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STUDIES ON ANTIOXIDANT ACTIVITY OF INDIAN GOOSEBERRY FRUIT AND SEED

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ABSTRACT

Fruits and seeds of Indian Gooseberry (*Phylanthus emblica*, S. Nelli) were investigated to evaluate their antioxidant activity. Antioxidant activity was evaluated by the estimation of peroxide values (PV) of shark liver oil, treated with fruit and seed extracts incubated at 60^0C for a period of nine days. Seed extracts showed higher antioxidant activity than fruit extracts. The ethanol and ethyl acetate extracts of the fruit and all extracts of the seed were found to have higher antioxidant activity than the synthetic antioxidant butylated hydroxy toluene (BHT).

Key words: antioxidant activity, peroxide value, synthetic

INTRODUCTION

There is a growing demand for natural antioxidants because of toxicological and carcinogenic (Wanasundara *et al.*1997; Halliwel & Gutteridge, 1989) effects of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), on animals (Amarowicz, 2000). Antioxidants are substances which help defend the body

against cell damage caused by various free radicals leading to ailments such as heart disease, hardening of the arteries, inflammatory conditions, cataracts & other visual problems, arthritis & rheumatism, cancer and diabetes (Halliwel & Gutteridge, 1989; Nenadis *et al*., 2003; Papas 1999).

A large number of phytochemicals, not recognized as conventional essential nutrients, apparently play an important antioxidative role in the body (Amarowicz *et al.*, 2000; Papas, 1999; Ng 2000). Studies have demonstrated that plant phenolics are a major source of natural antioxidants. (Marinova & Yanishlieva, 2003).They can be distributed in fruits, seeds, leaves, vegetables, barks, roots, and flowers (Wanasundara *et al*., 1997; Wang & Lin, 2000) of plants.

The fruit of Indian Gooseberry (*Emblic myrobalam*) (*Phylanthus emblica* S. Nelli) is acidic, bitter tasting and rich in vitamin C (Kalra, 1988; Singh *et al*., 1987). The fruit possess pronounced expectorant antiviral, antibacterial, antioxidative activities.The known antioxidants, Gallic acid, Catechol, Ellagic acid, Phloroglucinol, Pyrogallol, Trigalloylglucose, Indol acetic acid (IAA),Vitamin C, ß-carotene, superoxide dismutase enzyme have been reported to be present in the fruit (Kalra; 1988 Singh *et al.*, 1987).

The objective of this study was to evaluate and compare the antioxidant activity of different solvent extracts of fruits and seeds. In this study the measurement of lipid peroxidation of shark liver oil was used to evaluate antioxidant activity.

MATERIALS AND METHODS

Sample Preparation

(a) Fruits and Seeds

Fresh fruits of Indian Gooseberry were collected from Narammala area in the Kurunegala district. Seeds were separated from the fruit and oven dried separately at a temperature of 55°C for 5 days. The dried samples were finely ground using a mixer

grinder (Singer Super, domestic) and stored in stopped glass bottles at room temperature and in the dark.

(b) Shark Liver Oil

Bligh & Dyer (1959) extraction method was used.

Preparations of Extracts for Evaluation of Antioxidant Activity

Dried, finely ground samples (each 25g) were separately refluxed with water to obtain the aqueous extracts which were freeze dried. The products obtained were stored in glass bottles at a temperature of 10°C. Samples of freeze dried fruit extracts of Indian Gooseberry (0.5g) were extracted with 50 ml of each solvent, of hexane, dichloromethane, ethyl acetate and ethanol. The solutions were concentrated in vacuum and flushed with nitrogen to remove the last traces of solvents.

Evaluation of Antioxidant Activity

Freeze dried extracts of fruits and seeds were dissolved in ethanol and separately added to the fish oil maintaining concentration at 1000 ppm and stored at 60°C in an oven. Treated fish oils were analyzed for their peroxide values (PV) (AOCS, 1987) during a nine day period. The values obtained were compared with that of the known synthetic antioxidant BHT 200 ppm and of control sample containing fish oil and ethanol in the same proportion. The above procedure was carried out for different solvent extracts of fruits and seeds.

Fish oils are more susceptible to oxidation. Rate of fish oil peroxidation decreases in the presence of pronounced antioxidants. PVs were used to evaluate the antioxidant activity. PV is the quantity of those substances in the sample, expressed in terms of milliequivalents (meq) of peroxide per 1000 g of sample that oxidize potassium iodide (KI) under the condition of the test. It measures the amount of peroxides, which are the primary products of lipid peroxidation or other similar products of fat oxidation. BHT was used as the reference compound.

Data Analysis

Data were analyzed for variance by statistical analysis system, where four replicates were taken for each chemical analysis.

RESULTS AND DISCUSSION

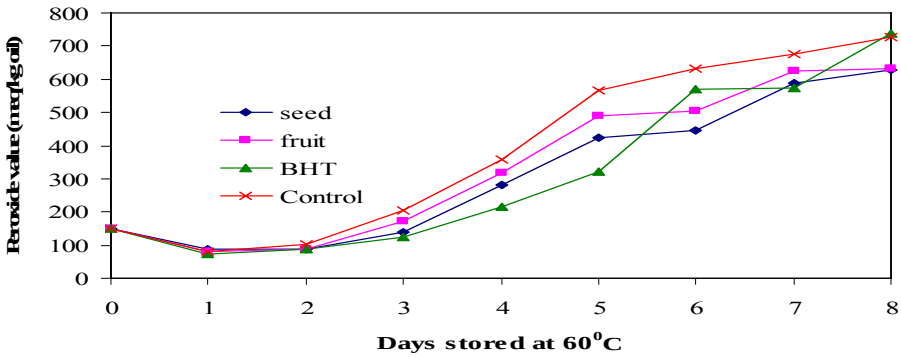

Figure 1. Changes in peroxide value (meq/Kg) during storage at 60°C of testing of antioxidant activity.

Table 1. Changes in peroxide value (meq/kg) during storage at 60°C of solvent extracts of seed

	Peroxide value(meq/kg)/Day							
Treatment	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
Seed	88.16	89.40	139.57	281.19	422.08	444.11	587.14	627.54
Fruit	81.37	87.14	172.25	318.81	488.47	505.07	625.53	632.04
BHT	71.57	71.91	124.99	215.43	320.17	568.67	572.83	736.79
Control	80.52	103.96	204.86	358.17	566.29	631.09	675.89	727.29
Level of significance	*	***	*	***	*	ns	ns	ns
LSD(P≤0.05)	10.60	9.97	40.22	32.65	119.17	-	-	-

*** - significant - * - % level ns – not significant

Fig. 1 and Table 1. show the changes in peroxide value (meq/Kg) during storage at 60°C of water extracts of seed and fruit. Figure 1 shows that there was a slight increase in Peroxide values (PV) of seed and fruit during the first three days followed by a rapid increase. This shows (Edirisinghe *et al*., 1996) that there is an increase of primary products (peroxides) followed by an increase in secondary products (aldehydes and ketones) etc.

According to the graph and analyzed data the PV of control was significantly higher, than the PV of all other treatments. Treatment containing fruit (1000 ppm) was significantly higher in PV than the treatment containing seed (1000 pm) and treatment containing BHT (200 ppm).Treatment containing BHT (200 ppm) maintained a significantly lower PV than all other treatments, until the fifth day of storage.

The above observations indicate that oxidation of fish oil was decreased by the treatment containing BHT (200 ppm), seed (1000 ppm) and fruit (1000 ppm). Susceptibility of oxidation of shark liver oil was decreased by BHT, followed by the seed extract. According to this observation BHT had the highest antioxidant activity, followed by the seed extract, and the fruit extract of Indian Gooseberry. The fruit of Indian Gooseberry had less antioxidant activity than the seed.

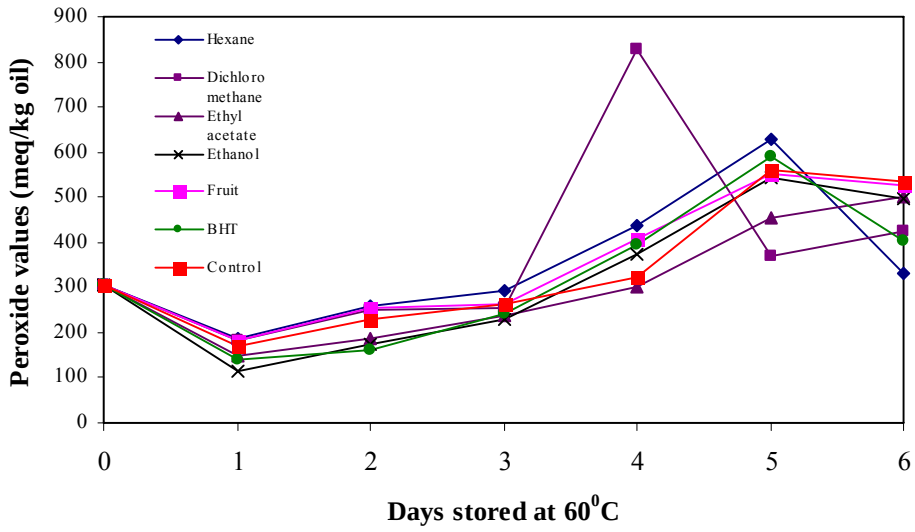

Figure 2. Changes in peroxide value (meq/kg) during storage at 60°C of solvent extracts of fruit

Table 2. Changes in peroxide values (meq/kg) during storage at 60°C of solvent extracts of fruit

Time/	Peroxide Values (meq/kg) /Day						
Days	H	D	EA	E	W	BHT	Control
0	306.34	306.34	306.34	306.34	306.34	306.34	306.34
1	185.75	181.62	148.67	115.51	181.71	138.86	169.52
2	257.85	248.78	185.02	176.09	255.10	160.80	229.99
3	293.52	256.64	236.29	230.97	265.26	240.29	262.00
4	435.52	828.32	301.43	375.43	408.23	392.69	323.57
5	628.74	370.87	452.60	544.57	552.44	589.03	562.29
6	331.37	424.78	499.56	497.19	528.24	404.02	536.83

H – Hexane D - Dichloromethane EA – Ethyl acetate

E – Ethanol W - Water

Fig. 2 and Table 2 show changes in peroxide values (meq/Kg) of different solvent extract of the fruits during storage at 60°C. Treatment containing BHT (200 ppm) maintained a significantly lower PV than all other treatments, until the third day. Treatment containing the ethyl acetate extract (EA) (200 ppm) showed a lower PV than the treatments except BHT (200 ppm), followed by ethanol (E) extract. Treatments containing E and EA showed similar PVs to the treatment containing BHT (200 ppm). Treatments containing dichloromethane (D) and hexane (H) extracts showed a higher PV than other treatments.

Therefore it could be stated that the treatments containing E (200 ppm) and EA (200 ppm) have a greater antioxidant activity than the treatments containing D (200 ppm) and H (200 ppm).

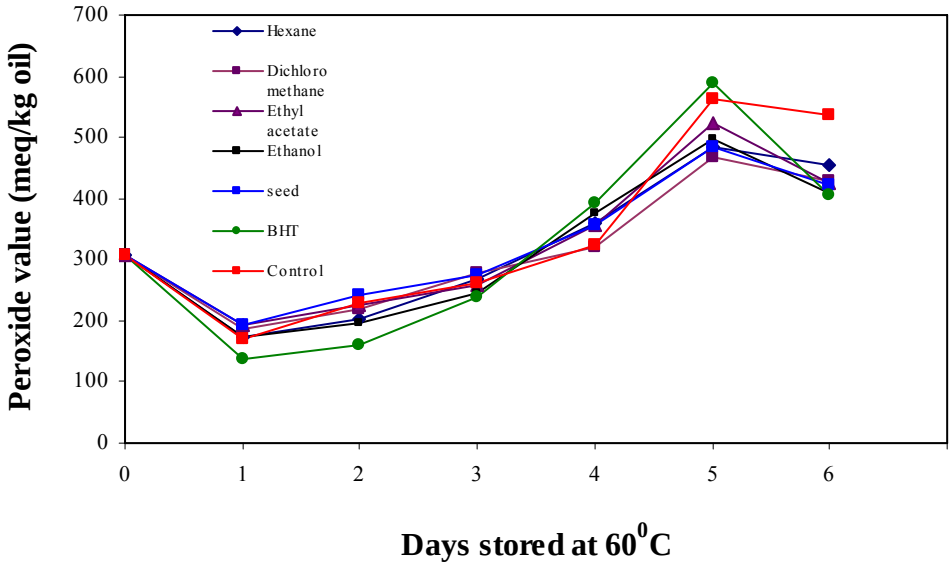

Figure 3. Changes in peroxide value (meq/kg) during storage at 60°C of solvent extracts of seed

Table 3. Peroxide values (meq/kg) during storage at 60°C of solvent extracts of seed over six days

Time/ Days	**Peroxide Values (meq/kg)/Day**						
	H	D	EA	E	W	BHT	Control
0	306.34	306.34	306.34	306.34	306.34	306.34	306.34
1	174.98	187.22	191.90	172.33	191.57	138.86	169.52
2	202.26	219.11	226.40	196.57	242.1	160.80	229.99
3	269.04	278.72	258.63	244.35	275.44	240.29	262.00
4	359.99	322.15	357.48	376.19	355.63	392.69	323.57
5	482.48	467.93	522.98	498.19	485.57	589.03	562.29
6	455.92	427.98	424.84	407.81	421.41	404.02	536.83

H – Hexane D - Dichloromethane EA – Ethyl acetate

E – Ethanol W - Water

Fig. 3 and Table 3 show the changes in peroxide values (meq/Kg) of different solvent extracts of seeds during storage at 60°C. Shape of the graph is similar to that of the Fig 2.

CONCLUSIONS

Peroxide values (PV) provide information regarding the antioxidant activity of substances. According to the results obtained in this study Indian Gooseberry appears to be a good natural source of antioxidants. The study indicated that the seed show more antioxidant activity than fruit. The polyphenolic substances that were shown to be present in this fruit may be the compounds that is responsible for this exhibited antioxidant activity. Treatment containing water extract of seed (1000 ppm) shows higher PV than all other treatments, until the third day of storage. After the third day, treatment containing the water extracts (1000 ppm) and hexane extract (200 ppm) showed similar PV. The antioxidant activity of the seed extract of Indian Gooseberry is higher than that of the fruit extracts of Indian Gooseberry. The overall results show that all extracts of Indian gooseberry show antioxidant activity.

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