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Studies on the Effects of Extracts of Fresh Khat/*Catha edulis* Leaves on the Oxidation of Niger Seed Oil

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Abstract: The methanolic extract of the fresh leaves of khat/*Catha edulis* was investigated using established procedures for its phytochemical constituents and effect on lipid oxidation. The phytochemical analysis revealed the presence of alkaloids, tannins, phenols, and flavonoids. The effects of the extract on oxidation of locally produced edible NSO (niger seed oil) was assessed through the measurement of peroxide value, iodine value and conjugated diene concentration for a period of sixteen days. Control setup involving NSO without the extract was also kept for the same period of time under similar conditions. It was observed that the peroxide value and conjugated diene value of the oil containing varying concentrations of the extracts of khat leaves increased significantly, relative to the control, showing that the extract of the khat leaves promoted deterioration of the oil. In a similar manner, the iodine value of NSO with and without added khat leaves extract was assessed. It was observed that the iodine value of the oil containing the khat leaves extract decreased more rapidly than the control, suggesting that the double bonds in the fat constituting the oil were diminished by the extract of the khat leaves. It is thus inferred from the present study that methanolic extract of fresh khat leaves leads to deterioration of NSO. It can also be suggested from the results of the present investigation that the health problems khat chewers face may be related to the oxidative damaging effects of these leaves exert on biomolecules found in the body.

Key words: Khat plant, lipid oxidation, oxidative stress, prooxidant.

1. Introduction

Khat (*Catha edulis*, family Celastraceae) is an evergreen plant grown in East-Africa and the South-West of the Arabian Peninsula. Khat leaves are chewed largely for their stimulant effect in several countries including Somalia, Kenya, Djibouti, Yemen, and Ethiopia [1, 2]. Previously known to grow mainly in the eastern part of Ethiopia, the khat plant is now widely cultivated in all parts of the country. The traditional way of khat leaves usage in Ethiopia used to be socially highly regulated: adult males (more seldom females) would gather and chew khat together at a so-called “khat party”, usually on weekends and afternoons until sunset and beyond. Currently, people of various age groups chew khat leaves at all hours of the day at homes, tea shops, hotel rooms as well as khat shops [3].

Khat leaves are reported to contain several chemical substances including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, and trace quantities of vitamins. In addition, elements including calcium, iron, manganese, zinc, copper and toxic metals like lead and cadmium have been reported to constitute the plant [1, 4, 5]. Fig. 1 depicted the alkaloids contained in the leaves of khat plant cathinone (A), cathine (B) and norephedrine (C) which are structurally and pharmacologically related to amphetamine [6]. Of these, the main active psychostimulant components are believed to be cathinone and cathine with cathinone being the most abundant and powerful stimulant.

While a typical khat chewing session may last between 3-7 hours, cathinone is released within 15-45 minutes of the onset of the chewing [4, 5]. The important

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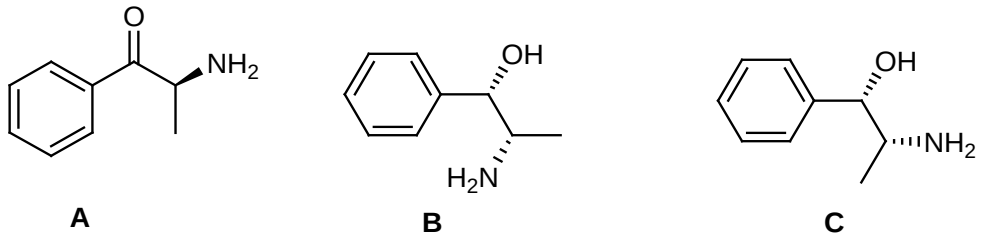

Fig. 1 Chemical structures of psychostimulants of the khat plant.

psychostimulants in the khat plant being related structurally to amphetamine, chewing khat leaves does have some health effects such as constipation, urine retention, increased alertness, heart attack, increased blood pressure, oral cancer, and suppressed appetite [7-9]. Free radicals and oxidants are now seriously implicated in khat toxicity despite the presence of different antioxidants as chemical components of khat, although the decreased activity of antioxidant enzymes due to ROS (reactive oxygen species) and oxidative stress have been reported in rats and humans [10]. ROSs are potentially very damaging to cells, leading to oxidation of essential cellular constituents including proteins, lipids and DNA [11].

Several health problems are now-a-days being associated with the habitual chewing of leaves of *Catha edulis* plant in several countries. As a result, the use of this plant is banned by law in some countries [12]. It is felt that the health problems emanating from Khat chewing are intrinsically related to the chemical constitution of the plant. This effect is likely to be aggravated by pesticides like DDT, and malathion sprayed on the khatleaves [13-15]. Currently, several million people are estimated to be frequent khat users for its euphoric/stimulant effects and other subjectively explained desirable effects. It is believed to enhance social interaction and usually used by individuals to improve their working capacity. Frequent users of the khat plant leaves include mainly students, drivers, female sex workers, military personnel, pastoralists, farmers, etc. Besides in some countries where the use of khat is widespread, the habit has a deep-rooted socio-cultural tradition. This is particularly true in Ethiopia where, chewing of khat in the eastern and south-eastern parts of the nation has a deep-rooted socio-cultural practice without knowing its bad effects. It was therefore thought desirable to look into the effects of extract of the leaves of this plant on the oxidation of edible oils which are known to contain lipids/polyunsaturated fatty acids that are similar to those found in the human body [16, 17], using locally produced NSO (niger seed oil) as source of the oxidisable substrates.

2. Materials and Methods

2.1 Collection of Khat/Catha edulis Plant Leaves

Fresh leaves of khat/*Catha edulis* (500 g) were purchased directly from farmers in February 2018 at a local farm land called “Gordema”, in Bahir Dar City, Ethiopia. The fresh leaves were packed in plastic bags and transported in an ice box to the laboratory and kept a deep freezer (Lyotrap plus) till needed.

2.2 Preparation of Khat/Catha edulis Leaves Extract

The extract was prepared as per a reported literature procedure [18]. A typical extraction experiment started with chopping the leaves to a fine powder in a dark place. The powder (300 g) so obtained was then soaked in an Erlenmeyer flask containing reagent grade methanol (1 L). The mixture was shaken using rotary shaker (120 rev/min) for two days at room temperature in a dark place. The extract was decanted and filtered (using Whatman No. 1 filter paper). The extract was placed in a hood under pressure for 3 days and then dried using freeze dryer at -30 °C. Extracts were kept in a small beaker and covered with aluminum foil and refrigerated until required for further analysis.

2.3 Preparation of the Analyzed Samples for the Determination of Oxidation (Peroxide Value, Iodine Value and Conjugated Diene Value)

The analyzed samples; NSO with and without khat leaves extract at different concentrations were prepared as per a reported literature procedure [19] with some modifications. Varying amounts of extracts of *Catha edulis* leaves (0.02 g, 0.04 g, 0.06 g, 0.08 g, and 0.1 g) were separately added to conical flasks each containing 0.02 g of NSO. Methanol (100 mL) was added to each flask and the contents were shaken thoroughly for proper mixing. Each flask was then kept in the laboratory at room temperature for all analysis. In a parallel control run, NSO (0.02 g/100 mL methanol) lacking the extract was thoroughly mixed and kept in the laboratory in a similar way. Those all prepared samples were kept at room temperature for the determination of oxidation of lipids with the parameters of peroxide value, iodine value and conjugated diene concentration.

2.4 Qualitative Phytochemical Screening Analysis of khat/Catha edulis Leaves Extract

The phytochemical analysis was carried out on the methanol extract using literature method [20]. One gram of methanolic extract of *Catha edulis* leaves was added to a 100 mL volumetric flask and dissolved in methanol. Methanol was then added up to the mark of 100 mL of the volumetric flask. The flask was gently shaken for uniform mixing. Aliquots of this were used for the phytochemical analysis.

Test for alkaloids: 5 mL of the *Catha edulis* leaves extract was added to 2 mL of 5% HCl. On addition of 1 mL of Wagner's reagent to this acidic medium, a red precipitate was produced immediately indicating the presence of alkaloids.

Test for flavonoids: On addition of a few drops of dilute sodium hydroxide (2 N) to 1 mL of the khat leaves extract, an intense yellow color was produced which becomes colorless on addition of a few drops of dilute 5% HCl indicating the presence of flavonoids.

Test for phenols: On treatment of 2 mL of the extract with few drops of neutral ferric chloride solution (5%) a deep blue color developed indicating the presence of phenols.

Test for tannins: 2 mL of the extract was added to 2 mL of water followed by addition of a drop of 5% diluted ferric chloride solution. A dark greencoloration appeared indicating the presence of tannins.

Test for saponins: 2 mL of khat leaves extract was diluted with 20 mL of distilled water and it was shaken in a graduated cylinder for 15 minutes. A layer of about 1 cm foam was formed showing the presence of saponins.

2.5 Determination of Effects of Khat Leaves Extract on the Oxidation of NSO

2.5.1 Determination of Peroxide Value

Peroxide value of an oil or fat is the amount of peroxides present and is expressed as milliequivalents of peroxide per 1,000 g of sample. The peroxide values were measured by AOCS (American Oil Chemist's Society) cd 8-53 official method (1990) [21, 22]. In a typical experiment, 5 g of samples; NSO with and without *Catha edulis* leaves extract at all concentrations were accurately weighed and added into separate 250 mL Erlenmeyer flasks containing 30 mL of a mixture of glacial acetic acid and chloroform in the ratio of 3:2 respectively. The solutions were swirled until the sample dissolves. Freshly prepared 0.5 mL of saturated potassium iodide solution was added to all solutions, stoppered, and gently shaken for exactly 1 min. Then 30 mL of distilled water and few drops of 1% starch indicator were added to the solutions. The solution turned blue due to formation of free iodine. Then this solution of liberated iodine was titrated with 0.1 N sodium thiosulphate solution until the blue color just started to disappear. A blank determination upon the same quantities of reagents (omitting the analyzed

samples), at the same time and under the same conditions was carried out.

$$PV\ (meq/Kg) = \frac{Nx\ (V_S - V_B)\ x\ 1000}{Wt.\ of\ sample\ (g)} \quad (1)$$

2.5.2 Determination of Iodine Value by Wij's Method

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100 g of the oil/fat, when determined by using Wij's solution [23]. Suitable quantity (0.25 g) of samples; NSO with and without *Catha edulis* leaves extract at all concentrations were accurately weighed and added into a 250 mL Erlenmeyer flasks containing 10 mL of CCl_4. The contents of the flasks were gently shaken to allow dissolution. Accurately 20 mL of Wij's solution (iodinemonochloride) was added. The flask was swirled once, closed with a stopper (moistened with minimum amount of 10% KI solution) and allowed standing at 15-20 ^oC for 30 min in the dark. Then, 15 mL of 10% KI solution and 100 mL distilled water were added sequentially to the solution. The solution was titrated with 0.1 N $Na_2S_2O_3$ using starch indicator until straw color appeared towards the end of the titration. On addition of two drops of starch solution, the mixture immediately changed to dark blue. The titration was continued until the blue color just disappeared. Blank test upon the same quantities of reagents (omitting the sample), at the same time and under the same conditions was analyzed.

$$Iodine\ value = \frac{(Blank\ titer - sample\ titer)\ ml\ x\ N\ of\ Na_2S_2O_3}{Wt.\ of\ sample\ (g)}\ x\ 12.69 \quad (2)$$

2.5.3 Determination of Conjugated Diene Concentration Using UV-Vis Spectrophotometer

Hydroperoxides react with poly unsaturated fatty acids (PUFAs) to form conjugated dienes that can be measured quantitatively by spectrophotometric UV measurement at wavelength 234 nm. Analyzed samples (0.25 g); NSO with and without khat leaves extract at all concentrations were added to separate 25 mL volumetric flasks and then dissolved with hexane. Hexane was then added up to the mark of each flask. Each flask was then shaken gently. Electronic absorption at 234 nm was then recorded [24]. Hexane was used as the blank.

$$E_{1cm}^{1\%} = \frac{A\ x\ V}{W\ X\ 100} = \frac{A\ x\ V}{\%\ oil\ in\ solution} \quad (3)$$

2.6 Data Analysis

All the data for oxidation determination methods were recorded in triplicate data as mean ± standard deviation and then analyzed with combination of Microsoft Excel 2010 and Origin software (version 8).

3. Results and Discussion

3.1 Qualitative Phytochemical Analysis

Phytochemical tests for alkaloids, flavonoids, phenols, tannins, and saponins were carried out on methanolic extract of *Catha edulis* leaves. Alkaloids, flavonoids, tannins and phenols showed positive results for their respective tests. On the other hand, the saponin content was not detected as shown below (Table 1).

3.2 Determinations of the Effects of Khat Leaves Extract on the Oxidation of NSO

Lipid oxidation products in analyzed samples of

Table 1 Qualitative phytochemical analysis of methanolic extracts of khat/*Catha edulis* leaves.

Sample	Phytochemical screening test results				
	Alkaloid	Flavonoid	Tannin	Saponin	Phenol
Khat extract	+	+	+	-	+

(+) = positive result/detected, (-) = negative result/not detected.

NSO with and without *Catha edulis* extract were prepared as per literature procedure [19] and studied using the parameters of the peroxide value, iodine value, and conjugated diene value.

3.3 Determination of Peroxide Value

Peroxides are the products formed at the initial stages of lipid oxidation and therefore, their values can be used as a measure of how lipid oxidation occurs. In the initial stage of lipid oxidation, conjugated double bonds rapidly form due to abstraction of hydrogen from bis-allylic positions of poly unsaturated fatty acids [25]. The peroxide content, expressed as milliequivalents of active oxygen per kg of oil (meq/kg), was determined by a standard procedure that entails reaction of oil contained in a 3:1 mixture of acetic acid and chloroform with a potassium iodide solution in the dark. The free iodine formed was then titrated with a sodium thiosulfate solution. The pertinent reactions are summarized in (Fig. 2).

The change in peroxide value of NSO with and without *Catha edulis* leaves extract was monitored every four days over a period of sixteen days at room temperature. It was observed that the peroxide values of all the samples increased during the entire period of experimentation in all the runs. During the first experimental period (i.e. first four days), the control run (i.e. NSO containing no *Catha edulis* leaves extract) showed no significant difference in peroxide value. However, in all the other runs involving NSO containing *Catha edulis* leaves extract significant increments in peroxide value were noted (Table 2). The increase in the peroxide value NSO containing *Catha edulis* leaves extract could be attributed to the formation of hydroperoxides, which are the initial products of oxidation. It is thus concluded that *Catha edulis* leaves extract lead to oxidative deterioration of the oil.

3.4 Determination of Iodine value by Wij's Method

Iodine value is a measure of the total number of unsaturated double bonds present in oils [26, 27]. Halogens add across the double bonds of unsaturated fatty acids to form addition compounds. Iodine monochloride (ICl) was allowed to react with the fat in the dark. The amount of iodine consumed is then determined by titration of the iodine released (after adding KI) with standard sodium thiosulfate solution used as a titrant. The pertinent reactions occurring in the test are shown in Fig. 3.

The iodine values were measured for all analyzed samples: control (NSO without *Catha edulis* leaves extract), and NSO containing *Catha edulis* leaves extract at different concentrations at room temperature at regular intervals of four days for sixteen days. The change in the iodine value of the analyzed samples is presented in Table 3. A decrease in iodine value of all analyzed samples was observed in all the runs during the experimentation period. The results of these experiments indicated that the iodine value of NSO containing *Catha edulis* leaves extract is lower than that of the control/run. In other words, *Catha edulis* leaves extract is able to lower the iodine value of NSO suggesting that the extract is acting as prooxidant thereby triggering peroxidation of the oil that produces a mixture isomeric conjugated dienes. The ultimate result is oxidative damage of the oil [1, 26].

3.5 Determination of Conjugated Dienes Using UV-Vis Spectrophotometer

Lipids exhibit a shift in their double-bond position because of isomerization and conjugated double bond formation during oxidation [27, 28]. The conjugated dienes (CD) formed show ultraviolet absorption at about 234 nm. In the present study, conjugated diene formation in all the analyzed samples (NSO lacking the extract, and NSO containing varying amounts of *Catha edulis* leaves extract) was monitored through measurement of electronic absorption at 234 nm regularly at intervals of four days during a period of sixteen days. The results revealed that the NSO containing *Catha edulis* leaves extract more amount of

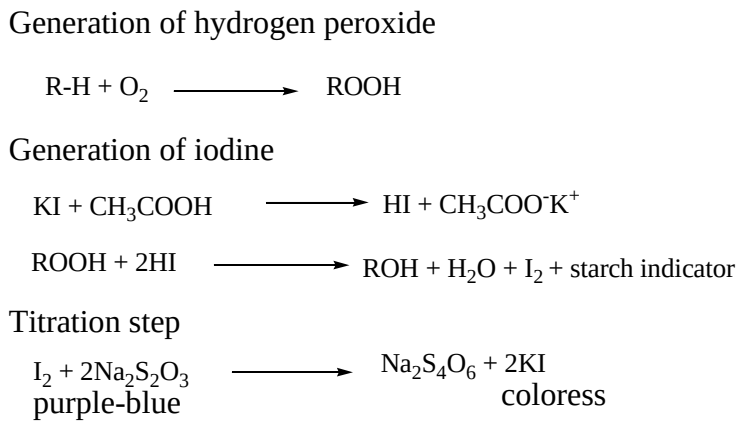

Fig. 2 Reactions occurring during peroxide value determination.

Table 2 Relative increase in means of peroxide values (meq/kg) of samples kept for 16 days at room temperature.

Days	Control/NSO	Khat extract + NSO				
		400 µg/mL	600 µg/mL	800 µg/mL	1,000 µg/mL	1,200 µg/mL
0	ND	0.6 ± 0.20	1.2 ± 0.20	1.6 ± 0.36	2 ± 0.00	5 ± 0.27
4	5 ± 0.44	8 ± 0.20	10 ± 0.70	12 ± 0.70	13 ± 0.36	14 ± 0.26
8	11 ± 0.17	15 ± 0.10	19 ± 0.62	19 ± 0.52	17 ± 0.26	19 ± 0.24
12	14 ± 0.36	17 ± 0.00	19 ± 0.20	19 ± 0.60	18 ± 0.79	20 ± 0.61
16	20 ± 0.87	20 ± 0.92	20 ± 0.92	20 ± 0.87	20 ± 0.36	22 ± 0.36

ND = not detected; all data are expressed as mean (n = 3) ± standard deviation.

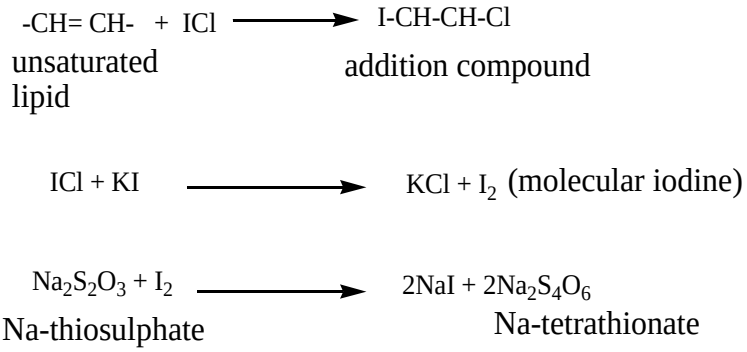

Fig. 3 Reactions on iodine value determination.

Table 3 Relative decrease in means of iodine values (g I_2/100 g oil/fat) of samples kept for 16 days at room temperature.

Days	Control/NSO	Khat extract + NSO				
		400 µg/mL	600 µg/mL	800 µg/mL	1,000 µg/mL	1,200 µg/mL
0	160.2496 ± 0.3675	152.6904 ± 0.4552	155.9184 ± 0.3289	154.8272 ± 0.5462	149.4320 ± 0.8847	126.0009 ± 0.7935
4	142.4596 ± 0.7069	140.6405 ± 0.4855	142.4219 ± 0.3892	139.4238 ± 0.5714	137.5432 ± 0.4594	119.9328 ± 0.2036
8	135.8848 ± 0.2158	130.9608 ± 0.1947	134.0640 ± 0.0633	131.9760 ± 0.0932	130.9608 ± 0.1989	115.7328 ± 0.5706
12	129.5434 ± 0.9064	122.8734 ± 0.5389	120.9473 ± 0.4845	123.4732 ± 0.1319	123.0312 ± 0.3505	122.4134 ± 0.5059
16	136.0368 ± 0.3913	137.0520 ± 0.2936	140.0976 ± 0.1168	142.1280 ± 0.1528	139.0824 ± 0.3428	140.0976 ± 0.3311

All data are expressed as mean (n = 3) ± standard deviation.

Table 4 Relative increase in means of conjugated diene concentrations ($g^{-1}·cm^{-1}·mL$) of samples kept for 16 days at room temperature.

Days	Control/NSO	Khat extract + NSO				
		400 μg/mL	600 μg/mL	800 μg/mL	1,000 μg/mL	1,200 μg/mL
0	0.808 ± 0.009	0.864 ± 0.133	0.934 ± 0.053	0.966 ± 0.054	0.988 ± 0.032	1.132 ± 0.134
4	0.808 ± 0.108	0.996 ± 0.207	1.008 ± 0.024	1.044 ± 0.060	1.134 ± 0.125	1.188 ± 0.199
8	0.808 ± 0.069	1.002 ± 0.124	1.088 ± 0.182	1.194 ± 0.196	1.138 ± 0.140	1.208 ± 0.208
12	0.808 ± 0.239	1.086 ± 0.194	1.146 ± 0.295	1.198 ± 0.205	1.156 ± 0.167	1.324 ± 0.136
16	0.808 ± 0.139	1.138 ± 0.152	1.154 ± 0.190	1.204 ± 0.217	1.244 ± 0.234	1.334 ± 0.126

All data are expressed as mean (n = 3) ± standard deviation.

conjugated dienes than the control (i.e. NSO lacking *Catha edulis* leaves extract), as shown in Table 4. This result again shows that *Catha edulis* leaves extract enhances oxidation of NSO thereby leading to its ultimate oxidative damage.

In summary, it is seen from the present study that measurement of peroxide value and iodine value of NSO containing varying amounts of methanolic extract of *Catha edulis* leaves was oxidized to a greater extent than the oil lacking the extract (control). Furthermore, measurement of UV-Vis absorption at 234 nm of the oil containing methanolic extract of *Catha edulis* leaves showed that the extract led to formation of conjugated dienes to a greater extent than in the control run. It is thus concluded that methanolic extract of *Catha edulis* leaves leads to oxidative damage of the oil. This finding may be taken as indicator of the health problems associated with chewers of *Catha edulis* leaves.

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