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Phytochemical, antioxidant and cytotoxicity studies of *Bambusa arundinacea* leaves

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Abstract

Medicinal plants, as a source of remedies, are widely used as alternative therapeutic utensils for the anticipation or treatment of many diseases. The Present study was carried out to investigate the medicinal importance of the plant *Bambusa arundinacea* leaves. The various extracts obtained from the plant leaves were analyzed for their phytochemical constituents, *in vitro* antioxidant, haemolytic acivities and stability of corn oil. The plant leaves contained appreciable levels of total phenolic contents (3.7-12.71 GAE mg/g of dry extract) and total flavonoid contents (2.31-6.71 CE mg/g of dry extract). DPPH radical scavenging (IC_{50}) and percentage inhibition of linoleic acid peroxidation was found to be in the range of 278-1536 µg/mL and 24.41-78.05% respectively. The antioxidant activity of plant extracts was also studied using corn oil as an oxidative substrate and found that it stabilized the oil. The haemolytic effect of the plant leaves was found in range of 0.70-2.88%. The results of the present investigation demonstrated significant ($p < 0.05$) variations. The correlation between the results of different antioxidant assays and oxidation parameters of oil indicated that plant leaves to be a potent source of antioxidants for stabilization of corn oil.

Keywords: Bambusa arundinacea, Antioxidants, Phytochemicals, Corn oil, Hemolytic activity.

Introduction

Medicinal plants are of great importance to the health of individuals and communities. Many of these indigenous medicinal plants are used as spices and food plants. Oxidation reactions can produce free radicals. The antioxidant action is supposed to protect living organism from oxidative damages resulting in the prevention of various diseases [1]. *Bambusa arundinacea,* locally known as *Bans* or bamboo, a perennial fastest growing plant on the earth is presumed to have origin in Asia. It belongs to the family Poaceae. It has more than 70 genera divided into about 1,000 species in the world, among which 130 growing in India [2]. In some parts of India and China young shoots of *Bambusa arundinacea* are used as food. Its leaves are given to horses suffering from coughs and colds.

The plant Bamboo is considered as a rich source of flavones glycosides having ability to interact with lipid bilayers by influencing their incorporation rate into cells. Different parts of bamboo plant possess various biological functions due to the presence of different compounds. The stilbene gulcosides from the root of bamboo has been reported to possess various medicinal properties [3]. Jeong *et al.* [4] reported the presence of poly phenols i.e. *p*-(hydroxyphenyl) propionic acid, ferulic acid, caffeic acid and chlorogenic acid in bamboo shoot. The phenolic compounds derived from the whole plant's extracts showed inhibition of Pglycoprotein in adriamycin resistant human breast cancer cells [5]. Bamboo leaves have been used clinically in the treatment of hypertension, arteriosclerosis, cardiovascular disease, and cancer [6]. Plants contain assortment of phytochemical such as phenols, flavonoids, terpenoids and vitamins which possess antioxidant activity [7].

The dietary roles of edible oils and fats are highly recognized. Vegetable oils and fats have wide application in foods where they are used in frying, salad dressing, shortening of pasty, margarine, cooking and ice cream manufacture. It is an established fact that different variables involved in oil shelf life, such as processing, storage conditions, light exposure, type of packaging material, availability of oxygen, and addition of antioxidants do affect the quality and characteristics of vegetable oils, fats and lipid containing products. The oils with higher contents of unsaturated fatty acids such as polyunsaturated fatty acid are more susceptible to oxidation. To overcome the stability problems of oils and fats,

synthetic antioxidants, such as BHA, BHT, and TBHQ have been used as food additives. But recent reports have shown that these compounds may be concerned in many health risks, including cancer and carcinogenesis [8]. As our efforts to explore the various flora of Pakistan [9-15] this study was an attempt to investigate Bamboo leaves as a potential source of natural antioxidants and to appraise their efficacy for stabilizing corn oil and to determine its cytotoxic effects against human erythrocytes.

Material and methods

Collection of plant material

The fresh leaves of the plant *Bambusa arundinacea* were collected from the local areas of Faisalabad, Pakistan and further identified by a Taxonomist, Dr. Mansoor Hameed from Department of Botany, University of Agriculture Faisalabad, Pakistan.

Sample preparation

The plant leaves were washed with distilled water and then shade dried. The grinded fine powder of leaves (1000 g) extracted with *n*-hexane (3x1.5 L) at room temperature. After 3 days the extract was filtered and concentrated through rotary vacuum evaporator (Eyela, Tokyo Rikakikai Co., Ltd., Japan). This process was repeated thrice to obtain a sufficient quantity of *n*-hexane extract (5.6 g). The remaining plant residue was further extracted with other different polarity based solvents and obtained successively chloroform (9.8 g), ethylacetate (9.2 g), acetone (7.3 g), *n*-butanol (8.9 g), absolute methanol (11.3 g) and methanol:water (9:1), (12 g) extracts per 100g of dry plant. All obtained extracts after drying were stored at -4°C till further analysis.

Evaluation of phytochemical activity *of Bambusa arundinacea*.

Qualitative screening of various phytochemicals in the methanolic extract of plant was carried out according to the methods described by Trease and Evans [16]. For quantitative analysis deffatation of the extract was carried out by taking 2 g of the extract in which 100 mL of diethyl ether was added by using a soxhlet apparatus for 2 hours. Alkaloids, tannins, saponins and flavonoid were quantitatively measured by following already described methods [17-20] and total phenols were quantitatively estimated by following spectrophotometric method.

Determination of total phenolic and total flavonoid contents

The plant leaves total phenolic contents (TPC), total flavonoid contents (TFC) were determined by following methods described by Rasool *et al.* [21].

Antioxidant activity determination in linoleic acid system

The antioxidant activity of the plant leaves was also determined in terms of measurement of % inhibition of peroxidation in linoleic acid system following a reported method of Iqbal and Bhanger [22]. Extracts (5 mg) were added to a solution mixture of linoleic acid (0.13 mL), 99.8 % ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH=7.4). Total mixture was diluted to 25 mL with distilled water. The solution was incubated at 40ºC and the degree of oxidation was measured following thiocyanate method [23] with 10 mL of ethanol (75 %), 0.2 mL of an aqueous solution of ammonium thiocyanate (30 %), 0.2 mL of sample solution and 0.2 mL of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5 % HCl) being added sequentially. After 3 min of stirring, the absorption values of mixtures measured at 500 nm were taken as peroxide contents. A control was performed with linoleic acid but without extracts. Synthetic antioxidants; butylated hydroxytoluene (BHT) was used as positive control. The maximum peroxidation level observed as 360 h (15 days) in the sample that contained no antioxidant component was used as a test point. Percent inhibition of linoleic acid peroxidation, 100 – [(Abs. increase of sample at 360 h/abs. increase of control at 360 h) x100], was calculated to express antioxidant activity.

Determination of reducing power

The reducing power of the extracts was determined according to the procedure described by Yen *et al.* [23] with little modification. Equivalent volume of leaves crude extracts containing 2.5-10 mg of dry matter was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0 %); the mixture was incubated at 50 °C for 20 min. Then 5 mL of 10 % trichloroacetic acid was added and centrifuged at 980 *x*g for 10 min at 5ºC in a refrigerated centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1 %), and absorbance noted at 700 nm (Hitachi U-2001 Spectrophotometer). The measurement was run in triplicate and results averaged.

DPPH radical scavenging assay

The antioxidant activity of the plant was assessed by their ability to scavenging 2, 2-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The DPPH assay was performed as described by Mimica- Dukic *et al.* [24].

Determination of antioxidant efficacy using corn oil as oxidation substrate

The crude concentrated various extracts of the plant were separately added into the preheated (50 °C) refined, bleached and deodorized corn oil at concentration of 600 ppm (w/w). The oil samples were stirred for 30 minutes at 50 °C for uniform dispersion. All oil samples were separately stabilized and stored in 100 mL airtight bottle. A control sample was also prepared (without extract) under the same set of analytical conditions. Samples were stored at room temperature. Synthetic antioxidant (BHT) was employed at

its legal limit of 200 ppm to compare the efficacy of extracts. Stabilized and control oil samples (100 mL) were placed in dark brown airtight glass bottles with narrow necks and subjected to accelerated storage in an electric hot air oven (IM-30, Irmeco, Germany) at 60 °C (8 hours heating cycle per day) for 28 days. All oil samples were prepared in triplicate. Oil samples were taken after every 7 days intervals.

Measurement of oxidation parameters of corn oil

The oxidative deterioration level was assessed by the measurement of peroxide value (PV), free fatty acids (FFA) conjugate dienes (CD), conjugate trienes (CT) and *p*-anisidine values. Determination of the FFA and PV of stabilized and control corn oil samples were made following the AOCS Official methods Cd 8-53 and F 9a-44 respectively [25]. The oxidation products such as conjugated dienes and conjugated trienes were analyzed by following the IUPAC method II D.23 [26]. The absorbance was noted at 232 and 268 nm respectively. The determination of the *p*-anisidine value was made following an IUPAC method II. D. 26 [26].

In vitro hemolytic activity for cytotoxicity assay

Hemolytic activity of the plant was checked by the reported method of Powell *et al.* [27]. 3 ml of freshly obtained heparinized human blood was gently mixed, poured into a sterile 15 mL polystyrene screw-cap tube and centrifuged 5 min, 850 *x*g. The supernatant was poured off and the viscous pellet washed three additional times with 5 mL of chilled (4 °C) sterile isotonic phosphate-buffered saline (PBS) solution, adjusted to pH=7.4, to stabilize the pH it was mixed for almost half an hour at room temperature. The washed cells were suspended in a final volume of 20 mL chilled, sterile PBS and the cells counted on a haemacytometer .The blood cell suspension was maintained on wet ice and diluted with sterile PBS to 7.068×10^8 cells mL^{-1} for each assay. Aliquots of 20 µl of plant extract/fractions were aseptically placed into Eppendrof. For each assay, 0.1 % triton X-100 was the positive, 100%lytic control and PBS was the negative control (0% lysis). Aliquots of 180 µl diluted blood cell suspension were aseptically placed into each 2 mL tube and gentlymixed three times with a wide mouth pipette tip. Tubes were incubated for 35 min at 37 °C. Immediately following incubation, the tubes were placed on ice for 5min then centrifuged for 5min at 1310 xg. Aliquots of 100 µl of supernatant were carefully collected, placed into a sterile Eppendrof, and diluted with 900 µl chilled, sterile PBS. All tubes were maintained on wet ice after dilution. Then 200µl into 96 well plates, and three replicates was taken in well plate which contain one positive control (100 % of blood lysis) and other negative control (0 % of blood lysis). After this absorbance at 576 nm was noted. The experiment was done in triplicate. % hemolysis was calculated by following formula:

% haemolysis = As (sample absorbance)/Ac (control absorbance) x100

Statistical analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p \leq 0.05$ was considered to denote a statistically significance All data were presented as mean values ± standard deviation (SD).

Results & Discussion

Phytochemical analysis

Qualitative analysis revealed the presence of alkaloids, saponins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins were present in the plant methanolic extract while the tannins were absent. Quantitative analysis showed that alkaloids, phenolics, flavonoids and saponins were present in 0.53±0.03, 1.41±0.01, 4.71±0.04, 0.64±0.01 mg/100g concentrations respectively.

Percentage yield, total phenolic contents (TPC), total flavonoid contents (TFC) and antioxidant analysis.

Percentage yield, total phenolic contents (TPC), total flavonoid contents (TFC), DPPH radical scavenging, IC_{50} and % inhibition of linoleic acid peroxidation of *Bambusa arundinacea* extracts are expressed in Table 1. In plants the TPC is a good index of antioxidantive compounds. The plant leaves showed good profile of TPC: methanol:water (9:1) (12.71) > absolute methanol (11.42) > chloroform(8.21) > ethyl acetate (6.45) > *n*-butanol (4.24) > acetone (4.12) > *n*-hexane (3.17 mg/g as GAE). Flavonoids are the example of aromatic compounds to which hydroxyl radical have high affinity. Flavonoids have the ability of scavenging free radical, chelating metal and inhibiting lipid peroxidation. Thus we can say that flavonoids were potent antioxidants. Various plant extracts Methanol:water (9:1) (6.71) > absolute methanol (6.52) > chloroform (5.53) > ethyl acetate (2.61) > *n*-butanol (2.53) > acetone (2.42) > *n*-hexane(2.31 mg /g as CE) extracted a reasonable amount of flavonoids from the plant leaves. DPPH radicals have been extensively used for measuring radical scavenging activity of different extracts of plants. Lower the IC_{50} value more effective extract for the inhibition of DPPH. Methanol:water, 9:1, absolute methanol, chloroform, ethyl acetate, *n*-butanol, acetone and *n*-hexane showed the IC_{50} value 278, 332, 746, 1056, 1103,1408 and 1536 µg/mL respectively

The extracts of *B. arundinacea* showed significantly lower antioxidant activity as compared to BHT. Percentage Inhibition of linoleic acid peroxidation (Table 1) showed by the extracts ranged from 24.41 to 78.05 %. The inhibition shown by BHT was 89.01 %. Methanol:water (9:1), absolute methanol, chloroform, ethyl acetate, *n*-butanol, acetone and *n*-hexane showed the % inhibition 24.41, 29.86, 35.60, 44.81, 56.78, and 78.05 % respectively.

Measurement of the reducing potential reflected some aspects of antioxidant activity. With the change of colour from yellow to bluish

Table 1. Percentage yield, total phenolic contents (TPC), total flavonoids contents (TFC) and antioxidant analysis of *Bambusa arundinacea* leaves.

Plant extracts	% yield (g/100g)	TPC[a] (mg/g of dry extract)	TFC[b] (mg/g of dry extract)	DPPH (IC_{50}) (µg/mL)	Inhibition in linoleic acid System (%)
Methanol:water (9:1)	1.2.0±0.11	12.71±0.12	6.71±0.14	278±2.71	24.41±0.21
Absolute Methanol	1.13±0.13	11.42±0.09	6.52±0.05	332±3.31	29.86±0.10
Chloroform	0.98±0.09	8.21±0.08	5.53±0.03	746±7.41	33.67±0.31
Ethyl acetate	0.92±0.08	6.45±0.06	2.61±0.02	1056±10.5	35.60±0.32
n-butanol	0.89±0.07	4.24±0.03	2.53±0.02	1103±11.0	44.81±0.42
Acetone	0.73±0.05	4.12±0.04	2.42±0.01	1408±12.0	56.78±0.53
n-hexane	0.56±0.05	3.7±0.01	2.31±0.01	1536±14.0	78.05±0.71
BHT	-	-	-	10.21±0.11	89.01±0.91

Values are mean ± SD of three separate experiments (n=2x3) (p<0.05).

Table 2 Reducing power of different extracts at different concentrations

Plant extracts	1 mg/mL	1.5 mg/mL	2.5 mg/mL	5 mg/mL
Methanol:water, (9:1)	0.45±0.03	0.91±0.05	1.00±0.04	1.51±0.05
Absolute methanol	0.44±0.01	0.71±0.02	0.95±0.05	1.42±0.03
Chloroform	0. 40±0.05	0.61±0.04	0.85±0.03	1.11±0.02
Ethyl acetate	0.35±0.02	0.57±0.01	0.75±0.04	0.89±0.01
n-butanol	0.32±0.04	0.49±0.02	0.74±0.05	0.79±0.04
Acetone	0.31±0.01	0.42±0.03	0.47±0.02	0.55±0.02
n-hexane	0.28±0.02	0.31±0.04	0.44±0.01	0.53±0.01

Values are mean ± Standard deviation of three separate experiments

green as the ferric ions reduced to ferrous ions. The intensity of color depends on the reducing potential of the compounds present in the medium. Greater the intensity of the color, greater was the absorption; consequently, greater the antioxidant activity [28]. The data for the reducing potential of different extracts presented in Table 2. The reducing potential of *Bambusa arundinacea* was measured for the concentration up to 5 mg/mL showed general increase in activity when concentration increased. Furthermore the study results indicated that the plant leaves were a good source of antioxidants.

Determination of antioxidant activity using corn oil as an oxidative substrate

The peroxide value (PV) which measure hydroperoxide products, may a good indicator of the primary oxidation products of the oils [28]. During the storage period of 35 days a rise in PV from 0.24 meq/kg of oil to 22.83 meq/kg of oil was observed in case of *n*-hexane (Figure 1). Control exhibited the highest PV while corn oil stabilized with synthetic antioxidant BHT exhibited least PV. All used extracts of the plant controlled peroxide value appreciably; revealing good antioxidant efficacy of extracts in stabilization of oil.

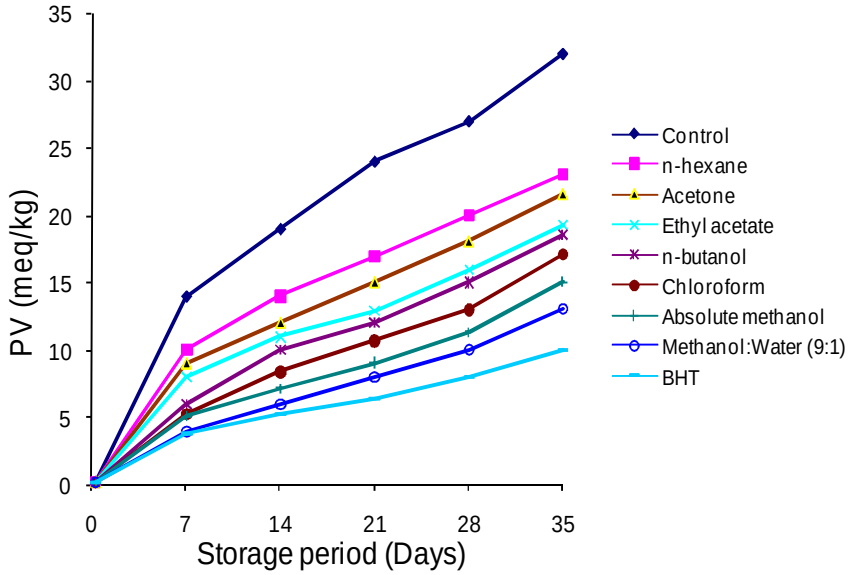

Fig. 1 Relative increase in peroxide value (PV) of treated corn oil sample under storage.

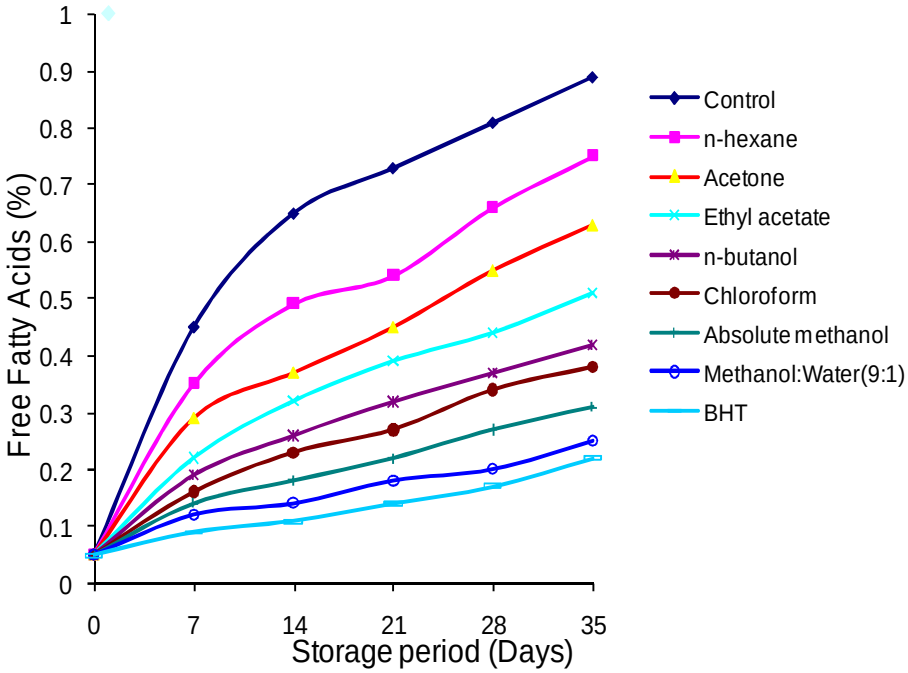

Fig. 2 Relative increase in free fatty acid content (FFA) of treated corn oil samples under storage.

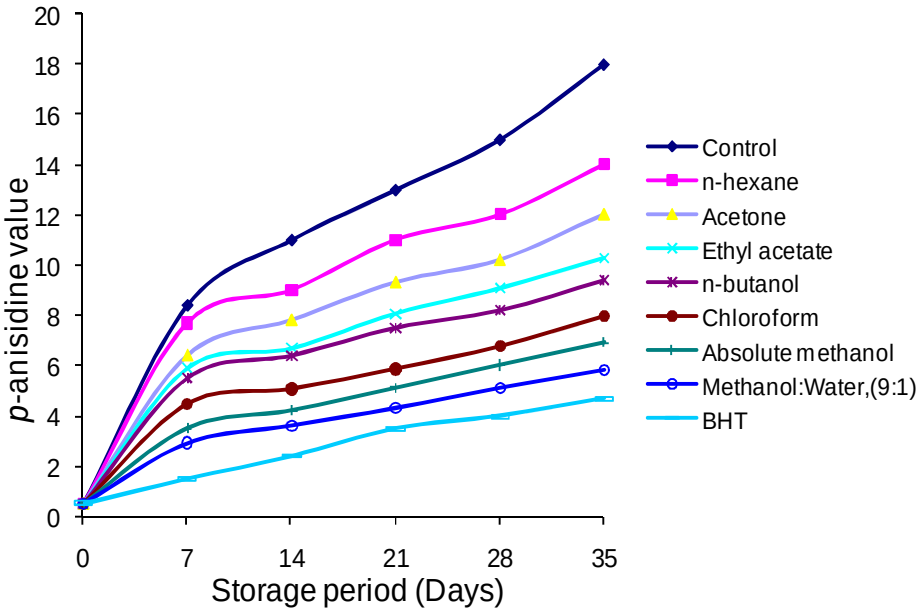

Fig. 3 Relative increase in *p*-anisidine value of treated corn oil samples under storage

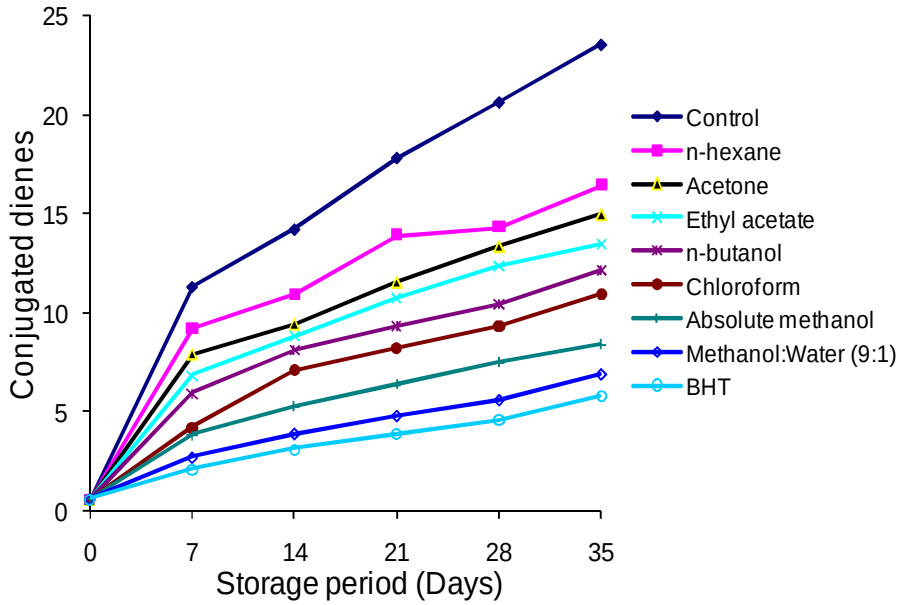

Fig. 4 Relative increase in conjugated dienes (CD) value of treated corn oil samples under storage

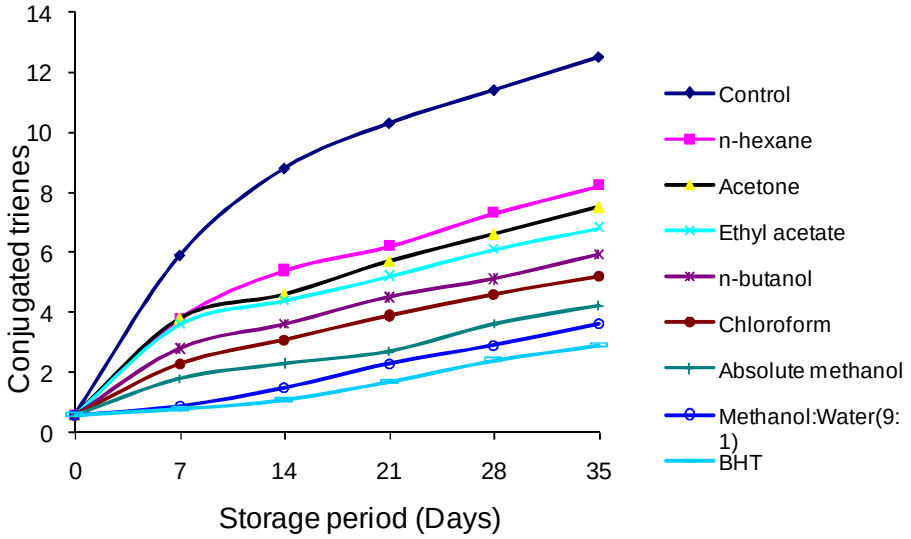

Fig. 5 Relative increase in conjugated trienes (CT) value of treated corn oil samples under storage

Formation of free fatty acids (FFA) might be an important measure of rancidity of foods. FFAs are formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture [29]. FFA content went on increasing with the increase in storage period for all the samples. Control exhibited the highest FFA while corn oil stabilized with BHT exhibited least (Figure 2). The lower values of free fatty acid contents of stabilized oil samples than control indicated the effectiveness of leaves extracts as natural antioxidant in retarding the free fatty acid contents. The results for *para*-anisidine values (PAV) which usually determines the amount of aldehyde in oils presented in Figure 3. The control sample showed the maximum increase in *para*-anisidine values indicating a higher rate of secondary product formation. A slow increase in PAV of stabilized corn oil as compared with the control indicating the antioxidant potential of the plant leaves. A decreasing order of stability of oil treated with different extracts of plant regarding *para*-anisidine values was found to be: BHT > methanol: water (9:1) > absolute methanol > chloroform> *n*-butanol > ethylacetate >acetone> *n*-hexane > control.

The formation of conjugated dienes (Figure 4) and trienes (Figure 5), analyzed for the control and stabilized corn oil at 232 and 268 nm respectively. Highest contents were observed for control, indicating greater intensity of oxidation. Formation of high contents of CD might be related to the presence of higher contents of polyunsaturated fatty acids [30] in corn oil. Conjugated trienes may be produced by dehydration of conjugated diene hydroperoxides. The periodical analysis of the incubated samples revealed a typical pattern of rise in the CD and CT contents. . A slow increase in CD and CT of the stabilized sunflower oil as compared with those of the control indicated the antioxidant potential of the plant leaves. During the storage period a rise in CD and CT from 0.6 to 16.4 and from 0.6 to 8.2 was noted for CD and CT respectively in case of *n*-hexane. Absolute methanol extract showed the lowest values. All extracts of the played a prominent role for stabilization of corn oil but after standard BHT, methanol extract was most efficient to stabilize the oil.

In vitro Hemolytic activity of plant extracts

Hemolytic activity was performed because the plant possessing potent antioxidant potential may not be useful in pharmacological preparations if they possess hemolytic effect. The total hemolysis (%) was obtained using 20 μL of Triton X-100 (0.1%). Methanol:water,(9:1) extract showed highest haemolytic effect (2.88%), followed by chloroform (1.94%), *n*-butanol (1.81%), ethylacetate (1.46%) and *n*-hexane (1.66%) and absolute methanol (0.70%) extracts respectively. The stability of the red blood cells membrane is a good indicator of the effect of various *in vitro* studies by various compounds for the screening of cytotoxity. The percentage lysis of human erythrocytes was less than 5.0 % for all samples, so it can be predicted that the extract and fractions have a minor cytotoxity [31]. All these results were in safe range. So pharmacologically this plant may be safe to use for human beings as a source of potential drug.

Conclusion

The results of the present study concluded that the plant possessed considerable antioxidant potential and it may also be used to stabilize the edible corn oil. Hence, the plant leaves investigated can be explored as a potential antioxidant source of natural origin. Cytotoxity of plant extracts against human

erythrocytes was checked and it was in safe range so the investigated plant may be safe to use for pharmaceutical and naturaltherapies.

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