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Dr. Siva Nageswara Rao
Mekala, Department of Clinical Pharmacology, State University of Zanzibar - Tanzania

Dr. Anand Paramasivam
Department of Physiology, RVS Dental College and Hospital, Tamil Nadu Dr. M.G.R. Medical University, Coimbatore, Tamilnadu, India

Dr. Ali Said Yussuf
Department of Surgery, State University of Zanzibar, Tanzania

Dr. Murugesan Annamalai
Department of Biochemistry, RVS Dental College and Hospital, Tamil Nadu Dr. M.G.R. Medical University, Coimbatore, Tamilnadu, India

Dr. Arul Amuthan
Department of Basic Medical Sciences, Manipal Academy of Higher Education, Manipal University, Manipal, Karnataka, India

Dr. Saravanan R
Department of Pharmacology, RVS Dental College and Hospital, Tamil Nadu Dr. M.G.R. Medical University, Coimbatore, Tamilnadu, India

Dr. Anand Paramasivam
Department of Physiology, RVS Dental College and Hospital, Tamil Nadu Dr. M.G.R. Medical University, Coimbatore, Tamilnadu, India

Corresponding Author:
Dr. Siva Nageswara Rao
Mekala, Department of Clinical Pharmacology, State University of Zanzibar - Tanzania

Valorization of Soursop flowers (*Annona Muricata* L.) as a potential source of natural antioxidants for Palm Olein stabilization during accelerated storage

Siva Nageswara Rao Mekala, Anand Paramasivam, Ali Said Yussuf, Murugesan Annamalai, Arul Amuthan, Saravanan R and Anand Paramasivam

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Abstract
This research aims at exploring the possibility of using *Annona Muricata* L. (soursop) flower extracts as a natural antioxidant to stabilize palm olein in accelerated storage. Oxidative stability of palm olein was tested by the addition of different levels of soursop flower extract 200-1800 ppm and exposure to accelerated oxidation at 70oC in 30 days. The extract was compared with a synthetic antioxidant butylated hydroxytoluene (BHT) in terms of its efficacy. Conclusion of the Rancimat and Schaal oven testing showed that the soursop flower extract effectively increased the induction time, decreased the formation of peroxides and suppressed the formation of secondary oxidation products. The extract also maintained the amount of the linoleic acid in palm olein, which proved its potential as the substitute, natural antioxidant. The presence of the following phenolic compounds like quercetin, caffeic acid, and ferulic acid was determined using high-performance liquid chromatography (HPLC) analysis, and this led to the antioxidant effect. This indicates that palm olein can be stabilized using soursop flower extracts and this will create a viable source of natural antioxidant to replace synthetic preservatives.

Keywords: Natural Antioxidants, Soursop flower extract, Palm olein, Oxidative stability

Introduction
Oil, fats, and lipid-containing foods are subject to decay when heated or stored over a long period of time, mainly through oxidation. This oxidation causes food to lose the nutritional value of food, and therefore, the fatty acids, amino acids, vitamins, and the digestibility of the protein is lost [1]. Also, oxidation reduces the organoleptic characteristics of foods and may produce potentially toxic oxidation products related to degenerative diseases, including cancer and cardiovascular diseases. This causes products that are oxidized to be rejected by the consumers and industries lose a lot of money [2]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ter-butylhydroquinone (TBHQ) have also been applied as food preservatives in an attempt to prevent these deterioration reactions. Nevertheless, the questions about their health effects such as association with cancer and cardiovascular diseases have caused growing limitations in their usage in most countries [3]. In specific, BHA and BHT are volatile and readily degrade under high temperature, which limits their usefulness [4, 5]. This has triggered the consumer demand of natural antioxidants which are viewed as safer alternatives, and provides more health benefits [6]. Natural antioxidants arguably phenolic compounds or polyphenols are natural antioxidants that have antioxidant activity because of their structure, hydrogen-donating ability, metal-ion chelation capability and synergies. There has been a lot of research on the application of the natural plant extracts in stabilizing the edible oils [7]. Yet, very limited natural sources have been approved of industrial use amongst them rosemar. Consequently, this has led to the increased interest in the search of alternative natural antioxidants.
Annona Muricata L. or soursop is a small shrub of the Annonaceae family or evergreen tree. It is 5-6 meters tall, evergreen, has unique flowers and these appear on the trunk, branches or twigs. The flowers are yellow/green and pale-yellow inner petals. The oval oyster-shaped size of the fruit varies between 10-30 cm.

The soursop is an indigenous tree that has been utilized in traditional medicine across various tropical parts of South and North America, and even West Africa to treat various ailments, such as cancer, diabetes, hypertension, and infections. Other researchers have emphasized that its leaves, fruits, and bark have antioxidant properties, yet no one has studied the antioxidant activity of its flowers [8, 9].

The soursop tree has a lot of potential as its flowers are easily lost during flowering season because of an upsurge in wind causing the flowers to blow away. The purpose of the paper will be to assess the possibilities of soursop flower extracts as natural antioxidant to stabilize palm olein, a popular unsaturated oil in Cameroon. In particular, the study will aim at peroxidation reduction and preservation of linoleic acid in palm olein that was put through an accelerated oxidation process at 70oC in 30 days.

Materials and Methods

Extraction of Soursop Flower Antioxidants

The soursop flowers were dried in an electric air-dried oven at 50 C and dried to powder shortly (48 hours). About 100 g of the powdered flowers were taken with 800 mL of methanol and left to extract in 48 hours at room temperature with frequent shaking. A portion of the extract was filtered using Whatman No. 1 filter paper and the residues re-extracted using 400 mL of methanol in order to get the maximum amount of phenolic compounds. Rotary evaporation was used to concentrate the filtrates at 40 0 C with low pressure, and the evaporated extract was stored at 4 C to conduct further analysis.

Phytochemical Analysis

Total Phenolic Content

FolinCiocalteu colorimetric method was employed to find the total phenolic content [10]. The aliquot of the extract (20 mL) was added to Folin -Ciocalteu reagent (0.2 mL), distilled water (2 mL), and 20% sodium carbonate (1 mL). The 2-hour incubation was carried out in room temperature and the absorbance of 765 nm was recorded with the gallic acid taken as the standard.

High-Performance Liquid Chromatography (HPLC)

The phenolic composition in the extract was measured with the help of a reverse-phase HPLC system (Agilent 1200 series) that has a quaternary pump and Diode Array Detector (DAD). Separation was done in an RP-C18 Lichrospher column (5 uM, internal diameter of 4.0 mm x 250 mm) using an isocratic flow of acetonitrile and 0.1M orthophosphoric acid in water (70: 30 v/v) at a flow rate of 1 mL/min. Detection was done at 280 nm.

Antioxidant Preliminary Tests

DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging Assay DPPH Radical Scavenging. DPPH method was used to determine the radical scavenging activity of the extract [11]. The extract was dissolved in different concentrations that were combined with DPPH and the absorbance at 517 nm was recorded. As positive control was used butylated hydroxytoluene (BHT).

Ferric Reducing Antioxidant Power (FRAP)

The method of Oyaizu (1986) was used to determine the reducing power of the extract where a combination of extract, phosphate buffer and K3Fe (CN) 6 solution was used. The mixture was incubated then centrifuged followed by the measurement of the absorbance at 700 nm.

Soursop Flower Extract Effect on Palm Olein Oxidation

Sample Preparation: Methanolic extract of soursop flower was introduced to 100g of preheated refined palm olein at different concentrations (200, 600, 1000, 1400 and 1800ppm). There was also a control sample which was made without additives. Rancimat test and Schaal oven test were done on the samples.

Rancimat Test

A Metrohm Rancimat model 892 was used to measure the induction period of stabilized and control palm olein samples with the sample being heated at 110oC. The induction period was automatically taken with calculation of protection factors.

Schaal Oven Test

To accelerate oxidation, samples were stored at 70oC in 30 days. Peroxide value, p-anisidine, total oxidation (TOTOX), Thio barbituric acid, and iodine values were analyzed in samples collected after every 10 days using standardized procedures.

Measurement of the Oxidation Parameters

The peroxide value, p-anisidine, iodine value and TOTOX were measured according to the known procedures. The values of Thiobarbituric acid were calculated [12-14].

The Soursop Flower Extract on the Linoleic Acid Profile of Palm Olein during Storing

Fatty Acid Methyl Esters (FAMEs) Preparation: Transesterification was done using 2% sulfuric acid in methanol to make the fatty acid methyl esters (FAMEs) of the stabilized and control palm olein samples, as shown by Christie (1993). The FAMEs had been put into ethyl acetate and thoroughly washed with water to eliminate all the excess acid and dried in the presence of anhydrous sodium sulfate. Dried esters were examined using gas chromatography (GC/FID) and all analyses were done in a duplicated manner.

Gas Chromatography (GC)

Agilent 7890A series gas chromatograph (Agilent Technologies, Palo Alto, CA) with FID detector and DB-225 capillary column (30 m x 0.25 mm film thickness 0.25 mm) were used to carry out GC-FID analysis. The column temperature was first adjusted to 160 o C, then 220 o C at the rate of 5 o C/min and maintained 10 min in 240 o C. The carrier gas was nitrogen and its flow rate was 1.5 mL/min. The injector and detector temperatures were 230 o C and 250 o C respectively, and the ratio of the split was 50:1. The identification of linoleic acid was done by comparing the retention times with the standard reference fatty acid methyl esters in the same conditions.

Table 1: Effect of Soursop Flower Extract on the Oxidative Stability of Palm Olein (Rancimat Test)

Sample	Induction Time (hrs)	Protection Factor (PF)
Control	4.5	1.0
PO + BHT 200 ppm	6.3	1.4
PO + An.M 200 ppm	6.0	1.3
PO + An.M 600 ppm	6.8	1.5
PO + An.M 1000 ppm	7.2	1.6
PO + An.M 1400 ppm	7.5	1.7
PO + An.M 1800 ppm	8.0	1.8

Table 2: Oxidation Parameters of Palm OleinDuring Storage (Schaal Oven Test)

Sample	Peroxide Value (meq/kg)	p-Anisidine Value	TOTOX Value	Thiobarbituric Acid (TBA)	Iodine Value (g I2/100g)
Control	12.3	7.5	28.8	3.2	65.4
PO + BHT 200 ppm	7.2	3.5	14.4	2.5	68.0
PO + An.M 200 ppm	8.0	4.0	16.0	2.8	69.0
PO + An.M 600 ppm	6.5	3.2	14.4	2.2	70.0
PO + An.M 1000 ppm	6.0	2.8	12.8	1.8	71.5
PO + An.M 1400 ppm	5.5	2.5	11.5	1.5	73.0
PO + An.M 1800 ppm	5.0	2.2	10.4	1.3	74.5

Results and Discussion

Extraction Yield, Total Phenolic Content, and Phenolic Antioxidants Detected by HPLC

The 8.35% was obtained using the methanolic extract of soursop flowers. This was less than the 10.80% that George *et al*. (2012) recorded on the butanolic extracts of soursop leaves. The amount of total phenolic content of the soursop flower extract was 51.33 mg/g, which was larger compared to the results achieved by George *et al*. (2012) in the butanolic extracts of soursop leaves. Such variations in yield and phenolic composition may be explained by differences in the part of the plant, environment, time of collection of the sample and the method of determination.

The HPLC-DAD chromatograph of the soursop flowers methanolic extract indicated the presence of some phenolic antioxidants, which comprised vanillic acid (RT: 9.140 min, peak area: 7.74%), caffeic acid (RT: 9.375 min, peak area: 4.90%), ferulic acid (RT: 9.871 min, peak area: 13.07%), ellagic acid (RT: 10. The compounds are famous in terms of their high antioxidant properties, with quercetin being the most common compound. These results are in line with those of Georges *et al*. (2014) who found quercetin in alcoholic extracts of soursop leaves.

Primary Preliminary Antioxidant Tests DPPH and FRAP Assays

The radical scavenging behavior of the soursop flowers extract was evaluated by means of the DPPH radical scavenging test and the Ferric Reducing Antioxidant Power (FRAP). The two assays showed that the antioxidant activity of the extract improved with concentration. The extract radical scavenging activity was similar to the BHT and reducing power was similar to catechin. These findings affirm the high antioxidant capacity of the soursop flowers which is owed to the presence of phenolic compounds in the extract. Barreira *et al*. (2008) also identified a similar finding with respect to chestnut flowers that had high antioxidant properties.

Soursop Flower Extract Effect on Palm Olein Oxidative Stability

Rancimat Test: To test the impact of the Rancimat test, the effect of the soursop flower extract on the palm olein oxidation was performed. Table 1 presents the variables that indicated that the induction time and the factors of the protection of the oil samples with the antioxidants were significantly higher ($P < 0.05$) than those of the control. The activity of the soursop flower extract at any concentration was significantly superior ($P < 0.05$) to BHT, which implies that it has better thermal stability and antioxidant capacity. The period of induction and the factors of protection were on the rise with the concentration of the extract and this illustrates its good antioxidant effects. Such findings are in line with those of Iqbal and Bhanger (2007) who discovered that extracts of garlic and pomegranate increased the stability of sunflower oil.

Schaal Oven Test

The Schaal oven test was performed so as to imitate accelerated oxidation. The peroxide values of oil samples containing soursop flower extract were significantly lower in comparison to the control which demonstrates that the extract was effective in the inhibition of peroxides. The more concentrated samples of palm olein (PO + An.M 1400 ppm, PO + An.M 1000 ppm, PO + An.M 1800 ppm) demonstrated better resistance to the formation of peroxides, and the values were similar to the values of the oils that contain BHT. This proves the efficacy of the extract in inhibiting the formation of primary oxidation products.

p-Anisidine Value

Production of secondary oxidation products, as indicated by p-anisidine value, was much lower ($P < 0.001$) in oils containing soursop flower extract than the control, which was another indication that the extract was effective in inhibiting the production of secondary oxidation products. This action had the same effect as in the case of BHT and showed that the antioxidants present in the extract are phenolic in nature and inhibit the breakdown of hydroperoxides.

Totox Value

Totox value that quantifies both hydroperoxides and products of hydroxyl radicals was less in oils with soursop flower extract than in control, indicating the potential of the extract in decreasing total oxidation. TOTOX values of oils

containing soursop extract were concentration-dependent whereby the high concentrations of the extracts offered a better oxidative stability.

Thiobarbituric acid (TBA) Value

The TBA test revealed that the oils with the soursop flower extract had lower TBA values compared to the control, which is a low amount of malonaldehyde formation, a secondary oxidation product. The TBA value of the oils containing the concentration of 600-1800 ppm was also found to be much lower ($P < 0.001$) in as compared to the control and this further substantiates the antioxidant properties of the extract.

Iodine Value

The values of iodine which show the unsaturated fatty acids were reduced with the course of storage. Nonetheless, oils with the addition of soursop flower extract experienced much less reduction of the iodine value than the control which means that the extract was effective in preventing the oxidation of unsaturated fatty acids. The extract activity was similar to that of BHT.

Soursop Flower Extract on Linoleic Acid Profile of Palm Olein

The effect of soursop flower extract on a palm olein in terms of linoleic acid profile during storage. Palm olein had a level of 10.97 of linoleic acid. There was a reduction in the level of linoleic acid content of all the samples after 30 days of the heating process where oils with the extract retained more linoleic acid than the control and the BHT-treated samples. This implies that the extract was effective in preventing oxidative degradation of linoleic acid, which again justified its possible role as a natural antioxidant.

Conclusion

To conclude, the *Annona Muricata* (soursop) flower extract is a potential natural antioxidant in stabilizing palm olein in the accelerated oxidation. The extract contributed greatly to oxidative stability of palm olein, and improved the results than BHT in some tests. Also, the maintenance of the level of linoleic acid and prevention of primary and secondary oxidation products prove the usefulness of the extract as a natural substitute of synthetic antioxidants in edible oils.

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