Original Research Article

Effect of Natural Antioxidants Extracted from *Simarouba glauca* and *Radermachera xylocarpa* Deoiled Seed Cakes on the Oxidative Stability of Soybean Oil during Accelerated Storage

ABSTRACT

Aims: The aim of the present study was to evaluate the protective effect of Deoiled seed cakes extracts of *Simarouba glauca* and *Radermachera xylocarpa* toward oxidation of soybean oil during accelerated storage.

Study design: Seeds harvesting, oil extraction, natural antioxidants extraction from deoiled cakes, supplementation of soybean oil with the extract and evaluation of its oil stability during accelerated storages.

Place and Duration of Study: Council for Scientific and Industrial Research-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, India, from July 2015 to December 2015.

Methodology: Oils were extracted from the seeds on a Soxhlet apparatus. Deoiled cakes were then extracted with methanol. The obtained extract was used for phytochemical analysis, by colorimetry and high performance liquid chromatography (HPLC). After preliminary antioxidant tests, the extracts were respectively added in soybean oil at concentrations 200-1800 ppm. Oil containing butylated hydroxytoluene (BHT) and oil without antioxidants served as positive and negative controls respectively. The oxidative stability of these oil samples was evaluated by Rancimat and Schaal oven tests under forced storage conditions and measurement of oxidation parameters.

Results: The outcomes showed the total phenolic contents to be 19.22 and 9.87 mg GAE/g for *Simarouba glauca* (SG) and *Radermachera xylocarpa* (RX) respectively. Quercetine, Vanillic, caffeic, ferulic acids were the phenolic antioxidants detected in RX, while gallic acid and vanillic acid were those detected in SG. The induction times of soybean oil supplemented with the extracts of SG and RX were found to be in the range of 5.08-6.28 and 5.87-6.55 h respectively. Those of oil without antioxidant and oil supplemented with BHT were 4.84±0.03 and 6.42±0.12 h respectively. The extracts, at all concentrations, were efficient like BHT in delaying soybean oil oxidation during 21 days of storage at 65 °C.

Conclusion: These extracts are viable sources of natural antioxidants for stabilization of soybean oil.

Keywords: Accelerated storage; Natural antioxidant; Oxidative stability; Soybean oil; Simarouba glauca; Radermachera xylocarpa.

1. INTRODUCTION

The oxidation of vegetable oils and fats is a degradation process which leads to the deterioration of their nutritional value by reducing their content in essential fatty acids and vitamins. It also leads to alteration of the organoleptic characteristics of the products by destroying their texture, aspect and generating off flavour and rancid odor [1]. From these reactions, some molecules (free radicals and reactive oxygen species) which are reportedly associated with cancers and cardiovascular diseases in humans are generated in foods [2]. In order to overcome these problems, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallates (GP) have been widely used for the preservation and protection of lipid-based products against oxidation. However, in various countries, the use of synthetic antioxidants is becoming restricted, because of their implication in many health risks such as cancer and cardiovascular diseases [3]. As such, an immense deal of interest in the use of natural antioxidants derived from plants extracts has been developped and is expected to rise tremendously in the future. In fact, secondary plant metabolites such as phenolic compounds from plant sources are highly valuable for their therapeutic attributes as

antioxidants [4]. In many studies, these antioxidants extracted from herbs, spices and some agricultural wastes have been proven to be efficient in delaying oxidation of common vegetable oils during accelerated storages [5-9]. Among the natural sources explored, only few have been authorized for industrial purpose (case of rosemary) [1]. It is necessary to find other natural sources of antioxidants which could contribute to the solution of the above mentioned problems.

Radermachera xylocarpa is a middle size deciduous tree belonging to family Bignoniaceae [10]. It is mainly distributed in area of Deccan, Konkan, Khandesh and Western Ghats of India [11]. Sneha et al. [12] reported the presence of tannins, steroids and flavonoids in its barks which are generally known to significantly contribute to the antioxidant activity of plant extracts. Additionally, the roots and barks of this plant have been reported to have antimicrobial and anti-inflammatory activies respectively [11, 13], and the oil extracted from the wood efficient for treating various skin diseases [14]. The antioxidant activity of the stem and root barks against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals has already been reported [15]. The seed oil of *Radermachera xylocarpa* was studied for its fatty acid composition and minor constituents and it was found that the oil contained α-linolenic acid in considerable amounts [16].

Simarouba glauca is a medium sized tree that grows up to 20 m high, with a trunk 50-80 cm in diameter. It produces bright green leaves 20-50 cm length, small white flowers, and small red leaves. The seeds, generally exploited for oil extraction are 1.5-2 cm size and it is reported that the seed kernel contains around 60% oil [17]. This plant is indigenous to the amazon rainforest and other tropical areas in Mexico, Cuba, Haiti, Jamaica and Central America [18]. It is one of the important medicinal plants with wide use. The bark and leaf extract are well known for their pharmacological properties such as haemostatic, antihelmentic, antiparasitic, antidysenteric, antipyretic and anticancerous [19]. The phytochemical composition of its leaves (extracted in methanol) revealed the presence of phenolic compounds, flavonoids, terpenoids and tannins [20]. It is well known that these compounds are powerful antioxidants. Umesh [21] has reported that the methanolic extracts of the leaves of these plants contain 250 µg Gallic acid equivalent (GAE)/mg of phenolic compounds, 18.87 µg Catechin equivalent (CE)/mg of flavonoids and 134.93 µg CE /mg of Tannins. They also showed the extract to be efficient in scavenging DPPH radical, in reducing ferric iron and chelating transition metals.

It can be observed that various plant extracts of both the plants have antioxidant activity and literature survey suggests that these two plants have not been investigated for their antioxidant activity in oils and fats till now. Both the plant seeds yield oil from their seeds with residual deoiled seed cakes in considerable amounts. It would be interesting to examine and investigate the role of deoiled seed cakes for application as antioxidants in oils. Therefore, the present research work was aimed to study the antioxidant properties of methanolic extracts of *Simarouba glauca* and *Radermachera xylocarpa* deoiled seed cakes in delaying soybean oil oxidation during accelerated storage. Soybean oil was chosen as substrate because of its high content of polyunsaturated fatty acids which are known to be oxidized faster than other fatty acids.

2. MATERIAL AND METHODS

2.1 Material

The reagents and chemicals used in this work were of analytical grade. They were procured from HiMedia Laboratories Pvt. Ltd, Sd Fine Chemicals, Mumbai, India and Sigma-Aldrich, St; Louis, USA. Concerning the standards fatty acids methyl esters, they were provided by Sigma-Aldrich, St. Louis, USA.

Seeds of *Simarouba glauca* and *Radermachera xylocarpa* were supplied by AP Forest Department, Hyderabad, India, and collected in March 2015.

2.2 Methods

2.2.1 Extraction of antioxidants from deoiled seed cakes

The seeds of *Simarouba glauca* and *Radermachera xylocarpa* were separately ground to powder and subjected for oil extraction using Soxhlet apparatus to obtain oil and deoiled seed cakes. The dried

Simarouba glauca and *Radermachera xylocarpa* deoiled seed cakes were ground to pass through a 1 mm sieve. After this, they have been ground to pass through a 1 mm diameter sieve. 100 g of that powder was macerated at room temperature in 800 ml of methanol with regular shaking, during 48 h. After filtration using the N° 1 Wattman paper, the residues were again macerated in 400 ml of methanol, this in order to maximize the extraction of phenolic antioxidants. The obtained filtrate was mixed to the previous one, before elimination of the solvent on a rotatory evaporator at 40 °C under reduced pressure. The concentrated extract was stored in the refrigerator at 4 °C prior to further analysis.

2.2.2 Total phenolic content

The total phenolic content of the extract have been evaluated using the Folin-Ciocalteu colorimetric method, as described by Gao et al. [22]. Briefly, in a test tube of 5 ml volume, 20 µl of a 2 mg/ml extract solution was added, followed by the Folin-Ciocalteu reagent (0.2 ml) and distilled water (2 ml). After 3 min incubation of the solution mixture at room temperature, 1 ml of 20% sodium cabonate solution was added and the mixture re-incubated for 2 h under the same conditions. The absorbance of the resulting blue color solution was measured at 765 nm using a spectrophotometer. The total phenolic content of the extract was calculated from the gallic acid standard curve, and expressed as milligrams equivalents gallic acid per gram of extract (mg GAE/g).

2.2.3 High performance liquid chromatography analysis of the extracts

Reverse-phase High performance liquid chromatography (HPLC) was used to analyse the composition of phenolics in the extracts (1 mg/ml in methanol) [9]. The HPLC Agilent system 1200 series used was equipped with a quaternary pump model G11311A and diode array detector (DAD) model G11315B in combination with Chemstation software. The column type was an RP-C18 Lichrospher column, 5 µm, 4.0 mm internal diameter × 250 mm length. Separations were done in the isocratic mode, using acetonitrile-1% orthophosphoric acid in water (70:30 v/v) at a flow rate of 1 ml/min; with an injection volume of 20 µl. DAD detection was at 280 nm. Identification of the antioxidants was achieved qualitatively by comparing their retention time to those of standards.

2.2.4 Preliminary antioxidant test: DPPH radical scavenging assay

The radical scavenging ability of the extract was determined according to the method of Braca et al. [23]. 4.5 ml of 0.002% alcoholic solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was added to 0.5 ml of different concentrations (125, 250, 500, 1000 and 2000 µg/ml) of samples and standard solutions separately, in order to have final concentrations of products of 25-200 µg/ml. The samples were kept at room temperature in the dark and after 30 min, the absorbance (Abs) of the resulting solution was measured at 517 nm. The absorbance of the samples, control and blank was measured in comparison with methanol. Synthetic antioxidant, butylated hydroxytoluene (BHT), which is a recognized powerful hydrogen donor, was used as positive control. The antiradical activity (AA) was determined using the following formula:

$$AA\% = [(Abs\ control - Abs\ sample) \times 100/Abs\ control]$$

2.2.5 Effect of the extracts on the stability of oil

2.2.5.1 Sample preparation

The samples have been prepared according to technique described by Iqbal et al. [24]. The concentrated methanolic extract has been dissolved in 1 ml of solvent (methanol) and individually added in 100 g of preheated soybean oil (50 °C during 3 h) at five differents concentrations (200, 600, 1000,1400 and 1800 mg/Kg or ppm). Butylated hydroxytoluene used at its recommended concentration (200 mg/Kg or ppm) [25] served as positive control in order to compare the preservative property of the extract. Soybean oil without antioxidant, also prepared as previously described was the negative control. After shaking the oils for 30 min for a good dispersion of antioxidants, they were stored in the oven at 45 °C for 48 hours in order to reduce the amount of methanol to a value less than 50 mg/kg as recommended by the

regulations [26-28]. After this storage, each sample was separated into two portions, 10 and 90 g, for Rancimat and Schaal oven tests respectively.

2.2.5.2 Rancimat test

Induction times of control and soybean oil samples supplemented with antioxidants were determined using an automated Metrohm Rancimat instrument (Model 892). Each oil sample (≥ 5 g) was separately weighted in a Rancimat test tube. The instrument was switched on and the heating block temperature set at 110 °C. After reaching the 110 °C, the measuring vessels filled with 60 ml deionized water were connected to the instrument via electrodes. The tubes containing the sample were sealed using the appropriate caps, and connected to both instrument and measuring vessels via the appropriate cables. After starting the gas flow (20 l/h), the reaction tubes were individually placed in their respectives heating blocks and the reaction started.Induction time, the time elapsed from the beginning until the oil starts to become rancid, was automatically recorded by the instrument, and the protection factors calculated following the formula: Protection factor = Induction time of oil with antioxidant/Induction time of oil without antioxidant [29].

2.2.5.3 Schaal oven test

The method used by Sultana et al. [30] has been used with slight modifications. Stabilized and control oil samples (90 g) were subjected to accelerated storage in an electric hot air oven at 65 °C (8 h heating cycle per day) for 21 days. Samples were collected every 7 days and stored in the refrigerator for further analysis. The oxidative deterioration level of oil samples was assessed by measuring peroxide, and TOTOX values.

2.2.5.4 Measurement of oxidation parameters

Peroxide value of each oil sample was determined according to the spectrophotometrical IDF standard method, 74A: 1991 [31]; the secondary oxidation products was determined as value as described in the AOCS official method guide Cd 18-90 [32]. The total oxidation (TOTOX) of different treatments was calculated from their peroxide and values, using the following equation: TOTOX = 2PV + AV, as reported by Shahidi and Wanasundara [33].

2.2.6 Statistical analysis

All the experiments were done in triplicates. The obtained results were subjected to one-way analysis of variance (ANOVA) with Dunnet and Student-Newman-Keuls tests using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. A probability value at $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Extraction and total phenolic content

The extraction yields of methanolic extracts of deoiled seed cakes of *Simarouba glauca* and *Radermachera xylocarpa* were found to be 11.35 and 8.55% respectively, while their oil yields of the soxhlet extraction were 57.2 and 12.65% respectively. Their total phenolic content were respectively 19.22 and 9.87 GAE mg/g. So, *Simarouba glauca* extract has exhibited highest yield and total phenolic content than that of *Radermachera xylocarpa*.

3.1.2 HPLC-DAD analysis of the extract

The HPLC analysis of *Simarouba glauca* and *Radermachera xylocarpa* deoiled seed cake methanolic extracts is presented in Figure 1. The HPLC analysis showed the presence of vanillic acid (Retention time or RT: 8.957 min), caffeic acid (RT: 9.493 min), ferulic acid (RT: 9.931 min) and quercetine (RT: 11.977)

in *Radermachera xylocarpa* extract; and gallic acid (RT: 7.812 min), vanillic acid (RT: 8.928 min) in *Simarouba glauca* extract.

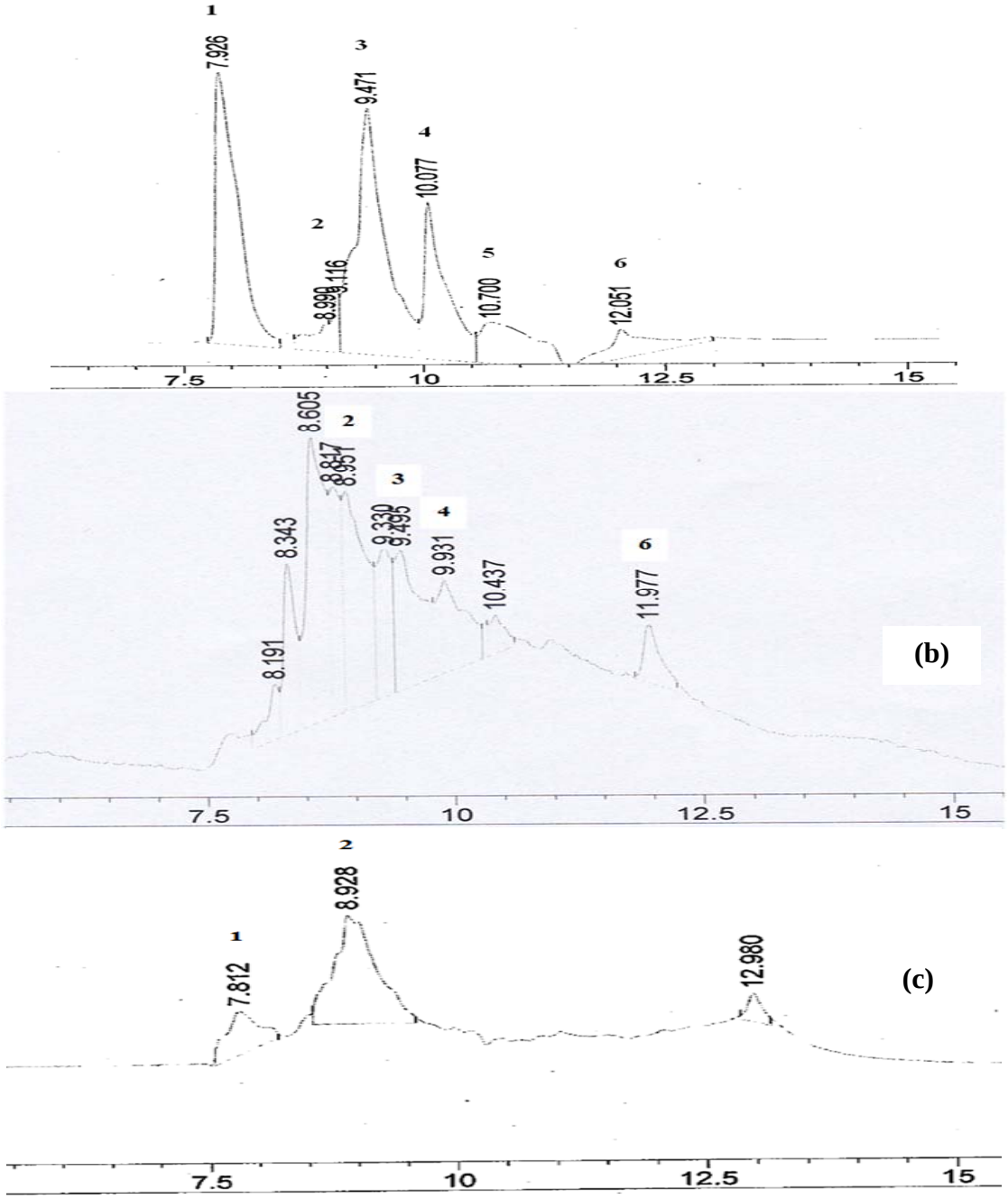


Fig. 1 (a-c). HPLC-DAD chromatograms of standards antioxidants (a) and methanolic extracts of *Radermachera xylocarpa* (b) and *Simarouba glauca* (c).
1 = Gallic acid, 2 = Vanillic acid, 3 = Cafeic acid, 4 = Ferulic acid, 5 = Ellagic acid, 6 = Quercetine

3.1.3 Diphenyl-1-picryl hydrazyl radical scavenging test (DPPH)

The property of *Simarouba glauca* and *Radermachera xylocarpa* deoiled seed cakes methanolic extracts and BHT in scavenging DPPH radical is presented in Figure 2. No significant difference ($P > 0.05$) has been observed between the antioxidant activity of *Radermachera xylocarpa* extract and BHT at all the tested concentrations. Similar observations were made with *Simarouba glauca* extract, but only from 50 µg/ml, while at 12.5 and 25 µg/ml, it activity was significantly lower ($P < 0.01$) compared to that of BHT and *Radermachera xylocarpa* extract.

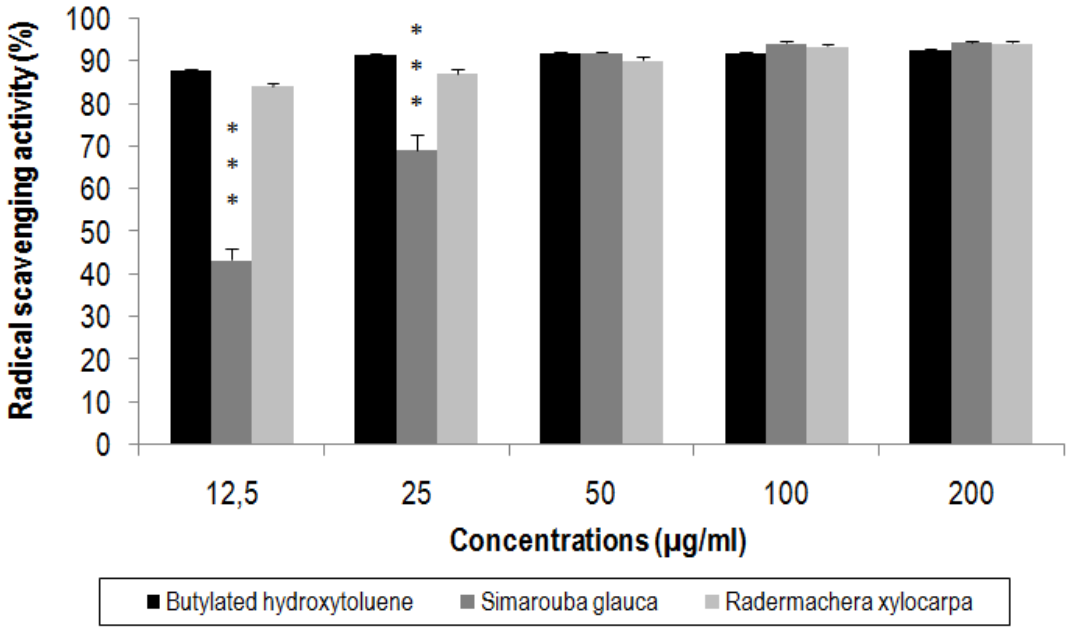


Fig. 2. DPPH radical scavenging activity of *Simarouba glauca* and *Radermachera xylocarpa* extracts in comparison with Butylated hydroxytoluene.
*** Significantly different ($P < 0.001$) to Butylated hydroxytoluene and the others at the same concentration

3.1.4 Effect of the extracts on the oxidative stability of oil

3.1.4.1 Rancimat test

The antioxidant efficiency of *Simarouba glauca* and *Radermachera xylocarpa* deoiled seed cake extract on the oxidative stability of soybean oil measured using Rancimat is presented in Table 1. It can be observed that the antioxidant activity of oil samples containing the extracts as antioxidants was increasing with their concentration. The lowest induction time was recorded in soybean oil without antioxidant (SBO) (4.84 h) and was similar to that of SBO+SG_{200ppm} (5.08 h). Apart from these samples, the induction time of the other oil samples was significantly higher ($P < 0.05$) ([5.22-6.26 h] and [5.87-6.55 h] for oil samples supplemented with *Simarouba glauca* and *Radermachera xylocarpa* extracts respectively). The induction period of the oil enriched with *Radermachera xylocarpa* extract was significantly higher ($P < 0.05$) compared to that of SBO and those stabilized with *Simarouba glauca* extract. At high concentrations, the activity of *Radermachera xylocarpa* extract was similar to that of BHT.

Table 1. Induction time of Soybean oil containing different concentration of *Simarouba glauca* and *Radermachera xylocarpa* extracts in comparison to BHT and Soybean oil without antioxidant (SBO)

Sample	Induction time (Hours)
SBO	4.84±0.03[a]
SBO + BHT_{200ppm}	6.42±0.12[bd]
SBO + SG_{200ppm}	5.08±0.00[a]
SBO + SG_{600ppm}	5.26±0.05[c]
SBO + $SG_{1000ppm}$	5.22±0.06[c]
SBO + $SG_{1400ppm}$	6.27±0.14[bd]
SBO + $SG_{1800ppm}$	6.28±0.06[b]
SBO + RX_{200ppm}	5.87±0.20[e]
SBO + RX_{600ppm}	6.13±0.07[bde]
SBO + $RX_{1000ppm}$	6.12±0.11[bde]
SBO + $RX_{1400ppm}$	6.34±0.00[b]
SBO + $RX_{1800ppm}$	6.55±0.23[b]

(Data are presented as mean (± SD) (n = 3) (a-e) Means within the column with different superscripts are significantly (P < 0.05) different. (SBO: Soybean oil without antioxidant; SBO+BHT_{200ppm}: Soybean oil containing BHT as antioxidant at concentration of 200 ppm; SBO+SG_{200ppm}: Soybean oil supplemented with the extract of Simarouba glauca at concentration of 200 ppm; SBO+RX_{200ppm}: Soybean oil supplemented with the extract of Radermachera xylocarpa at concentration of 200 ppm)

3.1.4.2. Peroxide value (PV)

The effects of butylated hydroxytoluene, deoiled seed cake extracts of *Simarouba glauca* (SG) and *Radermachera xylocarpa* (RX) on the peroxide formation in refined soybean oil during the storage is illustrated in Figure 3 (a, b). A typical pattern in the rise of peroxide was registered in almost all the samples during the storage. Soybean oil without antioxidants (SBO) has exhibited a significantly higher ($P < 0.001$) peroxide value compared to oils containing antioxidants and extracts. After 14 days of storage, a significant decrease ($P < 0.05$) in PV in SBO was registered, while those of oil containing antioxidants were still increasing.

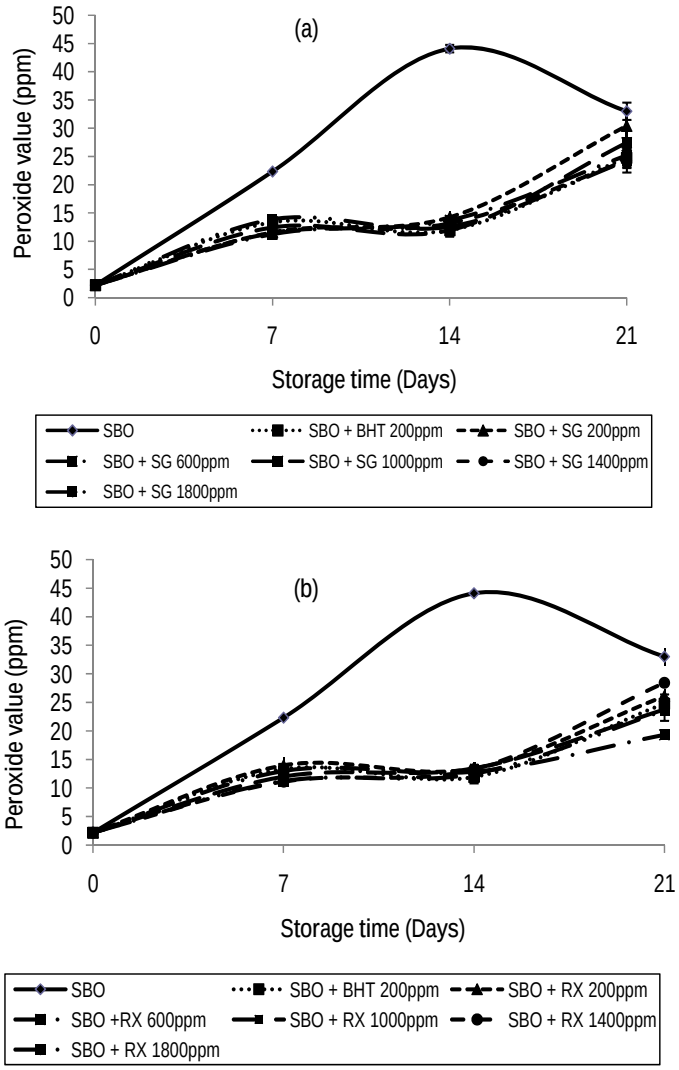
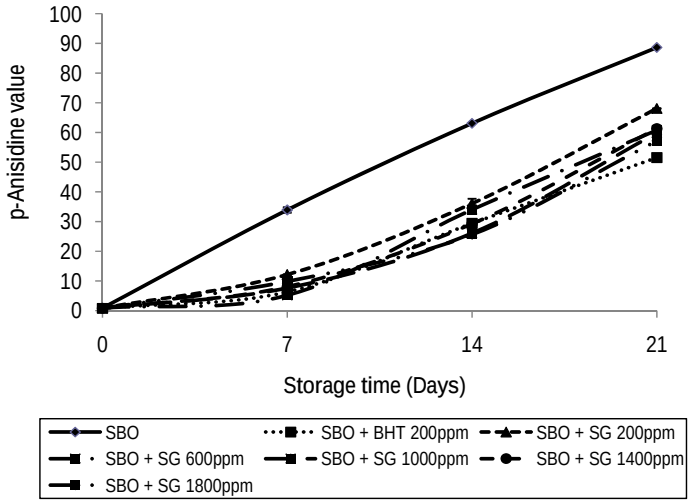


Fig. 3 (a and b). Relative changes in peroxide value of soybean oil samples supplemented with *Simarouba glauca* (a) and *Radermachera xylocarpa* (b) extracts during the storage
(SBO: Soybean oil without antioxidant; SBO+BHT$_{200ppm}$: Soybean oil containing BHT as antioxidant at concentration of 200ppm; SBO+SG$_{200ppm}$: Soybean oil supplemented with the extract of Simarouba glauca at concentration of 200 ppm; SBO+RX$_{200ppm}$: Soybean oil supplemented with the extract of Radermachera xylocarpa at concentration of 200 ppm)

3.1.4.3 p-anisidine value

The changes in *p*-anisidine values of stabilized and control (SBO) oil samples are presented in Figure 4 (a and b). It is clearly observed that the *p*-anisidine value in all the samples has significantly increased ($P < 0.01$) during the storage. SBO has exhibited a significant higher ($P < 0.001$) *p*-anisidine value compared to oils supplemented with antioxidants. The analysis of changes in values of oil samples supplemented with natural extracts showed that after 21 days storage, the value of oil stabilized with *Radermachera xylocarpa* and *Simarouba glauca* extracts at concentrations 600-1800 ppm were ranged between 25-35 and 50-70 respectively. It is also seen that at high concentrations (600-1800 ppm), the efficiency of *Radermachera xylocarpa* extract was higher than that of Butylated hydroxytoluene, while that of *Simarouba glauca* was similar or less than that of this synthetic antioxidant.

(a)



(b)

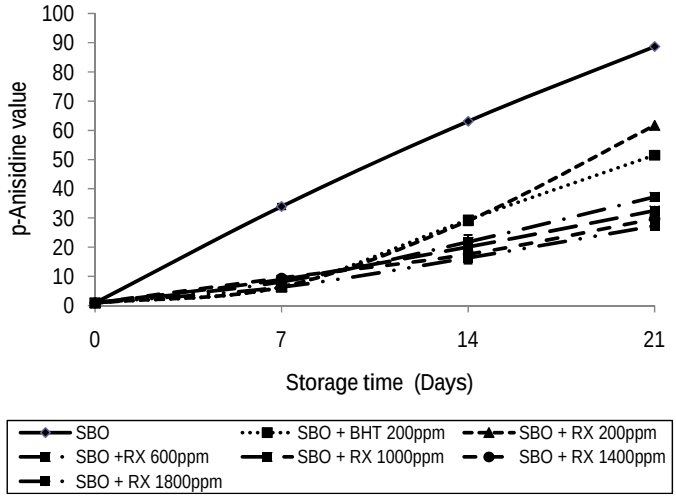


Fig. 4 (a and b). Relative increase in *p*-anisidine value of soybean oil samples supplemented with *Simarouba glauca* (a) and *Radermachera xylocarpa* (b) extracts during the storage.
(SBO: Soybean oil without antioxidant; SBO+BHT$_{200ppm}$: Soybean oil containing BHT as antioxidant at concentration of 200 ppm; SBO+SG$_{200ppm}$: Soybean oil supplemented with the extract of Simarouba glauca at concentration of 200 ppm; SBO+RX$_{200ppm}$: Soybean oil supplemented with the extract of Radermachera xylocarpa at concentration of 200 ppm)

3.1.4.4 TOTOX value

The TOTOX values of control (SBO) oil samples and those supplemented with antioxidants is shown in Figure 5 (a and b). A significant increase ($P < 0.05$) in TOTOX value was registered in all the samples. Control has exhibited the highest total oxidation, because it TOTOX value was significantly higher ($P < 0.001$) than that of oil containing natural or synthetic antioxidants. *Radermachera xylocarpa* extract was most efficient in delaying oxidation of soybean oil because it TOTOX value at the day 21 was ranged between 65-115 while those of oil supplemented with Simarouba glaucawas between 90-125. As

previously seen with *p*-anisidine value, at concentrations 600-1800 ppm, *Radermachera xylocarpa* extract was most efficient in limiting oxidation of the oil than BHT.

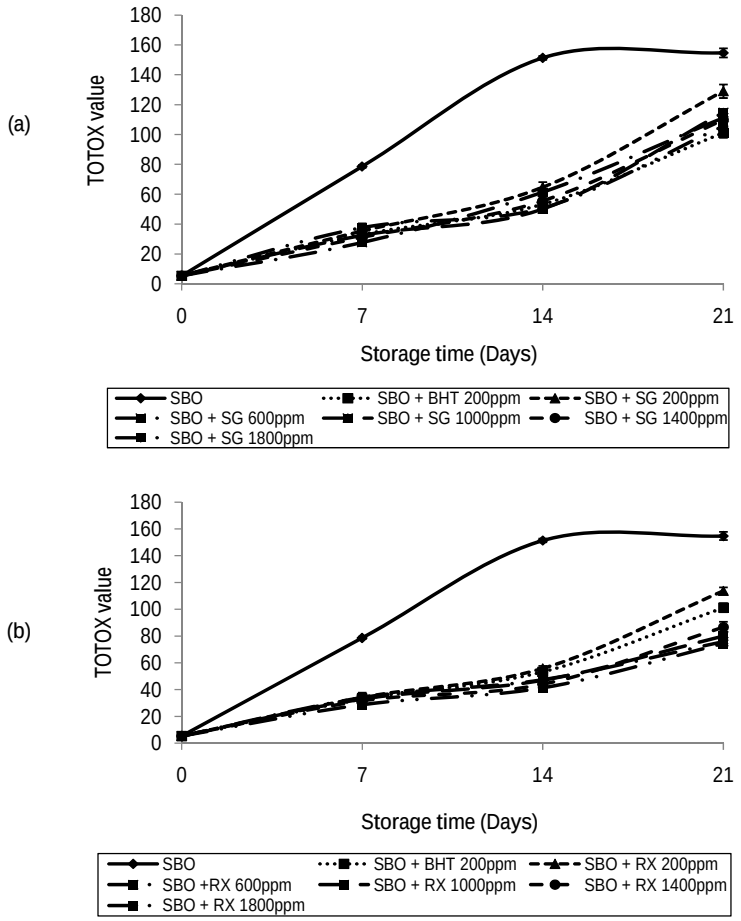


Fig. 5 (a and b). Relative increase in TOTOX value of soybean oil samples supplemented with *Simarouba glauca* (a) and *Radermachera xylocarpa* (b) extracts during the storage
(SBO: Soybean oil without antioxidant; SBO+BHT$_{200ppm}$: Soybean oil containing BHT as antioxidant at concentration of 200 ppm; SBO+SG$_{200ppm}$: Soybean oil supplemented with the extract of Simarouba glauca at concentration of 200 ppm; SBO+RX200$_{ppm}$: Soybean oil supplemented with the extract of Radermachera xylocarpa at concentration of 200 ppm)

3.2 Discussion

Results of the phytochemical analysis showed that the total phenolic content of *Simarouba glauca* and *Radermachera xylocarpa* extracts are respectively 19.22 and 9.87 mg GAE /g. The total phenolic content of *Simarouba glauca* seed cake extract was significantly lower than that reported by Umesh [21] in the methanolic extract of the leaves of this plant, which was 250 µg GAE/mg. The observed difference might be due to the part of the plant used for extraction, as this parameter, together with environmental differences (climate, location, and harvest period), nature of the solvent used, extraction procedure and the state of maturity of the plant can affect the phytochemical composition of the same plant [24, 34]. Concerning the extract of *Radermachera xylocarpa* seedcake, the total phenolic content has not yet been reported. However phenolic compounds of the bark of this plant have already been detected qualitatively [12]. Among the various phenolic antioxidants present, some have been detected by HPLC and were

vanillic acid (RT: 8.957 min), caffeic acid (RT: 9.493 min), ferulic acid (RT: 9.931 min) and quercetine (RT: 11.977) in the extract of *Radermachera xylocarpa*; gallic acid (RT: 7.812 min) and vanillic acid (RT: 8.928 min) in that of *Simarouba glauca*.

The 2, 2-Diphenyl-1-picryl hydrazyl is a kind of stable organic radical. The capacity of a substance or product to scavenge this radical can be expressed as its magnitude of antioxidant ability; especially its ability to donate hydrogen atoms for stabilization of free radicals [9]. This test has been used to check the preliminary antioxidant activity of the extracts in comparison with BHT and results showed no significant difference ($P > 0.05$) between the antioxidant activity of *Radermachera xylocarpa* (at all concentrations), *Simarouba glauca* (50-200 µg/ml) extracts and BHT. This could be attributed to the presence of additional phenolic antioxidants in the extract of *Radermachera xylocarpa* compared to Simarouba glauca. The antioxidants previously detected by colorimetry and HPLC could be responsible for the good radical scavenging properties of these extracts, as it has been demonstrated that phenolic antioxidants in plants are responsible for their antioxidant activity. These results are in accordance with those of Umesh [21] and Babu et al. [35], who showed that the methanolic extracts of *Simarouba glauca* leaves and *Radermachera xylocarpa* roots and barks were powerful radical scavengers by the DPPH method.

The antioxidant efficiency of *Simarouba glauca* and *Radermachera xylocarpa* deoiled seed cake extracts on the oxidative stability of soybean oil measured using Rancimat demonstrated that the induction times of oil samples supplemented with *Simarouba glauca* and *Radermachera xylocarpa* extracts were between 5.22-6.26 h and 5.87-6.55 h. Those of control and SBO+SG_{200ppm} were 4.84 h 5.08 h respectively. So, the extract of *Radermachera xylocarpa* was found to be more efficient than that of *Simarouba glauca* in prolonging soybean oil shelf-life at all concentrations, because the induction period of the oils enriched with *Radermachera xylocarpa* extract was significantly higher ($P < 0.05$) compared to that of SBO and those stabilized with *Simarouba glauca* extract. At high concentrations, the activity of *Radermachera xylocarpa* extractwas similar to that of BHT. It can be observed that the radical scavenging activity test has also shown that *Radermachera xylocarpa* extract has exhibited similar activity at all concentrations, while at concentrations 12.5 and 25 µg/ml, the antioxidant activity of *Simarouba glauca* was significantly lower ($P < 0.05$). Similar observations have also been made in their total phenolic content measured colorimetrically and by HPLC-DAD. The phenolic antioxidants present in the deoiled seed cake could be responsible for the long induction times recorded in the samples enriched with the extracts. The fact that some natural plant extracts can prolong the induction period of vegetable oils has previously been reported by other authors [5, 9].

The oxidative stability of control and soybean oil samples supplemented with antioxidants was also evaluated by the Schaal oven test and results showed a typical pattern in the rise of peroxides. The significant increase in peroxide value could be attributed to the formation of hydroperoxides, which are principal indicators of the primary oxidation state of oils and fats. The formation of these compounds can be limited by adding efficient antioxidants [9]. So, the highest peroxide values registered in control could be due to the absence of antioxidants which can stabilize the free radicals formed during heating. The decrement in peroxide value observed in that sample from day 14 to 21 might be attributed to the transformation of hydroperoxydes into secondary oxidation products, such as aldehydes, ketones…In fact, it has been proven that, peroxides rapidly formed in polyunsaturated oils without antioxidants are easily decomposed above 50 °C into secondary oxidation products [6, 36]. From these results, it is clear that the methanolic extracts of *Simarouba glauca* (SG) and *Radermachera xylocarpa* (RX) at all concentrations can significantly reduce the formation of hydroperoxides in soybean oil during heating at the same level as BHT. The lowest peroxide values registered in oil samples supplemented with these extracts can be attributed to the presence of phenolic antioxidants as shown by colorimetry and HPLC-DAD. These compounds have been previously shown as good hydrogen donors for the stabilization of the DPPH radical so, they could also delay the hydroperoxides formation in soybean oil during the storage through the same mechanism. Similar observations have been previously made by other authors in soybean, palm olein and sunflower oils supplemented with plant extracts as antioxidants during accelerated storage [5-9].

Concerning *p*-anisidine value, it helps to measure the secondary oxidation products formed when hydroperoxides decompose to aldehydes, carbonyls and other compounds which are responsible for the rancidity of the oil [8, 9, 24]. Results of this parameter showed that secondary oxidation in control was significantly higher compared to that of soybean oil samples supplemented with antioxidants. The extract of *Radermachera xylocarpa* was most efficient in retarding secondary oxidation of soybean oil compared

to that of *Simarouba glauca*; the maximum value in oils stabilized with *Radermachera xylocarpa* was in 20-35, while it wasranged in 45-65 in *Simarouba glauca*. However, the extracts, at all concentrations, were efficient in delaying secondary oxidation of soybean oil during the storage of 21 days at 65 °C. The antioxidants present in these extracts might be responsible for the lowest secondary oxidation product formation in these samples. So, the lack of antioxidant in control could explainits high secondary oxidation state during the storage. These results are in agreement with those of Mei et al. [8] and Sultana et al. [30] who showed that the methanolic extracts of Rambutan (*Nephelium lappaceum*. L) and some agricultural wastes were efficient in delaying secondary oxidation of sunflower and corn oil respectively during accelerated storage.

Total oxidation measures the primary oxidation products, hydroperoxides, and its breakdown products such as carbonyls. Therefore, it provides a better estimation of the progressive oxidative deterioration of the oil. Results of this parameter also demonstrated the control to be significantly more oxidized than soybean oil samples supplemented with antioxidants. This means that all the stabilized oils showed positive effects in delaying oxidative rancidity of soybean oil. The lowest TOTOX value registered in oils containing *Simarouba glauca* and *Radermachera xylocarpa* extracts after 21 storage days could be attributed to the presence of phenolic antioxidants. The significantly higher oxidation of SBO samples compared to those supplemented with the antioxidants could be due to the lack of antioxidants which can reduce its oxidation rate, by limiting anarchic production of free radicals. It can be deduced from the TOTOX value that *Simarouba glauca* and *Radermachera xylocarpa* extracts at all the tested concentrations were able to retard soybean oil rancidity during 21 days of storage at 65 °C.This is the equivalent of at least 2 years at room temperature, as it has been demonstrated that 8 h storage at 65°C is the equivalent to one month storage at room temperature [37]. These results are in accordance with those reported by Nyam et al. [7] and Mei et al. [8] in sunflower oil with the extracts of Kenaf and roselle seeds and that of Rambutan (*Nephelium lappaceum*. L) respectively during accelerated storage.

4. CONCLUSION

Results of this study showed that the deoiled seed cake methanolic extracts of *Simarouba glauca* and *Radermachera xylocarpa* extracts contain a good amount of phenolic antioxidants. At least three phenolic antioxidants have been detected in each extract by HPLC. The extracts were also found to be good radical scavengers, but activity of *Radermachera xylocarpa* extract was higher than that of Simarouba glauca. *Radermachera xylocarpa* extract was also the best in prolonging the induction period of soybean oil by Rancimat. The Schaal oven test revealed that both extracts were able to delay oxidation of soybean oil at the same level as BHT during 21 days storage at 65 °C. *Simarouba glauca* and *Radermachera xylocarpa* cakes can be exploited as alternative source of antioxidants for the stabilization of soybean oil.

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