Antioxidant Activity of Buchananina Lanzan Seed Extract

[1]Dr. Hemant Lariya, [2]Dr. Shailendra Lariya
[1]Forest Department Sagar, [2]Professor
Radharaman College Bhopal

Abstract
Antioxidants protect cell damage caused by free radicals- liberated under environmental influences. Commonly used antioxidants are synthetic. The present attempt is from a natural sources- Buchanania lanzan seed extract. Defatted material after extraction with methanol was subjected to chromatographic separation, which indicated presence of flavonoids as major components. The activity was measured using β-carotene as spray solution. Unhydrolysed extract prossessed marked antioxidant activity. Paper chromatography of methanol extract, using butanol : acetic acid : water as solvent, developed a distinct fluorescent band.

1. Introduction
Buchanania lanzan (Anacardiaceae) is a middle sized tree distributed in Bundelkhand. There is work on kernels and bark. Fat has been reported from the family· The raw material has collected from nearly forests and authenticated at the Botany Department of the University. It was extracted with petroleum ether (60-80˚) in a Soxhlet for oil content. Oil was obtained in an yield of 30% the oil contains myristic, palmitic, palmitoleic, stearic and linoleic acids as revealed by GLC of methyl esters. Standard authentics from Sigma Chemical Company, U.S.A. were used for co-chromatography and spiking. Antioxidants are known for their effective inhibition of lipid peroxidation. Synthetic agents like Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT) – which are used as food preservatives, have toxic side effects, therefore, the emphasis is on natural sources. Vegetable oils are being screened in some laboratories for their possible antioxidant activity. With this in view, the oil of *Buchanania lanzan* – which has good yield, presence of unsaturated fat and nutritional compatibility as measures for possible edible utility-as tested in our laboratory, has been analysed for presence of antioxidants.

2. Related Work
(A) Fatty Acid Profile (Seed Oil)
Buchanania lanzan (Anacardiaceae)' is a middle sized tree distributed in Bundelkhand. There is work on kernels[2] and bark[3]. Fat has been reported from the family[4].

(B) Antioxidant Content
Antioxidants are known for their effective inhibition of lipid peroxidation[6]. Synthetic agents like Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT) – which are used as food preservatives, have toxic side effects[7], therefore, the emphasis is on natural sources. Vegetable oils are being screened in some laboratories for their possible antioxidant activity[8,9]. With this in view, the oil of *Buchanania lanzan* – which has good yield, presence of unsaturated fat and nutritional compatibility as measures for possible edible utility-as tested in our laboratory[10], has been analysed for presence of antioxidants.[11]

3. Experiment Result and Discussion
Experimental
The raw material has collected from nearly forests and authenticated at the Botany Department of the University. It was extracted with petroleum ether (60-80˚) in a Soxhlet for oil content. The oil was saponified[5] in presence of KOH. The saponified mixed fatty acids were converted to methyl esters (FAME) with methanol/ H_2SO_4 and analysed by TLC taking diethyl ether: hexane: acetic acid (20:80:1) as solvent system and charring at 120˚ for detection and GLC using OV 101 column with N_2 as carrier gas at a flow rate of 100ml/min F.1. Detector, chart speed of 4 mm./min and 1µ l of sample injection. Standard authentics from Sigma chemical company U.S.A. were used for co-chromatography and spiking.

Result and Discussion
Oil was obtained in an yield of 30%. The oil contains
Myristic (14 : 0) acid
Palmitic (16 : 0) acid

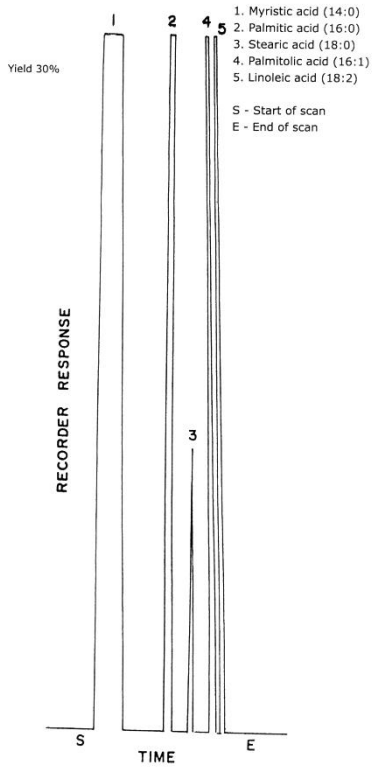


Fig. III / 1 : GLC profile of the mixed fatty esters of *Buchanania lanzan* seed oil

Pamitoleic (16 : 1) acid
Stearic (18 : 0) acid
Linoleic (18 : 2) acid
as esters (Fig. III/1)

(i) Tocopherol:

Experimental

A high performance liquid chromatographic (HPLC) method has been employed for detection of Tocopherol content. The HPLC analysis was performed with Shimadzu binary system (LC-10A) with LC-10AD pump, 7125 Rheodyne injector fitted with 20 µl sample loop, SPD-10 A UV-visible detector scanning between 190 and 600 nm and CR 7 Ae plus data processer. Shim-pack CLC NH_2 column (4.5 mm X 25 cm) with Hexane: Isoropanol (96 : 4 v/v) and flow rate of 1 ml/min with UV detection at 297 nm was used. Peak identification was based on comparison of RT values with authentic standards of Tocopherols (E Merck).

Result and Discussion

Fig. III/2 shows the HPLC profile of the Oil using normal phase and UV detection. 4 Peaks could be identified (Identity shown in the profile). This direct injection analysis has been found to be better than the conventional sample preparation method[6] – which includes saponification of the oil and separation of the unsaponifiable portion. The loss during saponification may be due to degradation of vitamin E compounds in alkaline medium and departure of the components of interest in the precipitates of fatty acid salts, generated during saponification. Thin-layer chromatography of the saponified sample may also attribute to loss of components of interest by adsorption of these on silica.

	R_1	R_2	R_3
α Tocopherol	Me	Me	Me
β Tocopherol	Me	H	Me
γ Tocopherol	H	Me	Me
δ Tocopherol	H	H	Me

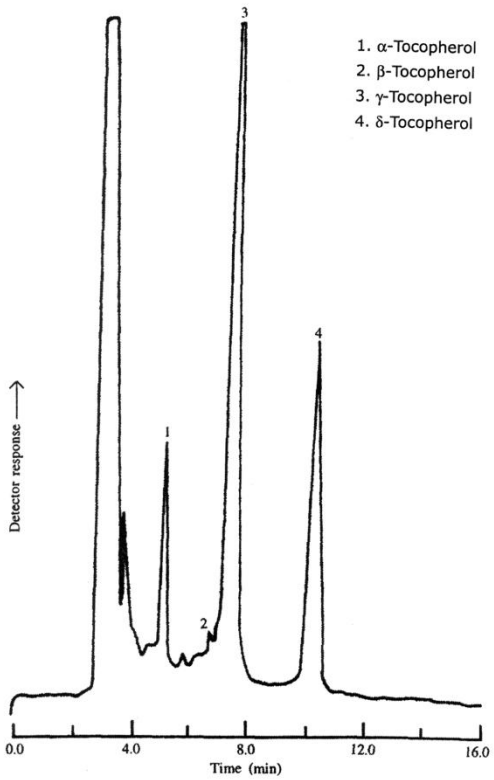


Fig. III/2 : HPLC profile of *Buchanania lanzan* seed oil

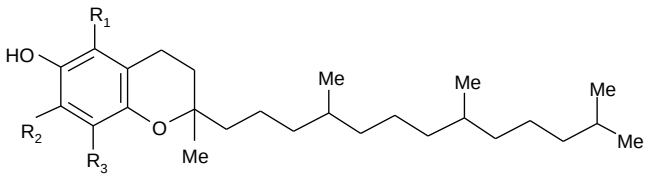


Fig. 3.2

(ii) Phenolics:

Plant phenolics have been reported to possess antioxidant properties.[7]

Experimental

The defatted seeds were extracted with methanol in a Soxhlet. The extract was concentrated by evaporation of the solvent.

The extract was tested for antioxidants on a TLC plate, using n-butanol : acetic acid : water (4 : 1 : 5 v/v) and ethyl acetate : methylethyl ketone : formic acid : water (5 : 3 : 1 :1) as developing solvent. β carotene (10mg) in chloroform (30 ml) + linoleic acid (2 drops) + alcohol (50 ml) was used as spray reagent. The plate was exposed to day light (1 hr.) Yellow spot indicated presence of antioxidant content.

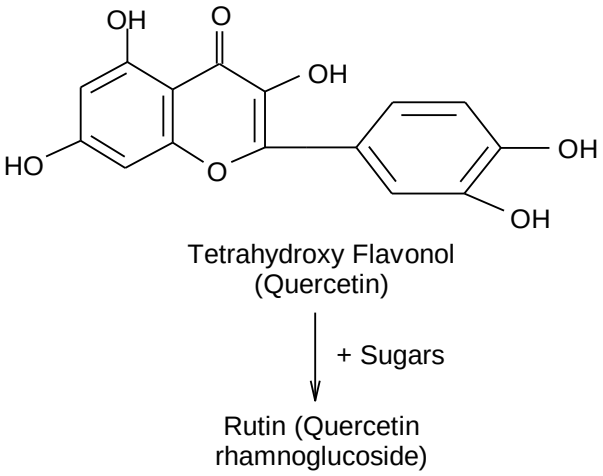
For further analysis, the sample was spotted on chromatographic (Whatman) paper-eluted with butanol : acetic acid : water (4 : 1 : 5) and a portion was sprayed with $FeCl_3 – K_3Fe(CN)_6$-giving blue colour (Phenol) and another with $FeCl_3$ (2% in alcohol) coloured (brown red) spot was obtained.

The sample was subjected to acid hydrolysis by first evaporating at to dryness, taking in minimum amount of methanol, adding concentrated HCl (1 ml), heating for 45 minutes, taking in minimum amount of ether and adding 1 ml water. Two fractions were obtained and both were subjected to chromatography using butanol : acetic acid : water (4 : 1 : 5). The results are given below : -

Results

S. No.	Experiment	Observation			Inference
		Analysed portion	Rf sample	Rf authentic	
1.	TLC of Methanolic extract (nbutanol : acetic acid : water 4 : 1 : 5 and ethyl acetone : methyl ethyl ketone: formic acid: water 5:3:1:1) β carotene spray, light exposure	Yellow spot			Presence of antioxidant content
2.	Paper chromatography Butanol : acetic acid : water (4 : 1 : 5), $FeCl_3$ spray	Whole sample (Brown red spot)	0.53	0.55	On the basis of co-chromatography with authentic standard, the compound has been identified to be Rutin
3.	Paper chromatography Butanol : acetic acid : water (4:1:5), $FeCl_3$ spray	Hydrolysed ext. (Upper portion)	0.69	0.70	Aglycone portion Quercetin (co-chromatography with authentic)
4.	Paper chromatography Butanol : acetic acid : water (4:1:5), $FeCl_3$ spray	Hydrolysed ext. (Lower portion)	0.22	0.21	Glucose
			0.59	0.61	Rhamnose

Thus,



4. Conclusion

In this research has been implemented of Antioxidant activity of Buchnania Lanzar by the TLC, HPLC, GLC, methods and paper chromotography TLC result yellow spot of presence of antioxidant content in buchnania Lanzar seeds. Antioxidants protect cell damage caused by free radicals-liberated under environmental influences. Commonly used antioxidants are synthetic.

5. References

1. "Useful plants of India", CSIR, New Delhi, 464 (1986).
2. Raikar, M.R. and Magar N.G., J. Ind. Chem. Soc., 2, 59 (1973).
3. Mitra, R. and Mehrotra, S., Herba Hung, 20 (1-2), 15 (1981).
4. Khabir, M., Ahmed, I. and Ansari, W.H., J. Ind. Chem., Soc., 64, 66 (1987).
5. Hildirch, T.P. and Williams, P.N. "Chemical Constitution of Natural Facts" 4th ed., Chapman and Hall, London (1964).
6. Quershi, A.A., Qureshi, N; Rapacz, J.O.H., Weber, F.E., Chaudhary, V., Crenshaw, T.D., Gapor, A.,Ong, A.S.H., Chong, Y.H., Peterson , D. and Rapaz, J. Am. J. Clin Nutr., 53, 1042 (1991).
7. Chang, S.S. Ostric-Matyasevic, B., Hsieh, O.A. and Huang, C.J., Food Sci., 42, 1102 (1977)
8. Diack, M. and Saska, M. J. Am. Oil Chem. Soc., 71, 1211 (1994).
9. Kramer, J., Blais, K.G.L., Fouchord, R.C., Melnyk, R.A. and Kallruy, K.M.R., Lipids, 32, 323 (1997).
10. Yadav, A. "Studies on lipids", Ph.D. Thesis (Supervisor, A.K. Banerjee), University, Sagar, 2000.
11. Laria, H., "Antioxidant activity of *Buchanania lanzan* seed extract", Presented at the 40th Annual Convention of Chemists, Bundelkhand University, Jhansi, E 5, 2003.