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EXTRACTION AND CHARACTERIZATION OF *LUFFA ACUTANGULA* VAR *AMARA* SEED OIL FOR ANTIOXIDANT ACTIVITY

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ABSTRACT
Phytochemical investigation of an indigenous seeds of *Luffa acutangula* var *amara* (Family: Cucurbitaceae) commonly known as Kadwi turai was carried out. The air-dried seeds were powdered and extracted with petroleum ether (40-60°C) in a soxhlet extractor for 24 hrs. The physico-chemical properties of the oil was determined by Official and tentative methods of the American Oil Chemists' Society, Chicago. Characterization of the oil was determined by Gas Liquid Chromatography. Oil was evaluated for free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method.
Key words: *Luffa acutangula* var *amara*, Antioxidant activity, Free radicals

INTRODUCTION

Luffa acutangula var *amara* (Family: Cucurbitaceae) commonly known as Kadwi turai in Hindi, is a fairly large climber found in Western, Central and Southern India and regarded as the wild form of the cultivated species. It resembles *L. acutangula* in every respect except that the leaves, flowers, fruits and seeds are smaller. All parts of the plant are highly bitter. The dried fruit is powdered and used as snuff in jaundice[1]. Practitioners of the indigenous system of medicine are using the leaf or fruit juice in the treatment of jaundice in the tribals of Western Madhya Pradesh of India[2]. The ethanol extract of *L. acutangula* var *amara* fruits was reported for its CNS depressant activity in mice[3]. The seeds are considered emetic, expectorant and demulcent. The present study was undertaken to investigate the composition of the seed oil and its antioxidant properties.

MATERIALS AND METHODS

Collection and characterization of seeds

The fruits of *L. acutangula* var *amara* (LA) were collected from Satara, Maharastra, and authenticated by Dr. B.D. Huddar, Professor, Department of Botany, HSK Institute of Science, Hubli. The fruits were shade dried and separated the seeds. Seeds were characterized by their physical properties such as seed index, moisture content, extractive values and determined oil content.

Extraction of oil

The air-dried seeds were powdered and extracted with petroleum ether (40-60°C) in a soxhlet extractor for 24 hrs. The petroleum ether extract was dried over anhydrous sodium sulphate and solvent was removed in vacuum to recover the oil. The oil contents were determined by following American Oil Chemists' Society Methods[4]. Official and tentative methods of the American Oil Chemists' Society, Chicago were followed for the determination for Acid value, Iodine value, Saponification value, Hydroxyl value, Halphen test[5], Picric acid test and 2, 4-DNP test[6] for an oil sample.

Preparation of methyl esters

Methyl esters of fatty acid was prepared by Methonol-Sulphuric acid method

The fatty acid sample was refluxed in a large excess of absolute methanol containing 1% sulphuric acid (v/v). The resulting mixture was diluted with water and then extracted repeatedly with ether. The combined ether extracts were dried over anhydrous sodium sulphate and solvent was removed in vacuum. The residual liquid was subjected to Gas Liquid Chromatography (GLC).

GLC was carried out using Schimadzu gas chromatography with dimethylpolysilazone column having length of 30 m and internal diameter 0.32 mm. The column temp was maintained at 200 to 225 C using nitrogen gas a mobile phase and flame ionization detector (FID) as a detector.

Antioxidant activity

The antioxidant activity was evaluated by the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical[7]. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turned from purple to yellow as the molar absorptivity of the DPPH radical reduced. The colour intensity was measured at 517 nm. The results were compared with standard butylated hydroxytoluene (BHT).

RESULTS AND DISCUSSION

Physical characters of the seeds, seed index, physico-chemical characterization of the oil and preliminary phytochemical tests for unsaponifiable matter were depicted in Table Nos. 1, 2, 3 and 4 respectively.

Spectral studies of the methyl esters of *L. acutangula* var *amara*.

UV spectra of the methyl ester of the oil showed the λ max at 285 nm in benzene. Infra red spectra of this oil showed the characteristic absorption bands at 1642.80 cm^{-1} due to carbonyl group of ester group and 2930.18 cm^{-1} due to the aliphatic chain of the fatty acids and other characteristic absorption bands present in the oil.

GCMS of this methyl ester showed the presence of different mixtures of fatty acids. They have shown the different retention time and percentage of the fatty acid. The important methyl esters of this oil are under Table-5.

The seed oil of LA was analyzed by GLC. It revealed the presence of lauric acid, myristic acid, palmetic acid, stearic acid, oleic acid and linoleic acid. The preliminary phytochemical tests for unsaponifiable matter showed the presence of steroids, triterpenoids, saponins, flavonoids and tannins.

The seed oil of LA was evaluated for antioxidant activity and has shown less activity than the standard by DPPH method (Table-6).

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REFERENCES

1. Anonymous, In. The Wealth of India, raw materials, Vol.VI, Publications and Information Directorate, CSIR New Delhi; 1988; p 179.
1. Samvastsar S, Diwanji VB. Plant sources for the treatment of jaundice in the tribals of Western Madhya Pradesh of India. J Ethnopharmacol; 2000;73:313-16.
2. Misar AV, Upadhye AS, Mujumdar AM., CNS depressant activity of ethanol extract of *Luffa acutangula* var *amara* C.B.Clarke fruits in mice. Indian J Pharm Sci; 2004;66:463-65.
3. Official and Tentative Methods of the American Oil Chem. Soc., 3rd edn., AOCS, Champaign, Illionis, 1973.
4. Halphen G., J. Pharm;1897; 6:390.
5. Fioriti JA, Bentz AP, Sims RJ., J. Am. Oil Chem. Soc., 1966;43:487.
6. Cuvelier ME, Richard H., & Berset, Biosci. Biotech. Biochem; 1992;56:324-325.

TABLE 1: PHYSICAL CHARACTERS OF SEEDS

Sl. No.	Parameters	Observations
1	Nature of Seeds	Oblong little flattened, deeply grooved on one side, more than 13mm. long.
2	Color	Dark yellowish to brown
3	Odour	Pungent
4	Taste	Bitter
5	Ash content	3.35

TABLE 2: SEED INDEX

Sl. No.	Characters	Observations
1	Wt. of 1000 seeds	820g
2	Volume of 1000 seeds	175 ml
3	Oil contents (%)	4.85
4	Moisture content(%)	5.53
5	Extractive values (%)	
	a) Pet. ether soluble extractive	0.16
	b) Benzene soluble extractive	0.12
	c) Chloroform soluble extractive	0.12
	d) Alcohol soluble extractive	0.68

TABLE 3: PHYSICO-CHEMICAL CHARACTERIZATIONS OF THE SEED OIL

Sl. No.	Physicochemical characters	Observations
1	Specific gravity	0.95
2	Refractive index	0.93
3	Acid value	2.04
4	Saponification value	150.82
5	Unsaponifiable matter(%)	3.56
6	Iodine value	57.91
7	Halphen test	Negative
8	Turbidity test	Positive
9	Picric acid test	Negative
10	DNP test	Negative

TABLE 4: PRELIMINARY PHYTOCHEMICAL TESTS FOR UNSAPONIFIABLE MATTER OF THE SEED OIL

Sl. No.	Chemical tests	Observations
1	Steroids	Positive
2	Triterpenoids	Positive
3	Saponins	Positive
4	Glycosides	Negative
5	Carbohydrates	Negative
6	Alkaloids	Negative
7	Flavonoids	Positive
8	Tannins	Positive
9	Proteins	Negative

TABLE 5: THE PRESENCE OF DIFFERENT MIXTURES OF FATTY ACIDS BY GCMS

Sl. No.	Retention time	Peak area	% Area	Molecular weight	Name of the methyl ester
1.	6.42	242404.90	1.62	C12	Lauric acid
2.	14.36	5397813.00	36.01	C14	Myristic acid
3.	18.73	881744.56	5.88	C16	Palmetic acid
4.	19.14	3967412.00	26.47	C18	Stearic acid
5.	19. 94	2010578.00	13.48	C18:1	Oleic acid
6.	23.198	224311.50	1.50	C18:2	Linoleic acid

TABLE-6: FREE RADICAL SCAVENGING ACTIVITY OF SEED OIL ON DPPH RADICAL

Conc (µg)	Seed oil	BHT
10	13.13	39.41
20	17.41	47.52
30	18.35	53.53
40	20.26	58.65
50	22.41	61.53
60	25.86	63.61
70	26.34	67.84
80	28.89	71.84
90	30.52	80.17
100	31.77	83.47
IC50	104.12 µg	25.54 µg

Values are mean of triplicate determinations.

IC_{50} - Inhibition concentration (the concentration producing 50% of maximal inhibition)

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