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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(5): 585-588, 2002

The Antioxidant Effect of *Sacoglottis gabonensis* Stem Bark Extract and Bergenin Isolate, Nigerian Alcoholic Beverage Additives on the Peroxidative Deterioration of Stored Vegetable Oils

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Abstract: The possibility of the antioxidant action of *Sacoglottis gabonensis* stem bark extract and its isolate, bergenin in peroxidative deterioration of three local vegetable oils, groundnut palm and soya bean was examined. The lipid hydroperoxide, malondialdehyde and malonaldehyde contents of the vegetable oils were determined before and after 30 days storage/incubation with bark extract or bergenin. Treatment of stored vegetable oils (palm oil and soya bean) with bark extract significantly reduced the level of peroxidative deterioration ($P< 0.05$) during storage compared with the bark extract free controls. Bergenin exhibited similar properties to a lesser extent. So, bergenin appears to be the phytochemical constituent that is largely responsible for the antioxidant property of the extract. The antioxidant properties of the bark extract and its isolate bergenin may find ready application as antioxidant additives in foods, beverage and pharmaceutical industries.

Key words: *Sacoglottis gabonensis,* bergenin, lipid peroxidation, vegetable oils, palm oil, kuli-kuli, soya bean oil, hydroperoxide, malondialdehyde, malonaldehyde

Introduction

Most data on cytoprotection *in vivo* and *in vitro* studies have been derived from plants, crops and their active ingredients. Some of these are those reports of Parasakthy *et al.* (1993) on the hepatoprotective property of eugenol, a-2 methoxy 4(2 - propenyl phenol) an active principle of various plant extracts such as ocimum, clove and nutmeg, of Aliyu on the hepatoprotective property of *Cochlospermum planchonii* against CCl4 induced jaundice (Aliyu, 1995), of Emodi on beta carotene of Maduka on r-glutamyl transferase activity and histopathological side effects during aflatoxin - B1- induced cytotoxicity (Maduka *et al.,* 2000), on metabolism, disposition and biological activity of aflatoxin B1 by Okoye and Neal (1988a, 1988b, 1991) and that of Madusolumuo on disposition of acetaminophen in human volunteers by *Sacoglottis gabonensis* treated palm wine (Madusolumuo *et al.,* 1993), of *Sacoglottis gabonensis* stem bark extract on anticoagulant properties and serum levels of acetylsalicylic acid and acetaminophen by Madusolumuo and Okoye (1993a). Notwithstanding, the current level of information on the antioxidant potentials of natural products is inadequate thus making it difficult to establish scientific basis to justify their use in folkoric medicinal practice. It has also limited their ready application as antioxidant additives in foods and drugs. Thus, there is need to study these natural products with a view to establish the inhibitory roles they may play in cytotoxic disorders. *Sacoglottis gabenensis,* a rain forest tree of Southern Nigeria, has been commonly used among the rural communities in this region as an additive to palm wine. Among other claims of its use, it has been reported to prolong the shelf life of palm wine thus suggesting antioxidant properties. The bark extract and bergenin its isolate, have been reported to act as antioxidant protectors of lysis of mammalian erythrocytes by peroxyl radicals of carbon tetrachloride *in vitro* (Maduka *et al.,* 1999, Maduka and Okoye 2000). We have shown that the bark extract reduced the formation of key intermediates of lipid peroxidation pathway (Maduka and Okoye, 2001a) as well as complemented the natural antioxidant defenses (Maduka and Okoye, 2001b) during experimental membrane peroxidation *in vivo.* In an attempt to ascertain whether or not the antioxidant properties observed in biological system also obtained in the non living system, the level of typical peroxidation products were determined in bark extract treated vegetable oils stored over long period of time in an environment conducive to lipid peroxidation . This is with a view to establish if the bark extract and its isolate bergenin can find ready application as antioxidant food additives.

Materials and Methods

The study was undertaken in the Nutritional Toxicology Research Section of the Department of Biochemistry, University of Jos, Plateau State, Nigeria. The project lasted for a total of five months with an additional one and half months for follow up studies.

Preparation of *Sacoglottis gabonensis* stem bark extract: Aqueous ethanol extract (4%) of *S. gabonensis* stem bark was prepared as described before (Maduka and Okoye, 2001a, 2001b; Maduka, 2000). Samples of fresh cuttings of *S. gabonensis* stem bark were purchased from Ekeapara, Aba, Abia State Nigeria. A portion of the extract was diluted with aqueous ethanol solution to give final working dilution (bark weight to volume) 1: 10 as described by Madusolumuo and Okoye (1993b). Similarly, pure bergenin powder was extracted from the *S. gabonensis* bark extract following the procedure of Maduka (2000).

Determination of the effect of *S. gabonensis* bark extract on peroxidative deterioration of stored vegetable oils: Twenty ml portions of each vegetable oil sample was transferred into clear properly labeled conical flasks (50ml capacity). There were also two sets of flasks for each vegetable oil marked test and control respectively. Twenty ml of *S. gabonensis* stem bark extract (1:10 w/v) was added to each conical flasks marked test. All the flasks were kept for three months at room temperature in the laboratory under conditions favourable for normal deterioration of the oils by lipid peroxidation. Thereafter, the extent of peroxidation was determined by estimating the concentrations of three marker products of lipid peroxidation (malondialdehyde, malonaldehyde and hydroperoxide). Statistical analysis between tests and their respective controls was done by Mann Whitney 2-pair sample Students t-test and set at $P< 0.05$.

Determination of lipid hydroperoxide: Lipid hydrogenperoxide formed was determined in the tests and control samples by the titrimetric method of Wills (1987). Two ml of samples of the oils were extracted with 10ml chloroform, shaken and allowed to settle on mechanical shaker. The mixture was centrifuged at low speed and decanted into a clean conical flask (50 ml) and stoppered. Fifteen ml glacial acetic acid was added to the supernatant to give an approximately 3:2 (w/v) acetic acid-chloroform solvent mixture of about a total volume of Twenty-five ml. The mixture in a conical flask was shaken on a mechanical shaker and for a fixed period of time (10 to 20 mins) and then continued with step II of the Wills method (Wills, 1987).

Approximately, 2.0g of solid sodium bicarbonate ($NaHCO_3$) was added and the stopper replaced as the evolution of carbon dioxide began to abate. Two ml of saturated solution of potassium iodide was added and the flask was transferred to dark. After one hour period, 100ml distilled water was added and titrated with standard sodium thiosulfate solution (0.01N). This was standardized using pure organic peroxide, 0.25M benzoyl peroxide (50, 100, 200, 300 and 400 liter). From standardization and calculations, 3.37ml of 0.01N sodium thiosulfate solution was equivalent to 3.13×10^{-3} M benzoyl peroxide. Results were resolved and presented as means standard deviations of three most reliable determinations.

Determination of malondialdehyde: The procedure of Hunter *et al.* (1963) adopted by Kirkova *et al.* (1995) was used to determine the concentration of malondialdehyde in all the oil samples. The 0.175 M KCl-0.02 Tris-buffer pH 7.4 was used as the medium for incubation. One ml oil was added to 3ml of buffer solution in a test tube of 40% TCA (0.25ml) and 0.125ml of 5N HCl were then added and after mixing, 0.25ml of 2% 2-Na-thiobarbituric acid was added promptly. In many experiments, TCA, HCl and thiobarbituric acid solutions were combined before addition. The tubes were stoppered with cotton and placed in boiling water for ten mins, cooled and centrifuged at 2500rpm for ten mins and the color absorbance read at 532nm. The Malondialdehyde formed was calculated using molar extinction coefficient 1.56×10^5 M^{-1} cm^{-1} and 46 as constant factor of lipid peroxidation. Results were presented as means of triplicate determinations with standard deviations.

Determination of malonaldehyde in the oils: The method of Gutteridge (1984) was followed in the determination of malonaldehyde in the bark extract treated stored vegetable oils and the bark extract free controls. Two ml of phosphate buffer (0.024M phosphate pH 7.4 in 0.15M NaCl) was added to 0.5ml oil samples followed by the addition of 0.5ml of 1% 2-Na-thiobarbituric acid in 0.05N NaOH and immediately prompt addition of 0.5 ml of 25% HCl. The tubes were mixed thoroughly, stoppered with cotton wool, and put in boiling water bath for fifteen mins at 100° C. Absorbance were read at 532nm against appropriate banks. Amount of malonaldehyde formed was calculated by the thiobarbiruric acid formula using absorbance and 1.56×10^5 cm^2 $mmol^{-1}$ as the molar absorbance coefficient. Results were presented as mean with standard deviations and statistical comparison set at $P < 0.05$.

Determination of the effect of bergenin on peroxidative deterioration of stored vegetable oils: The principle and procedure for the determination of the effect of samples were as described earlier for the study with crude extract except that bergenin was used in place of the bark extract. However, the bergenin was prepared 1:1 (w/v) in phosphate buffer pH 7.4 and dispensed in the ratio of 5:25 (w/v). Non-bergenin fractions of the bark extract were not used for comparison. Malonaldehyde was determined by the method of Gutteridge (1984) while Malondialdehyde was determined by the method of Hunter *et al.* (1963). Results were treated as means with standard deviations.

Results

The results of the effect of the bark extract on peroxidative deterioration of stored vegetable oils are summarized in Table 1. For each of the three-peroxidation products and each vegetable oil, the concentration in extract-treated oil was significantly lower than in the untreated controls. This would appear to suggest that the bark extract inhibited peroxidation of palm oil and soybean oil by exerting antioxidant action at the three points steps of lipid peroxidation pathway investigated namely, chain initiation and propagation stages leading to the first stable intermediate lipid hydroperoxide, the oxidation of hydroperoxide carbonyl carbons to dialdehyde and the oxidation of hydroperoxides to aldehydes (Wills, 1987). On the average, treatment with bark extract reduced the rate of production of lipid hydroperoxides the first stable intermediate of lipid peroxidation pathway by 83.33% and 56.76% in palm oil and soybean oil respectively. The corresponding values for malondialdehyde and malonaldehyde are 31.3, 48.24, 43.48, and 30.44%.

The concentrations of the indices of lipid peroxidation in locally processed ground nut oil (Kulikuli) and soybean oils were significantly lower in the bergenin - treated oils than their respective untreated oils (Table 2). For malonaldehyde, the concentrations in the treated palm oil was significantly lower than in untreated palm oil. This would suggest that bergenin exhibited antioxidant action against peroxidative deterioration of kuli-kuli and soybean oils at the levels of hydroperoxide carbonyls leading to oxidation of hydroperoxide carbonyl to dialdehyde. In the palm oil, the inhibition of propagation led to oxidation of hydroperoxide carbonyls to aldehyde.

The pattern of formation of malonaldehyde was also the same in kuli-kuli and soybean oils being less in bergenin treated tests than their respective bergenin - free controls though not significantly. This set of data demonstrates clearly that bergenin has antioxidant properties against deterioration of stored vegetable oils.

Table 1: Effect of *S. gabonensis* stem bark extract on various stages of peroxidative deterioration of commercial vegetable oils stored for three months

	Concentration of peroxidation products		
Vegetable oils	Lipid hydroperoxide (mol/l)	Hydroperoxide carbonyl (mg malonaldehyde/mg)	Hydroperoxide aldehyde (mg malondialdehyde/ml)
Palm oil	3.12± 0.18	1.06± 0.11	0.23± 0.04
Palm oil + Bark extract	0.52± 0.08[a]	0.73± 0.13[a]	0.13± 0.03[a]
Soybean oil	0.37± 0.07	0.85± 0.06	0.23± 0.03
Soybean oil + Bark extract	0.16± 0.02[b]	0.44± 0.04[b]	0.16± 0.02[b]

Values are means ± S. D. of triplicate determinations. [a]Significantly lower than untreated palm oil ($P < 0.05$)
[b]Significantly lower than untreated soya bean oil ($P < 0.05$)

Table 2: Effect of the level of malondialdehyde and malonaldehyde in palm and commercial vegetable oils during lipid peroxidation after 30 days

	Concentration of peroxidation products	
Oil samples	Hydroperoxide carbonyl (mg malondialdehyde/ml)	Hydroperoxide aldehyde (mg malonaldehyde/ml)
Kuli-kuli	0.157± 0.01	0.126± 0.02
Kuli-kuli + bergenin	0.127± 0.02[a]	0.098± 0.01
Palm oil	0.307± 0.02	0.074± 0.02
Palm oil+ bergenin	0.276± 0.05	0.033± 0.01[b]
Soybean oil	0.178± 0.04	0.239± 0.04
Soybean oil + bergenin	0.01± 0.03[c]	0.209± 0.06

Values are means + S. D of 3 most reliable determinations. [a]Significantly lower than untreated kuli-kuli oil ($P < 0.05$)
[b]Significantly lower than untreated palm oil ($P < 0.05$) [c]Significantly lower than untreated soya bean oil ($P < 0.05$)

Discussion

Free radicals can be originated endogenously from normal metabolic reactions or exogenously as components of tobacco smoke, and air pollutants and indirectly through the metabolism of certain solvents, drugs, pesticides and by exposure to radiations. These free radicals cause extensive damage to cells *in vivo* as seen in lysis of human erythrocytes where endogenous antioxidants like ascorbate, catalase and glutathione protect (Sugiyama *et al.,* 1993) or *in vitro* as seen in peroxidative deterioration of stored vegetable oils. Other defense systems against free radical damages include tocopherol (vitamin E), B-carotene, uric acid, bilirubin and several metalloenzymes such as catalase (Fe), superoxide dismutase (copper, zinc and manganese), glutathione peroxidase (selenium) and other proteins such as ceruloplasmin (copper) (Machlin and Bendich, 1987; Kumar *et al.,* 1988). Since the endogenous antioxidants acting as intracellular defense systems protecting cells from free radical damages and extensive lysis (Sugiyama *et al.,* 1993), scavenging and diminishing formation of oxygen - derived species are not 100% efficient, hence not sufficiently strong enough (Halliwell, 2012), dietary derived antioxidants or micro nutrients are particularly important in diminishing the cumulative oxidative damages. We recently reported the presence of core elements that are necessary in antioxidant enzymes in *S. gabonensis* stem bark extract (Maduka and Okoye, 2001c). For this and the above reasons, we have directed this research to the question of whether *S. gabonensis* stem bark extract and bergenin may exert antioxidant properties on the peroxidative deterioration of stored vegetable oils. This was with a further view of discovering antioxidant food additives. Deductions from indirect (Okoye and Neal, 1988a, 1988b, 1991) and direct evidences of experimental membrane lipid peroxidation (Maduka and Okoye, 2001a, 2001b) as well as *in vitro* evidences of peroxidation of human erythrocytes (Maduka *et al.,* 1999, Maduka and Okoye, 2000) suggested and later confirmed that *S. gabonensis* stem bark extract posses antioxidant properties against lipid peroxidation.

Treatment with the bark extract significantly reduced the formation of lipid hydroperoxide, the first major product of lipid peroxidation (Wills, 1987), ($P< 0.05$) over a storage period of three months in all the vegetable oils investigated compared with the bark extract free (controls). This observation particularly would suggest a possible role for the bark extract in preservation of foods as an exogenous antioxidant additive. The formation of lipid hydroperoxide carbonyls as determined by malondialdehyde and lipid hydroperoxide aldehyde determined as malonaldehyde were significantly reduced ($P< 0.05$) by pretreatment with the bark extract. Lipid hydroperoxides are formed at intermediate stages after oxidation of lipid peroxidation pathway in the reaction between isomerized fatty acid free radical (lipid peroxyl radical) and oxygen (Murray *et al.,* 1993). These carbonyl centers of lipid hydroperoxides combine with thiobarbituric acid to give a complex that absorbs maximally at 530nm and therefore, determined spectrophotometrically at that wavelength. The complex formed can be detected quantitatively as malonaldehyde (Dumelin and Tappel, 1977) which though unstable, correlates with other assays like determination of low molecular weight or volatile hydrocarbon gases, vitamin E used in assessment of biological antioxidant properties and lipid peroxidation. Malondialdehyde also formed as a stable lipid peroxidation product of polyunsaturated fatty acids undergoing peroxidation is reliably detected spectrophotometrically at 532nm. The formation of these lipid hydroperoxide carbonyls and aldehydes, malondialdehyde and malonaldehyde respectively were significantly reduced by the bark extract pretreatment as compared with the bark extract free stored vegetable oil controls. This set of data demonstrates clearly that pretreatment with *S. gabonensis* bark extract protected against lipid peroxidation deterioration of the vegetable oils over the period of storage. The same trend of results was essentially observed with bergenin, the isolate of the bark extract. Pretreatment with bergenin reduced the formation of thiobarbituric acid reactive substances, malondialdehyde and malonaldehyde significantly ($P< 0.05$) in the vegetable oils on a time course basis compared with the untreated controls suggesting that lipid peroxidation was inhibited. Plasma thiobarbituric acid reactive substances were measured to assess lipid peroxidation and free radical activity and thereafter, reported to be a better index of overall free radical activity (Hunniselt *et al.,* 1995). The results of this study have shown with additional evidences that even in vegetable oils, lipid peroxidation could be inhibited thus confirming the possibility of using *S. gabonensis* bark extract and bergenin as antioxidant additives in foods and drugs. This result would emphasize the need to compare the bark extract and bergenin with known commercial food additives like butyl hydroxyl toluene (BHT) and butyl hydroxyl anisole (BHA) to establish the limits of efficiency of the former. Soybean oil was chosen as one of the stored vegetable oils for investigation because saturated hydrocarbon gases were shown to arise early during the auto-oxidation of oil from soybean and methyl linoleate (Dumelin and Tappel, 1977). Pentane was also the predominant short chain hydrocarbon gas product noted during composition of linoleic acid (Dumelin and Tappel, 1977). The release of hydrocarbon gases (pentane, ethane), presence of fluorescent products that arise in part by malonaldehyde reactions with other biological compounds and release of ethane by rats fed vitamin E deficient diets were used to determine the rancidity of oils and oxidation processes in dehydrated food systems with good correlations in results. These were sensitive indices of evaluation of lipid peroxidation and mechanism of operation.

The results of this series of investigations have encouraged us to conclude that the bark extract and bergenin mechanism of action included inhibiting the propagation of lipid peroxidation by definitely reducing the rate of formation of key intermediate products of lipid peroxidation pathway (lipid hydroperoxide, malondialdehyde and malonaldehyde) responsible for peroxidation of stored vegetable oils.

Acknowledgment

The investigations reported here were partly funded by Senate Research Grant No SRGC/1992/93/001 to Professor Z.S.C. Okoye at the University of Jos and the Governing Council of University of Maiduguri, who awarded study fellowship to Dr. H. C. C. Maduka under their staff development programme for Ph.D. degree in Biochemical Toxicology.

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