Science Letters ISSN 2345-5463 – An International Triannually Journal

Research Article
August 2017 | Volume 5 | Issue 2 | Pages 173-179

ARTICLE INFO

Received
February 16, 2017
Accepted
April 24, 2017
Abstract Published
May 04, 2017

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Keywords
Bauhinia racemosa
Sunflower oil
Extraction
Antioxidants
Efficacy

How to Cite
Inam-u-llah, Akhtar S, Aadil RM, Piao FY, Ismail T, Riaz M, Suleman R, Roobab U, Ramzan M. Use of Bauhinia racemosa flower extract as an antioxidant agent for the stabilization of Sunflower oil. Sci Lett 2017; 5(2):173-179

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Use of *Bauhinia racemosa* Flower Extract as an Antioxidant Agent for the Stabilization of Sunflower Oil

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Abstract

The study was designed to assess the efficacy of *Bauhinia racemosa* flower in the stabilization of sunflower oil. The concentrated water and ethanol extracts of the *B. racemosa* flowers at varying doses were used with the sunflower oil. Ethanol (100%) and distilled water (100%) were used for the preparation of ethanolic and aqueous extracts of dried powder of *B. racemosa* flowers by using rotary evaporator, respectively. Both extracts were used at the concentration of 200, 400 and 600 ppm separately and compared with butyl hydroxyl toluene (100 ppm) and ascorbic acid (300 ppm). Both extracts were effective in controlling free fatty acids, acid values, and peroxide values. Most significant activity was seen with the ethanolic extracts while aqueous extract also showed a potent activity towards the stabilization of sunflower oil. The 200 ppm dose of aqueous extract was found to be the most efficient while both the extracts exhibited variable patterns against the lipid peroxidation, while for the thiobarbituric reactive substances, ascorbic acid was the most effective. The results of this study showed that *B. racemosa* could be used as a natural source of antioxidants in the stabilization of oils and also for increasing their nutritive profile due to natural bioactive components.

Introduction

Oils from plant origin are considered as an important part of our diet. These oils are vital in providing the essential fatty acids, which are components of various lipids in the body and hormones [1]. Nowadays, many degenerative diseases such as inflammation, neurogenesis, carcinogenesis, cardiovascular diseases and premature aging in human and animals occurred after the processing of fats and oil deterioration due to the production of free radical and active oxygen species [2]. Presently, the oil industry has concerns on preventing this phenomenon as it can cause a number of problems in food products containing oils and fats [3]. Polyunsaturated fats are the main reason of oxidation in oils; their higher amount initiates this process. To overcome this problem, a number of synthetic antioxidants have been used in oils, like butyl hydroxyanisole (BHA), butyl hydroxyl toluene (BHT) and tertiary butyl hydroxy quinine (TBHQ). But all these have been reported to cause carcinogenesis and mutagenesis effects with continuous use in food products [4, 5]. Native or advanced varieties of *Bauhinia* (*Fabaceae*) trees are widely consumed in Brazilian cities as food for human, animal forage, and also have ornamental characteristics and diuretic properties which can also be used as phytomedicines in the treatment of diabetes [6, 7].

Previous studies have proven *B. variegate* as a free radical scavenging agent. The dose-dependent effects of their aqueous and alcoholic extracts have been studied using several oxidation systems [8, 9]. Data on the antioxidant potential of Bauhinia is available, but no study has been found to imply this antioxidant activity for edible oil preservation. The current study is an attempt for this implication. In Pakistan, sunflower (*Helianthus annus*) is one of the major crops that are commercially cultivated for their edible, aesthetic or ornamental possessions. It produces sunflower oil, as an edible ingredient that is used for food preparation processes like cooking, frying, etc. This oil has a major portion of linoleic acid (60–75%) and less than 90% of combined oleic and linoleic acids with the exception of linolenic acid. A major portion of that oil consists of triacyl glycerol as 39% of linoleic, linoleic oleic, 19% of linoleic oleic oleic, 14% of linoleic linoleic linoleic, 14% of linoleic linoleic stearic, 11% of linoleic oleic stearic and 3% of other triacyl glycerols. It is also known as cooking oil; valuable product consumed in various products like soft spreads. Sunola or Highsunare, high oleic varieties have been reported in the USA, comprises of about 85%-90% of oleic acid, a tremendous source of oleic oil to meet the growing demand. In the United States, another variety called NuSun with ≈60% oleic acid has also been cultivated [10]. The purpose of the current study was to explore the efficacy of natural antioxidants for shelf life stability of sunflower oil in an attempt to replace the synthetic ones with natural one which are said to be healthy.

Materials and Methods

Collection of flower samples

The fresh flowers of the *B. racemosa* were collected in early April from the local vicinity of Multan, Pakistan. The sepals were removed and remaining flowers were dried in shade at room temperature [11]. These dried flowers were ground using a grinder, sieved (1 mm sieve) and stored in a moisture proof and airtight transparent plastic jars.

Proximate analysis

The petals of the *B. racemosa* were analyzed for the proximate analysis using the method as described by the Association of Official Analytical Chemists (AOAC) manual [12].

Preparation of extracts

Aqueous and ethanolic extracts were prepared by using powder and dissolving in a ratio of 1:3 (25g powder was mixed with a solvent to make 100 ml solution) and without using any mixture of solvents, i.e., only distilled water and ethanol were used separately. The mixture was later filtered using Whatman filter paper No 42. Then the filtrates were concentrated using rotary evaporator [11]. The concentrates prepared were dried in an oven set at temperature 40ºC. The dried extracts were preserved in air tight plastic bottles and stored at -80ºC.

Preparation of sunflower oil samples

To determine the stability of sunflower oil, refined, bleached and deodorized (RBD) sunflower oil was bought from a well-known edible oil processing plant in Multan, Pakistan. The sample was divided into 11 parts and every part was subjected to a separate treatment. Each treatment was applied to 200 ml sunflower oil. After uniform mixing, every treatment was divided into 50 ml volumes. In this way, there were four subsamples for every treatment. About 50 ml oil was poured into the tan colored glass bottles and closed with a lid. These bottles were placed at 65ºC for accelerated storage. After every week, one bottle of every treatment was obtained from storage and subjected to analysis. The treatments were as follows: (T0) Control; (T1) BHT as positive control, 100 ppm; (T2) ethanolic extract, 200 ppm; (T3) ethanolic extract, 400 ppm; (T4) ethanolic extract, 600 ppm; (T5) water extract, 200 ppm; (T6) water extract, 400 ppm; (T7) water extract, 600 ppm; (T8) ascorbic acid, 300 ppm; (T9) mixture of 25% ascorbic acid + 75% ethanolic extract, 200 ppm; (T10) mixture of 25% ascorbic acid + 75% water extract, 200 ppm

Total phenolic content

Total phenolic contents (TPC) of the extracts were determined in terms of gallic acid equivalent (GAE) by using a spectrophotometer as mentioned by Chew et al. [13].

Diphenyl-picrylhydrazyl (DPPH) radical scavenging assay

The extracts were analyzed for antioxidant activity by DPPH radical scavenging assay according to the method described by Blois [14] with some modification. The IC_{50} value or percentage reduction was calculated from a graph drawn and the equation generated was (y = -0.001x+0.1854), while ascorbic acid was used as standard

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant activity of the extracts was measured by ferric reducing antioxidant power (FRAP) assay using the method of Benzie and Strain [15]. $FeSO_4$ was used as standard and results were calculated in µM/g.

Analysis of stored sunflower oil

Free fatty acids

Free fatty acids were determined by the method of Awan and Rehman [16]. Five g sample was taken and 30 ml ethanol was added, swirled to dissolve fatty acids. 1-2 drops of phenolphthalein indicator were added. Then, the sample was titrated against 0.1 N NaOH until the pink color persisted for 15 seconds. Then the amount of FFA was calculated according to the following formula:

FFA (µM/ml) = Titer × normality of NaOH × 28.2 / weight of sample

Acid value

The acid value was calculated from free fatty acid value. It was assumed that oil is free from oryzanol as oil was sunflower oil and it was freshly processed. The total free fatty acid value was multiplied by 1.99 to determine the acid value of oil [17].

Acid value = free fatty acid × 1.99

Peroxide value

Peroxide value is the indication of the oxidation suffered by oil. Peroxide value was determined by the method of Association of Official Analytical Chemists (AOAC) [12]. The value of peroxide was calculated by the formula given below:

Peroxide value = Titer× N (normality of $Na_2S_2O_3$) × 100 / weight of sample

Thiobarbituric acid reactive substances (TBARS)

In order to determine the extent of lipid oxidation, thiobarbituric acid (TBA) reactive substances were determined by the method of Hodges et al. [18]. Two ml of sample was added to a test tube containing 2 ml of either of the two tubes containing following solutions:

1. −TBA solution: 20% (w/v) trichloroacetic acid and 0.01% butylated hydroxy toluene
2. +TBA solution: 20% (w/v) trichloroacetic acid, 0.01% butylated hydroxytoluene and 0.65% TBA.

Samples were heated at 70 ºC in a water bath for 30 min, cooled and centrifuged at 1300g. The

absorbance at 440 nm, 532 nm, and 600 nm were measured.

TBARS were calculated by following formula:

$A = [(Abs_{532} + TBA) – (Abs_{600} + TBA) – (Abs_{532} – TBA – Abs_{600} – TBA)]$

$B = [(Abs_{440}+TBA –Abs_{600}+TBA) 0.0571]$

MDA equivalents / TBARS (nM/ml) = $(A-B/157000) 10^6$

Results and Discussion

Physicochemical analysis

Physicochemical analysis of Bauhinia flowers showed that on the oven dry weight basis, these were a rich source of minerals and carbohydrates like other vegetables (Table 1). These results were relatively different from Verma et al., [19] probably due to the use of different commodities or different sample collection area. They studied fresh flower buds, but in the current study, whole dried flower was used except sepals.

Total phenolic content

The study showed that the total phenolic contents of extracts were increased in a concentration-dependent manner, i.e., increasing the concentration of sample increased the phenolic content (Table 2). This trend was seen with both types of extracts, i.e. ethanolic as well as water extract. This increase indicated that by increasing the amount of extract, the amount of total phenolic contents also increases, which would increase the antioxidant potential as suggested by Hatano et al. [20].

Free radical scavenging ability

The results of the DPPH assay showed that the inhibition of free radical scavenging ability was increased with the increase in the amount of extract and water extract showed the higher inhibition rate compared to ethanolic extract (Table 3). This type of trend was also shown in the extracts of *B. variegata* as reported by Negi et al. [21]. Generally, the free radical scavenging ability depends upon total phenolic contents and is directly proportional to it. But in this study, a different pattern was shown which showed that only total phenolic contents are not responsible for radical scavenging ability, but other interfering or affecting compounds may also be present in the extract.

Ferric reducing antioxidant power ability (FRAP)

The results regarding FRAP are shown in Table 3 which showed that by increasing the amount of extract FRAP reading was increased which suggested that higher amount of extract has the higher reducing ability. This trend was in line with the study of Tsai et al., [22]. They noted that the antioxidant capacity of Roselle extract was increased when extraction time or weight of petals increased. FRAP assay showed a linear relationship with anthocyanin, so higher the amount of extract higher FRAP value and hence the higher amount of anthocyanins [22].

Table 1 Physicochemical contents of dried Bauhinia flower powder.

Physiochemical contents	Percentage
Moisture	8.04 ± 0.8
Ash	7.26 ± 0.7
Fat	1.99 ± 0.2
Protein	6.56 ± 0.65
Carbohydrates	76.16 ± 7.61
Fibre	9.42 ± 0.94
Dry matter	91.96 ± 0.46

Table 2 Total phenolic contents of different concentrations (ppm) of water and ethanolic extracts of Bauhinia flower powder.

Extracts	Total phenolic contents (mg/100g)		
	100 ppm	200 ppm	300 ppm
Ethanol	60±6.7	155±8.4	260±10.2
Water	19±3.3	54±5.3	73±7.3

Table 3 Free radical scavenging ability inhibition and ferric reducing antioxidant power (FRAP) of water and ethanolic extracts Bauhinia flower powder.

Extracts	Inhibition at 25 ppm (%)	FRAP (µM/g)
Ethanol	38 ± 3.8	939.75 ± 9.4
Water	49 ± 4.9	985.25 ± 9.3

Effect of preservatives on stored sunflower oil

Free fatty acids and acid value

From Table 4, it is obvious that both types of extracts have been effective against free fatty acids. In water extracts, T2 (200 ppm) was the most effective in overall storage period and for 2nd and 3rd weeks; its values were even lower than the positive control (T1). This trend suggested that extracts required some activation time that is why FFA values were slightly higher in week 1, but lower than control. A similar trend was shown in T3 FFA; values were decreased from week 1 until week 2 and then gradually increased later on. While in T4, FFA values were decreased until week 3 which might be due to the higher amount of antioxidants due to the high

Table 3 Free fatty acid values (µM/ml) of sunflower oil treated with different treatments of water and ethanolic extracts of Bauhinia flower powder after different storage time.

Treatments	1st week	2nd week	3rd week	4th week
T_0	0.54 ± 0.05bc	0.5 ± 0.06b	0.63 ± 0.06b	0.62 ± 0.06cd
T_1	0.47 ± 0.05cd	0.52 ± 0.05bc	0.56 ± 0.06bc	0.56 ± 0.06d
T_2	0.51 ± 0.05cd	0.47 ± 0.05cd	0.51 ± 0.05cde	0.59 ± 0.06d
T_3	0.74 ± 0.08a	0.41 ± 0.05d	0.54 ± 0.05bcd	0.79 ± 0.08ab
T_4	0.56 ± 0.05bc	0.51 ± 0.05bc	0.46 ± 0.05def	0.60 ± 0.06cd
T_5	0.43 ± 0.05d	0.60 ± 0.06ab	0.43 ± 0.04ef	0.57 ± 0.06d
T_6	0.74 ± 0.08a	0.58 ± 0.06ab	0.57 ± 0.06bc	0.71 ± 0.07bc
T_7	0.61 ± 0.06b	0.67 ± 0.07a	0.38 ± 0.04f	0.57 ± 0.06d
T_8	0.53 ± 0.05bc	0.58 ± 0.06ab	0.53 ± 0.05cd	0.63 ± 0.06cd
T_9	0.53 ± 0.05bc	0.60 ± 0.06ab	0.56 ± 0.06bc	0.63 ± 0.06cd
T_{10}	0.52 ± 0.05bcd	0.58 ± 0.06ab	0.75 ± 0.07a	0.90 ± 0.09a

Treatments having same letters are not statistically different from one another.

Table 4 Acid values (%) of sunflower oil treated with different treatments of water and ethanolic extracts of Bauhinia flower powder after different storage time.

Treatment	1st week	2nd week	3rd week	4th week
T_0	1.07 ± 0.11bc	1.14 ± 0.11b	1.25 ± 0.13b	1.24 ± 0.12cd
T_1	0.94 ± 0.09cd	1.04 ± 0.1bc	1.12 ± 0.11bc	1.12 ± 0.11d
T_2	1.02 ± 0.1bcd	0.94 ± 0.09cd	1.01 ± 0.01cde	1.18 ± 0.12cd
T_3	1.47 ± 0.15a	0.82 ± 0.08d	1.07 ± 0.11bcd	1.57 ± 0.16b
T_4	1.12 ± 0.11bc	1.01 ± 0.1bc	0.92 ± 0.09def	1.19 ± 0.12cd
T_5	0.84 ± 0.08d	1.19 ± 0.12ab	0.86 ± 0.09ef	1.14 ± 0.11d
T_6	1.48 ± 0.15a	1.16 ± 0.12ab	1.13 ± 0.11bc	1.40 ± 0.14bc
T_7	1.21 ± 0.12b	1.34 ± 0.13a	0.76 ± 0.08f	1.14 ± 0.11d
T_8	1.05 ± 0.11bc	1.16 ± 0.12ab	1.05 ± 0.11cd	1.25 ± 0.13cd
T_9	1.05 ± 0.11bc	1.19 ± 0.12ab	1.12 ± 0.11bc	1.22 ± 0.13cd
T_{10}	1.03 ± 0.1bcd	1.15 ± 0.12ab	1.49 ± 0.15a	1.80 ± 0.18a

Treatments having same letters are not statistically different from one another.

amount of extract, while in week 4 an increase was recorded. Ethanolic extract showed a slightly different trend with time, in T5, after week 1, FFA values were increased until week 2 then decreased and then increased again. While in T6, values were decreased until week 3, later on, an increase was recorded. Similar to T5 trend was shown in T7, but at a slower pace, this suggested that higher amount of antioxidants did interfere with FFA. Ascorbic acid and its combinations with the extracts did not show any favorable results, although every treatment was different from the others. The efficacy of treatments was changed every week, which suggested that the natural antioxidants did have some positive time-dependent effects on sunflower oil stability. These results were supported by a previous study of Iqbal et al. [23]. They used garlic extract, but their results were not exactly following the trend of the current study. This difference might be due to the environment of the experiment or difference in the antioxidant profile of two commodities. In the current study, FFA did not increase with time but showed fluctuations. Acid value showed the same trend as shown by FFA. Here also the efficacy of treatments changed every week as is obvious from Table 4.

Peroxides

When peroxide values were studied, it was found that peroxide values of each treatment were lower than control, suggesting that every treatment had some role in controlling peroxide values (Table 5). But here also the efficacy of treatments changed with time. T5 was found to be the most effective in the first week, but not later. In week 2, T3 showed effectiveness, while in week 3. T6 and T7 were effective and their values were equal to positive control T1. It suggested that synthetic antioxidants could be replaced with natural ones. In the last week, T3 showed the lowest value, followed by T8 (ascorbic acid), while T6 and T7 also had resembling values, but those were higher than T8. This trend can be explained by the results reported by Iqbal et al. [23], which supports our findings. In their study, control samples showed an increase in peroxidase values, but a decline was observed after 20 days. A similar trend of decline was also observed in the current study, but after week 3, rest of the treatments

Table 5 Peroxide values of sunflower oil treated with different treatments of water and ethanolic extracts of Bauhinia flower powder after different storage time.

Treatment	1st week	2nd week	3rd week	4th week
T_0	0.216 ± 0.02a	0.271 ± 0.027a	0.347 ± 0.035a	0.206 ± 0.021c
T_1	0.053 ± 0.005de	0.164 ± 0.016c	0.065 ± 0.0065f	0.138 ± 0.014d
T_2	0.071 ± 0.007d	0.092 ± 0.009de	0.119 ± 0.012e	0.098 ± 0.01ef
T_3	0.034± 0.003ef	0.074± 0.007e	0.169± 0.017d	0.049± 0.005g
T_4	0.048 ± 0.005e	0.098 ± 0.010de	0.233 ± 0.023bc	0.113 ± 0.011de
T_5	0.016 ± 0.003f	0.169 ± 0.017c	0.202 ± 0.020c	0.242 ± 0.024b
T_6	0.115 ± 0.012c	0.147 ± 0.015c	0.071 ± 0.007f	0.107 ± 0.011e
T_7	0.130 ± 0.013bc	0.111 ± 0.010d	0.065 ± 0.0061f	0.111 ± 0.011de
T_8	0.143 ± 0.140b	0.230 ± 0.023b	0.25 ± 0.025b	0.075 ± 0.0075fg
T_9	1.05 ± 0.11bc	0.159 ± 0.016c	0.088 ± 0.009ef	0.228 ± 0.023bc
T_{10}	1.03 ± 0.1bcd	0.152 ± 0.015c	0.167 ± 0.01d	0.311 ± 0.031a

Treatments having same letters are not different from one another.

Table 6 Thiobarbituric acid reactive substances values (µM/ml) of sunflower oil treated with different treatments of water and ethanolic extracts of Bauhinia flower powder after different storage time.

Treatment	1st week	2nd week	3rd week	4th week
T_0	3.47 ± 0.35c	3.69 ± 0.37c	4.23 ± 0.429b	4.35±0.43c
T_1	1.71 ± 0.17d	1.92 ± 0.19de	2.27 ± 0.23d	3.50±0.35d
T_2	4.11 ± 0.41c	8.60 ± 0.86a	2.99 ± 3c	1.62±0.16f
T_3	5.12 ± 0.51b	5.19 ± 0.52b	3.75 ± 0.38b	1.65±0.17f
T_4	5.12 ± 0.51b	3.94 ± 0.39c	5.11 ± 0.51a	1.98±0.2ef
T_5	0.75 ± 0.08e	3.23 ± 0.32c	5.34 ± 0.53a	3.62±0.36d
T_6	0.92 ± 0.09e	2.09 ± 0.21d	3.96 ± 0.40b	1.80±0.18f
T_7	0.29 ± 0.03e	5.47 ± 0.55b	3.67 ± 0.37b	6.67±0.67a
T_8	4.02 ± 0.40c	1.46 ± 0.15de	1.50 ± 0.15e	0.46±0.05g
T_9	7.71 ± 0.77a	1.26 ± 0.13e	2.10 ± 0.21de	4.95±0.49b
T_{10}	5.29 ± 0.53b	4.91 ± 0.49b	3..83 ± 0.38d	2.52±0.25e

Treatments having same letters are not different from one another.

showed some fluctuations and results were not similar to the findings of Iqbal et al. [24]. This suggested that as control samples did not have any preservatives so in both studies their behavior was similar but the rest of the treatments were different so different trends were noticed.

Thiobarbituric acid reactive substances

When thiobarbituric acid reactive substances were tested, it was found that almost all the treatments had same behavior and the TBARS values were increased (Table 6) even positive control had the same effects. A general trend was observed in all treatments; increase in TBRAS and then decrease gradually, later on; this can obviously be seen in T1, T2, T3, T6, and T8. In other words, TBARS value first increased and then decreased. Their effects against TBARS was somewhat similar but not exactly similar, such as in T1, TBARS value were increased until week 3 and then decreased, but in T2 and T3 TBARS values were reached the maximum in week 2 and decreased later on, while T6 and T8 showed a similar trend like T1. From Table 6, it is obvious that in the 1st week there was no treatment effect against TBARS, later had a negative effect and later on, some treatments became effective as compared to control. T8 was most effective, except in week 1 while in week 2, T8 was most effective followed by T6, and none was effective in week 3 except T8. While in week 4, T10 was more effective, even more than the positive control (T1) and ascorbic acid T8. From this data, it might be observed that Bauhinia flower extracts might possess some preservative effect against TBARS but not in the initial stages, their effectivity changed with time and might increase with time as in week 4 most of the treatments showed a gradual decline in TBARS. These results are different from the previously reported results of Iqbal et al. [24]. This may be due to the difference in phytochemical profile and their effects. From all the above discussion, it is clear that in every treatment, affectivity of treatments changed with the passage of time, but in TBARS, T8 was effective up to three weeks while in week 4, T10 was most effective.

Conclusions

It can be concluded that natural antioxidants in

Bauhinia flowers have the preservative ability against lipoperoxidation and rancidity of oil. Water and ethanolic extracts possess antioxidant effects and the maximum effect was found against free fatty acid production, acid value, and peroxide values. But in the case of TBARS, results were not satisfactory which suggests further studies be carried out in this domain. At the end, it can be inferred that by increasing the dose, synthetic antioxidants can be replaced by natural ones keeping in mind that natural antioxidants may require high doses.

Conflict of interest

The authors declare no conflict of interest.

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