ISSN 0973-3450 (Print)
JUC Vol. 15(2), 9-17 (2019). Periodicity 2-Monthly
ISSN 2319-8036 (Online)

JOURNAL OF ULTRA CHEMISTRY
An International Open Free Access Peer Reviewed Research Journal of Chemical Sciences and Chemical Engineering
website:- www.journalofchemistry.org
Estd. 2005

Zanthoxylum rhetsa DC, a potential antioxidant preservative to control the rancidity of peanut oil

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http://dx.doi.org/10.22147/juc/150201

Acceptance Date 23rd February, 2019, Online Publication Date 2nd March, 2019

Abstract

Tribal people in India traditionally practiced to use available spice materials in cooking, medicine, food preservations and as well as in perfumes. Zanthoxylum rhetsa DC is thorny tree growing in different region of India. Its extract has multiple effect like antioxidant, anti-inflammatory, antimicrobial, antibacterial etc. The present work designed to study the antioxidant effect of zanthoxylum rhetsa DC dry fruit in controlling rancidity of groundnut oil. Peroxide value and para anisidine value of dried coarse powder and methanol extract in groundnut oil were found to be controlled in compare to the blank oil at incubated temperature 60°C. Duration of 20 days' incubation at 60°C, the 1 gm dry fruit powder and methanol extract of 1 gm powder in groundnut oil shown peroxide and para anisidine values 16.8meqO_2/kg, 16.5 meq O_2/kg and 17.4, 17.9 respectively to that of blank oil 19.4 meqO_2/kg as peroxide value and 20.3 para anisidine value. The control butylated hydroxyl toluene (BHT) and α-tocopherol recorded a peroxide and para anisidine value11.6 meq O_2/kg, 16.3 meq O_2/kg and 9.3, 10.4 resspectively. The ethanol, ethyl acetate and water extract have maximum peroxide and para anisidine values (19.7, 20.1, 20.9 meqO_2/kg and 21.1, 22.2, 22.3) with the control and blank oil. Dry fruit coarse powder as whole and 1gm methanol extract stabilize the groundnut oil by controlling primary and secondary oxidation. This study suggested that the dry fruits of Zanthoxylum rhetsa DC is natural antioxidant to control rancidity of groundnut oil

Key words: Zanthoxylum rhetsa DC, Peroxide Value, Para anisidine Value, Rancidity, Groundnut oil, Butylated hydroxyl toluene(BHT), α-tocopherol

Introduction

Zanthoxylum rhetsa is a tree of Rutaceae family growing very tall. It is well grown in the Western Ghats to a height of 33-37 metres. The bark is mottled with conical prickles. It is widely spread in warm, temperate and subtropical area worldwide. It is appeared in Konkan, Mysore, Malabar, Anamalai and Travancore at low elevation Orissa, Chitttagong, Pegu. Also found in Assam Meghalaya and in eastern Western Ghats of peninsular India[1]. Many researchers have reported that polyphenolic compounds exceptionally present in plants' extracts show multiple protective effects like antioxidant[2], anti-inflammatory[3], antibacterial[4] and anti-proliferative activities[5]. K. Poornima *et al.*[1] proposed that fruit extracts of Zanthoxylum rhetsa DC from the collected sample, during November, were tested for the presence of bioactive compounds. Various phytochemicals like carbohydrates, alkaloids, flavanoids, coumarins, cardiac glycosides, phenols, terpenoids, tannins, saponins, phlobatannins, steroids, anthraquinons etc were analysed. The modified ferric ion reducing method was used to assay antioxidant property[6]. Phytochemical analysis of methanol and ethanol extract of fruit indicated the presence of alkaloids, flavonoids, terpenoids, proteins, carbohydrates, triterpenes, saponins, coumarins, steroids and chalcons. Escherichia coli, Pseudomonas aeruginosa, Staphylococcos aereus, Klebsiella exhibited sensitivity towards methanol, ethanol and ethyl acetate extracts of fruit. The antioxidant property was promising and the study revealed that Z. rhetsa shows antioxidant as well as antibacterial property. A recent review of the literature on the topic total phenol content (TPC) and antioxidant capacity (AC) related data on different varieties of fruits, rice, vegetables and indigenous foods was well documented[7,8,9,10]. Avonti Basak Tukun., *et al.*[11] analysed 15 medicinal plants' TPC hydrophilic extracts. Among the assayed plants, TPC (mean±SD), expressed as gallic acid equivalent, varied from 0.04±0.01 (Amaranthus spinosus) to 6.01±0.04 (Zanthoxylum rhetsa) mg gallic acid equivalent/g fresh weight. AC (mean±SD), expressed as trolox equivalent, ranged from 0.14±0.00 (Alternanthera philoxeroides) to 7.54±0.00 (Zantho. xylum rhetsa) µmol trolox equivalent/g fresh weight. Shivprasad Mahadkar., *et al.*[12] reported an analysis of five species of promising wild edible fruits such as Gmelina arborea Roxb, Oroxylum indicum (L.) Vent, Bauhinia recemosa Lam. Caryota urens L and Zanthoxylum rhetsa (Roxb.) DC from Kolhapur district, M.S.(India) for their antioxidant activities using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity, ferric reducing antioxidant property(FRAP), reducing power ability, chelating activity on ferrous ion and total antioxidant capacity. The total phenol content varied from 0.061±0.29 (Zanthoxylum rhetsa) to 0.500±0.012 g/100g FW(Caryota urens).

The chemical composition of Zanthoxylum rhetsa DC was reported by many researchers. A crystalline compound obtained from the oil of Zanthoxylum rhetsa, called mullilam diol and formulated as p-menthane-2,3-dihydroxy-1,4-oxide has been conclusively verified to possess a revised structure, (±)-p-menthan-1α,2β,4β-triol [13]. Volatile constituents of Zanthoxylum rhetsa leaves and seeds were analyzed by GC and GC/MS which resulted in the identification of 118 compounds from the leaf oil and 77 compounds from the seed oil. Caryophyllene oxide (12.7%), β-caryophyllene (9.6%), β-copaene (5.3%) and spathulenol (3.3%) were the main components of the leaf oil while sabinene (66.3%), α-pinene (6.6%), β-pinene (6.3%) and terpinen-4-ol (3–5%) were the major components of the seed oil (14). Effect of extraction methods on the composition of Z. rhetsa seed essential oil was examined. The steam-distilled essential oil from the seeds of Z. rhetsa, obtained under three different conditions were analysed by GC and GC–MS, resulting in the identification of 43 constituents. Sabinene (66.74% and 72.7%), β-pinene (6.47% and 6.59%), α-pinene (6.08% and 6.08%) and myrcene (1.48% and 1.6%) were the major components obtained from aqueous and alkaline media, respectively, while α-terpinene (23.68%),

γ-terpinene (23.06%), terpinolene (5.66%) and limonene (4.67%) were the main components from acid medium[15]. A chemical fingerprint of various secondary metabolites of Zanthoxylum rhetsa (Roxb.) DC was screened. HPTLC of phytochemical screening for various secondary metabolites were done and profiles were developed for authentication. The ethanolic extract of the fruit showed the presence of eight Glycosides, ten Flavonoids, six Essential Oils, five Anthraquinones, nine bitter principles, seven Coumarins and eight Terpenoids[16]. Fresh and dried fruits provided a higher yield of volatile oil by approximately 10% and 20% respectively, compared to other studies. Twenty-eight compounds were identified and the major components of fresh and dried fruits were not distinctly different. The major component, α-limonene, was found in dried fruits from Phayao, southern Nan and Chiang Rai province. A high content of β-phellandrene was found in dried fruits from northern Nan province and the (+)-sabinene was found in high content of fresh fruits from southern Nan, Phayao and Chaing Rai province. Among the components investigated, fresh and dried fruits from southern Nan province showed to be the most potent[17].

The present work was focused on the antioxidant effect of various hydrophilic solvent extracts and dried coarse powder of the fruit in the preservation of groundnut oil to control the rancidity. Butylated hydroxyl toluene was used as control. Coastal people of Karnataka and Goa, India are using the fruits of zanthoxylum rhetsa DC as flavoring agents in food preparation especially in preparing fish curry.

Materials and Methods

Chemicals :

Methanol (LR), Glacial Acetic acid (LR), Ethanol (LR), Ethyl acetate (LR), Chloroform, Double distilled water, Potassium iodide(AR) Sodium thiosulphate (AR), Potassium dichromate (AR), Potassium hydroxide (AR), Oxalic acid hydrated (AR), Starch powder, Butylated Hydroxy Toluene (B.H.T). α-Tocopherol, Filtered Groundnut oil from the local market (Karwar, Karnataka, India).

Sample preparation :

Zanthoxylum rhetsa DC fruits were collected from the local market, Karwar, Karnataka. India. The fruits were washed and dried under sunlight and the seeds were removed. Seed free fruits were ground to coarse powder using a mixer and stored in the air tight bottle.

Methanol Extract :

Exactly one gram of coarse powder was homogenized in for 5 minutes in methanol and stirred well. The supernatant liquid was filtered through What Mann filter paper No 1 in to a 150 ml beaker. This process was repeated for another two times. The total solvent was evaporated on the water bath.

Ethanol Extract :

Exactly one gram of coarse powder was homogenized in for 5 minutes in Lab reagent grade ethanol and stirred well. The supernatant liquid was filtered through What Mann filter paper No 1 in to a 150 ml beaker. This process was repeated for another two times. The total solvent was evaporated on the water bath.

Ethyl Acetate Extract :

Exactly one gram of coarse powder was homogenized in for 5 minutes in Lab reagent grade ethyl acetate and stirred well. The supernatant liquid was filtered through What Mann filter paper No 1 in to a 150 ml beaker. This process was repeated for another two times. The total solvent was evaporated on the water bath.

Water Extract :

Exactly one gram of coarse powder was homogenized in for 5 minutes in Lab reagent grade ethyl acetate and stirred well. The supernatant liquid was filtered through What Mann filter paper No 1 in to a 150 ml beaker. This process was repeated for another two times. The total solvent was evaporated on the water bath.

Preparation of oil sample :

Each was prepared in 150 ml beaker as shown in the Table 1 and incubated at 400C.

Table 1 Sampling for antioxidant properties

Sample	Extract/ Substance	Groundnut oil (ml)
S_1	-	100
S_2	0.1gm B.H.T	100
S_3	0.01 gm α-Tocopherol	100
S_4	1gm Coarse powder of Z rhetsa DC	100
S_5	Methanol extract	100
S_6	Ethanol Extract	100
S_7	Ethylacetate Extract	100
S_8	Water Extract	100

Methods

Density :

Density of oil was determined specific gravity bottle method. The specific gravity bottle was washed well, rinsed with acetone and dried using hair drier. The bottle was cooled in desiccator and weighed for constant weight. The density of the sample was determined against distilled water as shown below.

$$\rho = \frac{Wo - W_e}{W_w - W_e}$$

ρ = Density of oil, Wo = Weight of specific gravity bottle with oil, W_e = Weight of empty specific gravity bottle, W_w = Weight of specific gravity bottle with water

Acid Value of oil (AV) :

Filtered peanut oil from local market was used to determine acid value of Acid value is an important indicator of oil quality. AV is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil. In the present work AV determination is based on the international standard method[18]. The chemicals used are analytical grade with 99% purity.

Exactly 1±0.05 gram of peanut oil was weighed into 250ml clean conical flask. To this 25ml of neutralized ethanol was added and mixed well. The resulting solution was titrated against standard 0.1N KOH using phenolphthalein as an internal indicator to the end point colourless to pink colour. KOH solution was standardized by standard 0.1N oxalic acid solution.

Peroxide Value (PV) :

Exactly 5gm oil was weighed into the Erlenmeyer flask with glass stopper. Acetic acid and chloroform mixture in the ratio 3:2 was added to the flask. To this 0.5 ml saturated KI solution was added, and peroxide value was determined by titrating against standard 0.01 M sodium thiosulfate solution using starch as indicator. The procedure was followed according to officially recommended method by AOCS[18].

$$PV = \frac{(S\text{-}B) \times N \times 1000}{\text{Weight of sample}}$$

B = Blank titre value. S = Sample titre value. N = Normality of sodium thiosulfate solution

p-Anisidine value (p-AV) :

The carbonyl content in oils was determined by standard method according to AOCS [18]. It measures the reactiveness of the aldehydes' carbonyl bond on the p-anisidine amine group forming a Schiff's base which absorbs at 350 nm. 2g (W) of soy bean oil were dissolved in 25 ml isooctane and absorbance A_1 was measured at 350nm against a blank isooctane. An aliquot (5ml) of this solution, respectively 5 ml of isooctane (as blank) was transferred to each of two test tubes of 10ml and 1ml anisidine solution (0.25% g/v glacial acetic acid) was added to each. After 10 minutes the absorbance A_2 was measured at 350nm

Table 2 Density versus storage duration

Duration (hours)	Densities							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
0	0.9112	0.9112	0.9112	0.9112	0.9112	0.9112	0.9112	0.9112
5	0.9089	0.9108	0.9107	0.9093	0.9097	0.9103	0.9105	0.9106
10	0.9059	0.9105	0.9103	0.9069	0.9082	0.9095	0.9091	0.9090
15	0.9019	0.9097	0.9096	0.9047	0.9077	0.9090	0.9096	0.9096
20	0.9051	0.9075	0.9073	0.8938	0.8988	0.9081	0.9085	0.9089

against isooctane containing p-anisidine. The p-AV is determined as; p-AV = 25 x 1.2 x (A_2-A_1) / W [19].

Results and Discussion

Density

Density of each incubated sample at 40 - 60°C was determined in five days' interval as shown in the Table 2. The incubated samples were cooled in the desiccator and used for the determination of densities.

A graph of density versus number of days of storage was plotted as shown in the Figure 1. The plot shows a significant decrease the density of incubated samples. Samples S_6, S_7, S_8, S_2 and S_3 were recorded a small decrease in the density whereas S_4 and S_5 shows a steady significant decrease in the density. The blank oil sample S_1 shows a decrease for fifteen days' storage duration and there was an increase after fifteen days. For the samples S_4 oil with zanthoxylum rhetsa coarse powder records a significant decrease in density from 0.9112 g/ml to 0.8938 g/ml. This indicates that some lighter compounds which responsible for anti-oxidation were extracted into the groundnut oil. Similarly, sample S_5 oil with methanol extract of zanthoxylum rhetsa shows a decrease of density from 0.9112 g/ml to 0.8988 g/ml. Samples S_2 and S_3 with BHT and α-Tocopherol attributed strong antioxidant effect having a gradual decrease in densities from 0.9112 to 0.9075 g/ml. This was assumed that the decrease in the densities was the cause of deformation of anti-oxidants. However, samples S6, S7, S8 showed minimum decrease in densities confirming oxidation of oil has been enhanced.

Acid value (AV) :

Free fatty acid value of peanut oil was recorded with storage duration at incubation temperature 60°C as shown in the Table 3

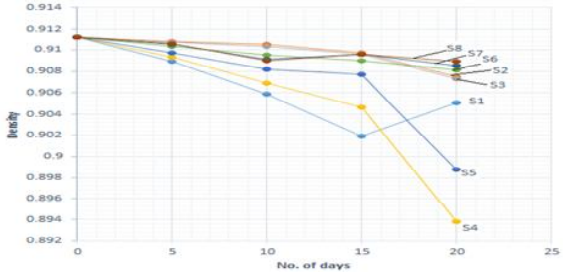

Figure 1 Density with storage duration at incubation temperature 60°C

Table3 Free fatty acid value with storage duration

Duration (hours)	Acid value							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
0	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
5	4.1	4.2	4.1	4.1	4.2	4.2	4.1	4.2
10	4.3	4.4	4.3	4.3	4.2	4.4	4.2	4.3
15	4.6	4.5	4.3	4.4	4.3	4.7	4.3	4.7
20	4.6	4.5	4.4	4.4	4.3	4.7	4.3	4.7

It is observed that the tree fatty acid value increases in small intervals and become constant 15 days of incubation at 60°C.The blank oil, oil with ethanol and water extract showed acid values 4.6, 4.7 and 4.7 mgKOH/g respectively. Samples S_2, S_3, S_4, S_5 and S_7 shows relatively less acid value. This indicates that there is no conspicuous hydrolysis of peanut oil during incubation at 60°C.

Peroxide value (PV) :

Peroxide values were determined against storage period at the interval of two days as recorded in the Table 4.

Table 4 Peroxide with incubation period

Duration (hours)	Peroxide values of samples							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
0	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
2	9.2	8.3	8.2	8.3	7.4	10.1	11.0	10.1
4	11.9	9.3	9.1	9.2	10.1	12.0	12.9	12.9
6	13.9	11.0	11.2	12.0	11.0	12.9	14.7	14.7
8	14.2	11.1	12	12.5	11.9	13.3	15.1	15.3
10	14.7	11.1	12.2	12.8	12.4	13.8	15.6	15.8
12	15.3	11.1	12.8	13.2	13.5	15.3	16.6	16.4
14	16.6	11.1	14.1	14.5	14.6	16.6	17.6	18.4
16	17.4	11.1	14.8	15.1	15	17.5	18.3	19.2
18	18.1	11.1	15.4	15.9	15.7	18.6	19.2	20.1
20	19.4	11.6	16.3	16.8	16.5	19.7	20.1	20.9

A graph of peroxide values versus number of days of storage duration was plotted as shown in the Figure 2

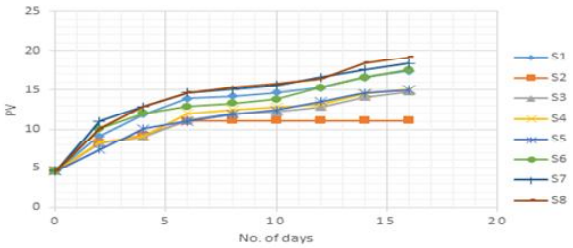

Figure 2 Peroxide value versus storage duration incubated at 60°C

The peroxide values gradually increase with storage duration at incubated temperature at 60°C. The control sample S_2 (with BHT) reached lowest peroxide value 11.6 meq/kg after twenty days at the incubated temperature acting as strong synthetic antioxidant. Sample S_3 with α- tocopherol have relatively recorded more peroxide value 16.3 meq/kg than the control and act as a natural antioxidant to control the rancidity of oil. The plot conspicuously attributed relatively comparable peroxide values for samples S_4 and S_5 16.8 and 16.5 meq/kg respectively. Samples S_6, S_7, S_8 have relatively high peroxide values 19.7, 20.1, 20.9 meq/kg respectively which is comparable with blank oil sample (19.4meq/kg). These results more confidently prove that the coarse powder and methanol extract of zanthoxylum rhetsa DC shows antioxidant effect to control the rancidity of groundnut oil. Since peroxide value is directly related with rancidity of oil, it is a factor to decide the quality of groundnut oil for hygienic usage or expulsion. The above results suggested to the dry fruit coarse powder and methanol extract can be used in the preservation of ground oil to control rancidity due to primary oxidation of oils.

p-Anisidine value (p-AV) :

p-Anisisdine value of each sample was determined with storage time duration as in the Table 5.

Table 5 p-Anisidine value with storage duration

Duration (hours)	p-Anisidine values of samples							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
0	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
2	8.2	6.1	6.2	6.2	6.4	8.4	8.9	9.0
4	10.4	6.9	6.8	6.9	7.0	10.7	11.0	11.3
6	11.8	7.3	7.4	7.5	7.7	12.1	12.8	13.1
8	13.1	8.7	8.9	8.8	9.0	13.9	14.2	14.5
10	14.0	9.2	9.4	9.7	9.9	14.6	14.9	15.1
12	14.8	9.2	9.7	10.4	11.8	15.8	16.2	16.9
14	15.7	9.2	10.0	11.3	13.7	16.1	16.9	18.2
16	17.2	9.2	10.1	11.9	14.9	17.6	18.0	19.0
18	18.7	9.3	10.3	14.8	16.1	19.2	19.9	20.4
20	20.3	9.3	10.4	17.4	17.9	21.1	22.2	22.3

A graph was plotted paraanisidine value versus number of storage days as given the Figure 3.

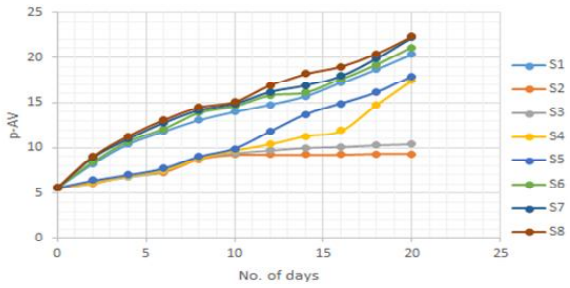

Figure 3 Paraanisidine value versus storage duration incubated at 60°C

Once in two days intervals, p-AV was determined and it was continuously increasing for samples S_1,S_6,S_7 and S_8 relatively with higher value than S_3,S_4,S_{15} in comparision with the control S_2 as shown in Figure 3. The para anisidine values for oil containig B.H.T. and α-Tocopherol (9.3 and 10.4) were lesser and control the secondary oxidation of peanut oil by limiting the rancidity of the oil. The oil sample with methanol extract and coarse powder of Zanthoxylum rhetsa DC shows lower p-AV (17.4 and 17.9) as compare to blank oil (20.3) and slightly higher than the oil sample with B.H.T and α-tocopherol. Thus Oils with B.H.T. and α-tocopherol shown strong antioxidant property. The coarse powder and methanol extract of Zanthoxylum rhetsa DC have shown significant antioxidant effect in controlling the secondary oxidation of peanut oil. The graph authentically proves that the oil samples with methanol extract and dry coarse powder of zanthoxylm rhetsa DC shows a limiting effect on p-AV. The ethanol extract, ethyl acetate and water extract increases the p- AV. From the overall results, the methanol extract and coarse powder of Z.rhetsa DC plays significant role in controlling secondary oxidation of the lipids there by limiting the rancidity. These results attribute to increase the life time of peanut oil during preservation.

Conclusion

The local people in the coastal region of south Indian states using Zanthoxylum rhetsa DC's fruits in fish curry preparation and other cooking as flavouring agent. The present study was carried out to evaluate the antioxidant ability in controlling the rancidity of peanut oil which is best consumable oil. 1 gm dry fruit coarse powder of Zanthoxylum rhetsa DC stabilize the peanut oil by controlling peroxide value and para anisidine value with 16.8 meqO_2/kg and 17.4

respectively in correlation with blank oil having 19.4meqO_2/kg and 20.3 after 20 days of incubation at 60°C. The decrease in peroxide and para anisidine value is the indication of the stability of peanut oil. A similar trend was observed with methanol extract of 1 gm dry powder having peroxide and para anisidine values 16.8 meqO_2/kg and 17.9. The evaluation peroxide and para anisidine values of peanut oil having Zanthoxylum rhetsa DC dry fruit coarse powder and methanol extract in comparison with control and blank oil samples indicate that the rancidity was controlled significantly. Since acid value is not conspicuous, peroxide value increases due unsaturation of the lipids. Thus, this study is a significant and cheapest means of controlling the rancidity of peanut oil.

Acknowledgement

Authors are sincerely acknowledging the moral support given by the beloved principal and the all staff members to carry out this research work.

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