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Antioxidant Activity of Ethanolic Kalonji Extract in Sunflower Oil, Oil Blends and Vanaspati

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ABSTRACT

The study reports the effect of ethanolic kalonji extract on the oxidative stability of sunflower oil, blend of sunflower oil and palm oil and Vanaspati. The oxidative stability of sunflower oil and its blend with palm oil was determined by schaal oven test at 60ºC. The oxidative stability study of vanaspati was carried out by Rancimat Test at 120ºC. The oxidation properties were compared to that of synthetic antioxidants like TBHQ. The ethanolic Kalonji extract was analysed for phytochemical screening and antioxidant assay. The phytochemical screening showed the presence of flavonoids and terpenoids in the ethanolic kalonji extract. The % inhibition for ethanolic kalonji extract at 5mg/ml was almost 97%. Curcuminoids in conjugation with kalonji showed the antioxidant results comparable to that of TBHQ. Ethanolic kalonji extract at 0.9% showed better antioxidant activity in Vanaspati. Thus, the results suggesting that the ethanolic kalonji extract can be preferred over the synthetic antioxidants.

INTRODUCTION

Vegetable oil which is sources of essential fatty acids are prone to oxidation. Unfotunately this auto oxidation is cause of food deterioration, giving rise to off flavours and odours to oil. During refining stages of oil like bleaching and deterioration, the natural antioxidants like tocopherol are removed. (De Greyt and Kellens 2005). As the residual tocopherol is insufficient to provide antioxidant activity to oil, synthetic antioxidants are added to the proceed oil. However, there is a concern regarding the safety of synthetic antioxidant. The prolonged consumption of synthetic antioxidants could lead to potential health problems. They could be promoting agents that target liver, lung and stomach issues to alter their gene expression. (Pitot and Dragon 2001) On the other hand, antioxidant phytochemicals from natural sources are considered GRAS – generally recognised as safe and no safety test are required by legislation. With this advantage and safety concern of artificial chemicals more and more consumers and food developers prefer using natural antioxidants to replace synthetic antioxidants.

There are array of evidence for the therapeutic effects of plant based components or foods and are gaining popularity all over the world. Among them, Kalonji an important carminative and spice, a common household spice have been shown to display various therapeutic effects, including antioxidant, antihypertensive, analgesic, anti-inflammatory, anticancer, antihistaminic, antiparasitic, antibacterial and protective effects against hepatotoxicity and nephrotoxicity (Daba and Rahman; Padhye *et al.*, 2008). It contains antioxidants like thymoquinone (~12%), thymol (~0.8%), carvacrol (~3.7%) (Singh *et al.*, 2005).

The objective of the present work was to study the oxidative stability of sunflower oil, blend of sunflower oil and palm oil and vanaspati by utilising the Kalonji extract. Curcuminoids, which is also a common household spice was used in conjuction to Kalonji to study the effect on the oxidative stability of sunflower oil. Several researchers have reported the anti-inflammatory properties (Woo *et al*., 2007), anticarcinogenic effects (Wahl *et al*., 2007; Zhang *et al*., 2007) and hypoglycemic effects in humans (Srinivasan 1972).

MATERIALS AND METHODS

Materials, Chemicals and Reagents

RSFO (Refined sunflower oil) was received as a gift sample from M/s Cargill India Pvt. Ltd., Pune while Palm oil was procured from Kamani Oils Industries Pvt. Ltd., Mumbai. Both the oils did not contain any synthetic antioxidants.

Kalonji seeds were purchased from the local markets in Mumbai. The seeds were ground to powdered form and stored. All the other chemical Reagents and solvents were obtained from Himedia, Mumbai.

Proximate Composition of Seeds

The seeds were analysed for proximate composition. Moisture content, protein content with a nitrogen-protein conversion factor of 6.25 and ash content were determined according to AOAC (1990).

Extraction of Oleoresin from Kalonji seeds

The Kalonjiin powdered form was extracted using soxhlet extractor. Ethanol was used as a solvent for extraction. The crude oleoresin was obtained after removal of solvent using rotary vacuum evaporator.

Phytochemical Screening

Phytochemical screening was performed using standard procedures (Sofowora, 1993; Trease and Evans, 1998).

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour change.

Test for anthroquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test

tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Tannin

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Terpenoids

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Flavanoids

A few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Test for saponins

0.5 g of extract was taken in a test tube and 5 ml of water was added. After shaking the solution was observed for a stable persistent froth. The frothing was mixed with3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Antioxidant Activity

The free radical scavenging activity was determined by according to the procedure of Brand-Willaims (1995). The absorbance of DPPH diluted in ethanol was considered as a control. The absolute ethanol was used as a blank. The absorbance was measured at 520nm. The antioxidant capacity to scavenge DPPH radical was calculated by the equation.

$$\% \text{ Inhibition} = [(C-S)/C] \text{ X } 100$$

C- Net absorbance of control

S-Net absorbance of sample

Percent inhibition was plotted against concentration, and the equation for the line can be used to obtain the IC50value. A lower IC50 value indicates greater antioxidant activity.

Stability Test

Schaal Oven Test

Preparation of Blends

A.) The blends of oil containing KEE (2% w/v) along with curcuminoids (50 ppm) and at other varying concentrations were prepared The oxidative stability of oil blends was checked at 60°C for 30 days at regular interval of 5 days according to the AOCS Official Methods (AOCS 1994) by peroxide value (PV, Method Cd 8-53).

B.) The blend of RSFO and palm oil was prepared and the oxidative stability of the blend was determined by schaal oven test at 60ºC. The oxidative stability of oil blends was checked at 60°C for 12 days at regular interval of 4 days according to the AOCS Official Methods (AOCS 1994) by peroxide value (PV, Method Cd 8-53).

Rancimat Test for oxidative stability

The oxidative stability of oil was determined by the rancimat assay (Metrohm 743 Rancimat). 3g of vanaspati (free of added antioxidants or preservatives) was taken in the reaction vessel. Ethanolic kalonji extract was added in varying concentrations. The test was carried out at 110^0C with airflow 20 $l.h^{-1}$. Secondary volatile reaction products which are formed are absorbed in the deionised water. The electrical conductivity of the water increases due to absorption of the reaction products. The induction time is the time until the secondary reaction products are detected and the graph conductance vs. time is recorded by Rancimat. The activity of ethanolic banana peel extract was compared with control sample and TBHQ, BHA (50ppm) under the same conditions.

RESULTS AND DISCUSSION

Proximate Composition of Kalonji Seeds

The proximate composition of Kalonji seeds is shown in the table below. Proximate composition is important in determining the quality of raw material. The Kalonji seeds contain 4.32±1.05, 20.65±0.58, 4.26±1.94, 14.56±2.34 % of moisture, protein, ash and fat content. Authors reported the composition of Kalonji seeds and moisture, fat, protein, ash contents in the range of 3.8-7.0, 22.0-40.35, 20.85-31.2, 3.7-4.7 %. (Dandik and Aksoy, 1992; Abdel-Aal and Attia, 1993; El-Dhaw and Abdel-Manaem, 1996; Takruri and Dameh, 1998; Atta, 2003; Salem, 2005). The proximate values in the present research were in close conformity with the values described in the literature with slight variations. This might be due to the differences in genetic makeup, environmental factors like climate and location.

Table No. 1: Proximate Analysis of Kalonji seeds

Parameters	Composition (%)
Moisture	4.32± 1.05
Protein	20.65±0.58
Ash content	4.26±1.94
Fat Content	14.56±2.34

Values are mean± standard deviations

Phytochemical Screening

Preliminary phytochemical screening of Kalonji seed oleoresin showed the presence of Terpenoids, flavonoids and saponins. Reducing sugars, tannins were absent in the Kalonji seed oil. In a study carried out by Purkayastha *et al*., (2012) the flavonoids were absent in Kalonji seed oil.

Antioxidant Activity

The antioxidant activity of Kalonji seeds, its ethanolic extract and synthetic antioxidant TBHQ is shown in Fig 1. In this test, IC 50 value was calculated through the interpolation of linear

regression analysis. Due to ease and convenience, DPPH assay has widespread use in determining the free radical scavenging activity (Wu *et al*., 2003; Thaipong *et al*., 2006; Erkan *et al*., 2008; Scherer and Godoy, 2009). On the basis of DPPH radical scavenging activity, the powdered Kalonji and the Ethanolic extract showed antioxidant activity equivalent to TBHQ. However, the IC50 value for Ethanolic extracts of Kalonji was 0.38 which was lower than that of TBHQ (IC50 value-0.78). Thus, the order of antioxidant activity follows: Ethanolic Extract of Kalonji> TBHQ> powdered Kalonji (Nigella Sativa). At 5 and 10 mg/ml the Nigella S. powder and its ethanol extract showed similar scavenging activity to that of TBHQ. The inhibition by ethanolic Kalonji extract at 5mg/ml was almost 97%. While Khattak *et al*. (2008) observed DPPH radical quenching activity in the range of 70-90% for 5mg/mL for methanolic extract. Further, Singh *et al*. (2005) suggested that antioxidant activity of Kalonji seeds is comparable to that of synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and propylgallate (PG).

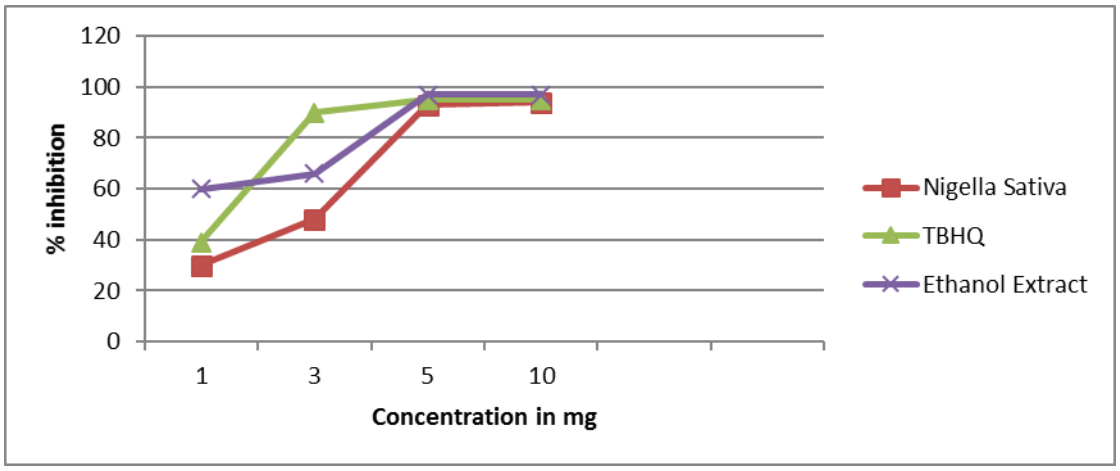

Figure No. 1: Antioxygenic activity of Kalonji seeds and its extracts using DPPH radicals Scavenging activity

From the graph, IC-50 value for kalonji seeds was 1.81, whereas for TBHQ the IC -50 value was 0.78 and for ethanolic Kalonji extract was 0.38.

Effect of Kalonji Extract (K.E) with Curcuminoids on Oxidative Stability of RSFO at 60°C

From Table 2, the peroxide value of the control sample (RSFO) increases to 27 up till 25th day which is higher than the RSFO+2% K.E, RSFO+2% K.E+ 50ppm curcuminoids, RSFO+TBHQ.

It can be posits that KE extract and curcuminoids are having good antioxidant activity. On 20th day, peroxide value for RSFO+2%K.E+ 50ppm curcuminoids was 12.5 equivalent to the peroxide value of RSFO+TBHQ *i.e.* 12. When Kalonji extract was added with curcuminoids, it showed synergistic activity in antioxidant efficacy. As curcuminoids are potent antioxidants it necessitates maintaining these curcuminoids in the keto form by employing acidic conditions to utilize their antioxidant activity. The acidic medium required to stabilize curcuminoids in the keto form can be achieved by using KEE.

If we compare the control sample and RSFO+50 ppm curcuminoids there is a subtle increase in peroxide value of sample RSFO+50 ppm curcuminoids. But **when curcuminoids used in combination to KEE (Kalonji Ethanolic extract) showed results equivalent to that of TBHQ and better than even the 2% KEE**.

Table No. 2: Effect of Kalonji Extract (K.E) with Curcuminoids on Oxidative Stability of RSFO at 60°C (Peroxide Values)

Time (Days)	RSFO	RSFO+2%K.E	RSFO+2%K.E+ 50ppm curcuminoids	RSFO+50ppm curcuminoids	RSFO+TBHQ
0	1.3	1.29	1.29	1.276	1.24
5	9	7	6.5	22.5	3
10	12.5	10	8	24	5.1
15	14.8	10.9	8.5	25	7
20	22	14.8	12.5	27	12
25	27	16	14.8	32	12.9

Effect of Kalonji extract on oxidative stability of RSFO + Palm oil blend (20:80) at 60ºC

From Table 3, it can be seen that on the 8th day the peroxide value for blend +0.9%K.E was 6.78 lower than the peroxide value of Blend+0.7%K.E. To achieve the antioxidant activity equivalent to TBHQ higher concentrations than 0.9% should be employed.

Table No. 3: Effect of Kalonji extract on oxidative stability of RSFO + Palm oil blend at 60ºC

Group A: Sunflower (20) + Palm oil (80)

Time (Days)	Blank	Blend+0.5% K.E	Blend+0.7%K.E	Blend+0.9% K.E	Blend+200ppm TBHQ
0	2.97	2.5	2.7	2.1	2.1
4	7.86	6.06	6	5.87	2.7
8	9.34	7.93	6.56	6.78	3.4

Effect of Kalonji extract on oxidative stability of RSFO: Palm oil (80:20) blend at 60ºC

From Table 4, it can be seen that on the 8th day the peroxide value for blend +0.9%K.E was 6.8 lower than the peroxide value of Blend+0.7%K.E. To achieve the antioxidant activity equivalent to TBHQ higher concentrations than 0.9% should be employed.

Table No. 4: Effect of Kalonji extract on oxidative stability of RSFO: Palm oil (80:20) blend at 60ºC

Group B: Sunflower (80) + Palm oil (20),

Time (Days)	Blank	Blend+0.5% K.E	Blend+0.7%K.E	Blend+0.9% K.E	Blend+200ppm TBHQ
0	2.27	2.2	2.17	2.03	2
4	8	6.5	6.9	6.4	2.3
8	9.8	8	7	6.8	3.8

Effect of kalonji extract on oxidative stability of Vanaspati at 120ºC by rancimate test

The induction time for varying concentrations of Kalonji extract and synthetic antioxidants in vanaspati shown in Table. From the graph (conductance vs time), it can be posist that 0.9 % Kalonji ethanolic extract shows better activity. Since the induction time for 0.9 % Kalonji

ethanolic extract is higher than the 1.5 % kalonji ethanolic extract. However, the induction time for TBHQ was 1 hour more than the 0.9 % kalonji ethanolic extract.

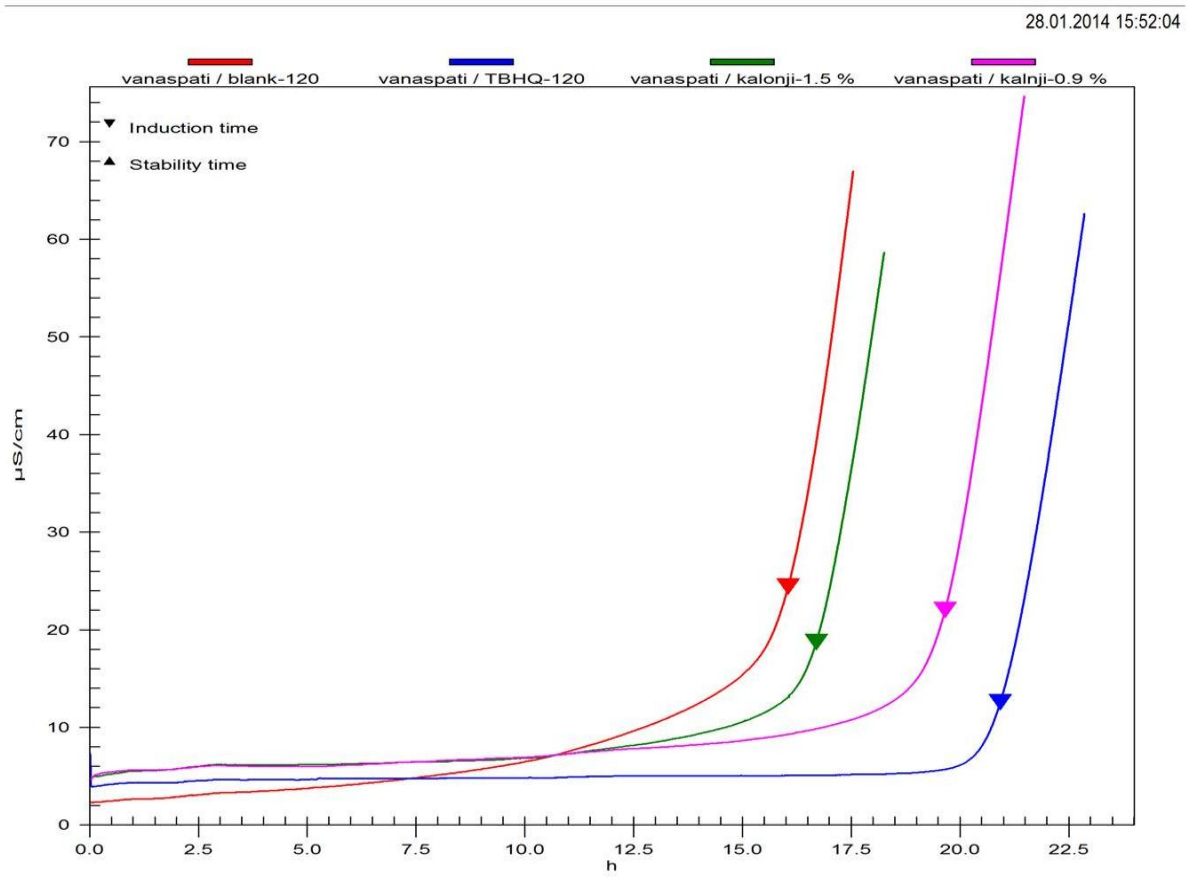

Figure No. 2: Electrical conductance vs. time graph for Kalonji extract in Vanaspati

Table No. 5: Antioxidant activity of ethanolic Kalonji extract in Vanaspati at 120°C

Composition	Induction time
Blank (Vanaspati without antioxidant)	16.05 hrs
Vanaspati + 0.9% Kalonji Ethanolic Extract	19.66 hrs
Vanaspati + 1.5% Kalonji Ethanolic Extract	16.70 hrs
Vanaspati + TBHQ	20.93 hrs

CONCLUSION

Ethanolic kalonji extract in conjugation with curcuminoids showed synergistic activity. A higher concentration of ethanolic kalonji extract is required to achieve the oxidative stability in oil blends comparable to that of synthetic antioxidants. In Rancimat test, higher thermal stability is observed in case of TBHQ, but in comparison with the cost to kalonji and for use as a spice in food preparation, kalonji can be preferred as a natural antioxidant.

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