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Fatty acid composition of *Alstonia scholaris* (Linn.) R. Br. seed oil having some antibacterial principles

MITA DUTTA[1], SUKANTA SEN[2] and SUBRATA LASKAR[3]*

[1]Department of Chemistry, S.B. College, Bagati, Magra - 712 148 (India).
[2]Department of Botany, Visva-Bharati, Santiniketan, West Bengal (India).
[3]Natural Product Laboratory, Department of Chemistry,
University of Burdwan, Burdwan - 713 104 (India).

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ABSTRACT

Fatty acid content of hexane extracted oil of mature seeds of *Alstonia scholaris* (Linn.) R.Br has been analyzed by GLC and its antibacterial property identified for the first time to determine the nutritional, pharmaceutical and industrial importance of the oil.

Result

Oil content of the seeds was found to be 239 gkg^{-1} (weight per kg of dry seeds). The oil contained four fatty acids accounting to 100% of total fatty acids. The unsaturated fatty acids identified were Oleic acid (65.66%) and Linoleic acid (12.15%), whereas saturated fatty acids were Palmitic acid (13.77%) and Stearic acid (8.42%). Moreover, the oil was found to show profound activity against *Bacillus subtillis*, *Eschericia coli*, *Salmonella* sp. and *Bacillus* sp.

Conclusion:

Seed oil of *Alstonia scholaris* contains four fatty acids. The presence of two unsaturated fatty acids, Oleic acid (C18:1 n-9) and Linoleic acid (C18:2 n-6) makes the oil important from nutritional point of view as well as for oil stability. High content of linoleic acid may render the oil interesting for cosmetic industry. The present study thus identifies the possibility of future domestication of the oil. Antibacterial assay establishes the antibacterial property of the oil. So the oil definitely has the potential to be used as medicine after toxicological studies. Further, all these informations can be used for taxonomic and evolutionary studies.

Key words: Fatty acid, seed oil, *Alstonia scholaris*, GLC, anti-bacterial activity.

INTRODUCTION

Plant seeds are common sources of oils which may have nutritional, industrial and pharmaceutical importances[1]. Suitability of an oil for nutritional purpose is however dependent to a considerable extent on its fatty acid composition. Generally, oils from different sources differ in their fatty acid compositions and most of the oils from single sources have been found to be unsuitable for all purposes. The patterns of fatty acid variation in plant seed oils have proven to be useful tools in taxonomic and phytogenetic studies[2]. Large number of non-conventional plants have been surveyed in search for new source of oils[3]. *Alstonia scholaris,* one of such non-conventional plants belongs to the family Apocynaceae, is widely found in the Indian subcontinent and has various folk medicinal uses. This plant is known for its healing and curative properties for a long time and it is an excellent and popular substitute for cinchona and quinine for the treatment of intermittent and remittent fevers[4]. The bark is regarded as a bitter tonic and a mild febrifuge, possessing astringent, antihelmintic and galactogouge properties[5]. It is also reported to be employed in heart diseases, asthma, chronic

diarrhea and to stop bleeding of wounds[5]. It is also administered in leprosy, dyspepsia, skin diseases and even in cancer like conditions[5]. Decoction of young leaves is used traditionally in Malaysia for Beri-beri, congestion of liver and dropsy[5]. No reports are presently available on *Alstonia scholaris* seed oil and its use for edible as well as for medicinal purpose.

The present study deals with some investigations for characterization of fatty acids and their composition of the seed oil and tracing existence of bioactive ingredients in the seeds of *Alstonia scholaris*. Such study will be potential for possible future domestication and safe use for human consumption[6]. Bioactivity studies will help to realize the potential of the seed oil as medicine. Furthermore, these informations may also be used for taxonomic and evolutionary studies.

MATERIAL AND METHODS

Plant material and chemicals

Fresh mature seeds of *Alstonia scholaris* were collected from the Burdwan Divisional Forest Department, Burdwan, West Bengal, India and authenticated by Prof. A. Mukherjee, Department of Botany, University of Burdwan, Burdwan, West Bengal, India. Voucher specimen Burdwan, Mita 199 has been deposited at the herbarium of the Department of Botany at the University of Burdwan, Burdwan, bearing acronym BURD.

Standard fatty acids used in the experiment were purchased from Sigma Chemical Co., USA. All reagents and chemicals used in this investigation were of analytical grades.

Isolation and Characterisation of Seed oil

The seeds were taken out of the pod and dried in air. The finely powdered air dried seeds (405 gms) were extracted with hexane in a soxhlet for 60 hrs and after complete removal of the solvent under vacuum, seed oil was obtained. The total oil was weighed and stored under nitrogen at 4°C for further analysis. The chemical analysis of the seed oil (including acid, iodine and saponification values) were performed according to the methods of Association of Official Analytical Chemists[7] and the results have been placed in Table 1.

Extraction and identification of fatty acids

The extraction of fatty acids and its methyl ester preparation were performed according to the method described by G.H. Wikfors *et. al*[8]. Methyl esters of fatty acid mixture of the seed oil was purified by preparative TLC using hexane: ethyl acetate (1:1) as chromatographic solvent and fatty acid methyl ester band was eluted with chloroform (Merck, India) and stored in refrigerator for further analysis.

GC analysis

Gas chromatographic analysis of the purified methyl ester of fatty acids of seed oil of *A. scholaris* was carried out with the aid of a Hewlett Packard (HP; Palo Alto, CA, USA Model, Agilent 6890 series plus) instrument fitted with HP-5 capillary column (30m long; 0.25 mm i.d) using a flame ionization detector (FID), the temperatures of the injection and detector ports were set at 250°C. The oven temperature was initially 160°C (held for 2 min), then raised at 3°/min to 220°C (held for 15 mins). The carrier gas was nitrogen (flow rate of 20ml/min); volume injected 1 ml; split ratio,1:20. Peaks were identified by comparision of their retention times with that of standard fatty acids methyl esters. The percentage composition of the samples were computed from the GC peak areas. The results obtained were placed in Table 2.

Inhibitory test with seed oil

Inhibitory test was performed with the total seed oil, saponified seed oil and acidic and neutral parts of the seed oil (after separating it into acidic, basic and neutral fractions).

Bacterial organisms (a few gram positive and gram negative organisms) taken in the experiment were *Bacillus subtillis*, *Eschericia coli*, *Salmonella* sp. and *Bacillus* sp. Solutions of total seed oil, saponified seed oil, acidic neutral fractions of the seed oil (2mg/mL in each case) were given separately to observe its activity against the aforementioned micro-organisms. The cup bore method[9] was used to perform susceptibility test[10]. The zones of inhibition were estimated visually and the data were placed in Table 3.

RESULTS

Oil (pale yellow in colour) content of this seed was found to be 239.5g/kg (weight per kg dry matter of seeds). The characteristics of the seed oil of *A. scholaris* is given in Table 1.

The acid value is indicative of the amount of free fatty acids present in the oil. From the GC analysis of the methyl esters of fatty acids present in the oil of matured seeds of A. scholaris, indicates four fatty acids were present in the oil. They were identified and quantified (Table 2) representing 100% of the fatty acids.

Results of the inhibitory test performed with the seed oil of *A.Scholaris*, saponified oil, acidic and neutral fractions of the seed oil as tabulated in Table 3 indicate that the seed oil, saponified seed oil and acidic fraction of oil has profound activity against the test microorganisms.

Table 1: Characteristics of the seed oil

S. No.	Parameters	*A.scholaris* seed oil
1.	Specific gravity	0.8519
2.	Acid value (mg KOH/gm)	3.703
3.	Iodine value (g/100gm)	118.25
4.	Saponification value (mg KOH)	165.2
5.	Water content (%)	32

DISCUSSION

Oleic acid (C18:1 n-9) was the principal unsaturated fatty acid (65.66%), followed by linoleic acid (C18:2 n-6) as the second main unsaturated fatty acid (12.5%). Palmitic acid (C16:0) and stearic acid (C18:0) were the other saturated fatty acids present their in. Their relative amounts being 13.77% and 8.42%. The proportions of unsaturated and saturated fatty acids are 77.81% and 22.19% respectively.

Table 2: Fatty acid compositions of the seed oil of *Alstonia scholaris*

S. No	Name of Fatty Acid	Retention time (in minutes)	Relative amount of Fatty acids present	Amount of Fatty Acid in seed oil per 100 gms
1.	Palmitic acid	11.523	13.77	1.54
2.	Linoleic acid	15.964	12.15	1.36
3.	Oleic acid	16.125	65.66	7.35
4.	Stearic acid	16.845	8.42	0.94
	Total		100.00	11.19

Table 3: Antibacterial activity obtained from Inhibitory test

S. No.	Test compound	Inhibition zone (mm)			
		E.Coli **Gram -ve**	***Salmonella*** **sp. Gram -ve**	***Bacillus subtillis*** **Gram +ve**	***Bacillus*** **sp. Gram +ve**
1.	Total seed oil	12	11	10	12
2.	Saponified seed oil	12	11	9	9
3.	Acidic part of seed oil	12	15	21	16
4.	Neutral part of seed oil	-	-	-	-

Cup diameter = 8 mm; Sample used = 0.1 ml/cup

Medium used for assay: Nutrient agar

Solvent as control: Hexane

The oil of this seed is rich in two unsaturated fatty acids i.e., oleic and linoleic acid. Both of these acids are important from the nutritional point of view as well as for oil stability. It is well known fact that dietary fats rich in linoleic acid prevent cardiovascular disorders like coronary heart diseases and high blood pressure. Its derivatives also serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds[11]. The result of the present investigation indicates that *A. scholaris* seed oil may be a good source of essential fatty acids and thus it may be nutritionally valuable. Moreover, high content of linoleic acid may render the oil interesting for cosmetic industry.

As evident from the inhibition zones from Table 3 it is clear that the acidic fraction of the seed oil showed highest activity against the test organisms.

CONCLUSION

The hexane extracted oil from the seed of *A. scholaris* contains four fatty acids of which two are very essential unsaturated fatty acids (oleic acid and linoleic acid). Moreover, the total oil and the acidic part of the oil showed profound activity against *E. coli*, *Salmonella* sp., *Bacillus subtilis*, *Bacillus* sp. Thus, it can be concluded that the oil may be important from nutritional as well as medicinal point of view after proper toxicological study.

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