "Phenolic compounds and their role in oxidative processes in fruits",*Food Chemistry*, vol. 66, pp. 401-436.1999.
[26] C. Ganthavorn, C and J.S. Hughes, "Inhibition of soybean oil oxidation by extracts of dry beans (Phaseolus vulgaris)". *Journal of the American Oil Chemists' Society*, vol. 74,pp. 1025-1030.1997
[27] T. Masuda, S. Yonemori, Y. Oyama, Y.Takeda,T.Tanaka,T. Andoh, A. Shinohara andM. Nakata,"Evaluation of the antioxidant activity of environmental plants, activity of the leaf extracts from seashore plants", *Journal of the Agricultural and Food Chemistry*, vol. 47,pp. 1749-1754.1999.
[28] R. Nakayama, Y. Tamura, H. Kikuzaki and N. Nakatani,"Antioxidant effect of the constituents of Susabinori (Porphyra yezoensis)",*Journal of the American Oil Chemists' Society*, vol. 76,pp. 649-653.1999.
[29] V. Fogliano, V. Verde, G. Randazzo, and A. Ritiene, "Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines",*Journal of Agricultural and Food Chemistry*, vol. 47, pp. 1035-1040. 1999.
[30] P. Simonetti, P. Pietta and G. Testolin, "Polyphenol content and total antioxidant potential of selected italian wines",*Journal of Agricultural and Food Chemistry*, vol. 45, pp. 1152-1155.1997.
[31] D. B McPhail, P. T.Gardner,G. G. Duthie,G. M. Steele and K. Reid, "Assessment of the antioxidant potential of scotch whiskeys by electron spin resonance spectroscopy, relationship to hydroxyl-containing aromatic components",*Journal of Agricultural and Food Chemistry*, vol. 47,pp. 1937-1941.1999.
[32] E.L. Copeland, M.N. Cliord and C.M. Williams, "Preparation of (ÿ)epigallocatechin gallate from commercial green tea by caffein precipitation and solvent partition",*Food Chemistry*, 61,pp. 81-87.1998.
[33] M. Suhaj, "Spice antioxidants isolation and their antiradical activity: a review",*Journal of Food Composition and Analysis, vol.* 19, pp. 531–537. 2006.
[34] M. Wang, Y. Shao, J. Li, N. Zhu, M. Rangarajan, E.J. LaVoie and C.T. Ho, "Antioxidative phenolic glycosides from sage (*Salvia officinalis*)",*Journal of Natural Products*, vol. 62, pp. 454-456.1999.
[35] C.A. Hall, S.L. Cupett and P. Dussault, "Hydrogen-donating mechanism of rosmariquinone, an antioxidant found in rosemary", *Journal of the American Oil Chemists' Society*, vol. 75, pp. 1147-1154. 1998.
[36] U.N. Wanasundara, R. Amarovicz and F. Shahidi, "Isolation and identifcation of an antioxidative component in canola",*Journal of Agricultural and Food Chemistry*, 42, pp. 1285-1290.1994.
[37] A. Moure, J.M. Cruz, D. Franco, J.M. Domínguez, J. Sineiro,H. Domínguez, M.J Núñez, and J.C Parajó, "Natural antioxidants from residual sources." *Food Chemistry, vol.* 72, no. 2. pp. 145-171. 2001
[38] U.N. Wanasundara and F. Shahidi, "Antioxidant and pro-oxidant activity of green tea extracts in marine oils",Food Chemistry,vol.63, no.3, pp. 335-342.1998.
[39] R.Carpenter, M.N. O'Grady, Y.C. O'Callaghan , N.M. O'Brien and J.P. Kerry,"Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork",*Meat Science*, vol. 76, pp. 604–610.2007
[40] J. Fernandez-Lopez, N. Zhi, L. Aleson-Carbonell, J.A. Perez-Alvarez and V. Kuri, "Antioxidant and antibacterial activities of natural extracts: application in beef meatballs", *Meat science*, vol.*69, no.*3, pp.371-380. 2005
[41] E.H. Mansour and A.H. Khalil,"Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties",*Food Chemistry*,vol. 69, no.2,pp. 135–141.2000.
[42] N.S. Hettiarachchy, K.C. Glenn, R. Gnanasambandamand M.G.Johnson,"Natural Antioxidant Extract from Fenugreek (Trigonella foenumgraecum) for Ground Beef Patties",*Journal of Food Science*, vol.61, no.3, pp. 516–519. 1996.
[43] S. Singh and I. Genitha, "Extraction of Antioxidants from Fruit Peels and its Utilization in Paneer." , *Journal of Food Processing and Technology.* Vol.5,no.7. 2014.