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PHARMACOLOGICAL STUDY AND PHYSICO-CHEMICAL CHARACTERISTICS OF *KHAYA SENEGALENSIS* SEED OIL

Karigar Asif A*[1], Alagawadi K R[2]., M Himaja [3], Sikarwar Mukesh S.[4], Sutar P S[1]

[1]Maratha Mandal's College of Pharmacy Belgaum, Karnataka, India
[2]K.L.E.S College of Pharmacy Belgaum, Karnataka-India
[3]V.I.T.University Vellore, Tamilnadu-India
[4]K.L.E. S College of Pharmacy Ankola, Karnataka-India

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ABSTRACT

Seed oil from species viz.,*Khaya senegalensis* plant belonging to Meliaceae family has been analyzed for physico-chemical properties by IR, NMR, TLC, and GLC techniques. The seed oil resembled the simple 'linoleic-oleic-palmitic' type. The chief components among unsaturated acids are oleic and linoleic together forming 18.20-72.50% of total components of acids. Among saturated acids, palmitic acid is present in the range of 10.90-40.90%. *Khaya senegalensis* seed oil containing 72.50% of oleic acid is very unusual. The seed oil is screened for LD50 studies, anti-inflammatory and antibacterial activities.

KEYWORDS*:* Minor seed oil, Physico-chemical, Pharmacological characteristics.

For Correspondence:
* Professor,
Maratha mandal's college of pharmacy
Belgaum-16, Karnataka,India.
E mail: asifmpharm@yahoo.com
Tel no: +91 - 09880219415

INTRODUCTION

Oils and fats are not only essential part of human and animal diet but also indispensable ingredients in numerous industries such as paints,varnishes,soaps, consmetics,textiles,leather,paper,pharmaceuticals and lubricants etc[1].

There has been a growing interest in minor seed oils [2] of tree or herbaceous origin to develop better substituents for conventional oils and also to study their biological activity. This report pertains to the species viz. *Khaya senegalensis* (Meliaceae). The genus Khaya A. Juss. Is the main source of African mahagony, and is closely related to the South American genus Swietenia, the original source of mahagony. *Khaya senegalensis (*Family: Melieceae) is probably the most distinctive of the species. It is a common tree in the savannah areas South of Sahara, and is found from Senegal to the Eastern Sudan and Uganda. It is often planted throughout the area as an avenue tree, and under favorable conditions reaches over 100 ft in height. The bark is very bitter and is traditionally used for medicinal purposes [3].

In present study oil extract of Khaya senegalensis was subjected to physico-chemical and pharmarmacological studies.

MATERIALS AND METHODS

Plant material

The seeds of *Khaya senegalensis* (Meliaceae) were collected from Dandeli (Dandakarannya) of Karnataka state and authenticated by the Botanist Prof. G. S. Naik, Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen numbers GCSAC/KS/01 were also preserved in the same college. The collected parts were dried under shade and powdered to coarse consistency in grinder mill. The powder was passed through 40 # mesh particle size and stored in an airtight container at room temperature.

Animals

Adult Albin mice (15-22g) of either sex were procured and housed in K L E S College of Pharmacy, Ankola with 12 h light and 12 h dark cycles. Standard pellets obtained from Goldmohar rat feed, Mumbai India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad libitum. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no.: 627/02/a/CPCSEA).

Preparation of Oil extract

The air dried seeds were ground, powdered and extracted with light petroleum ether (40-60°) in a Soxhlet extractor for 24 hrs. The petroleum ether extract were dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40° to recover the oil. The oil contents were determined by following American Oil Chemist Society Methods.

Experimental methods

Seeds were dried under shade and extracted for oil content by Soxhlet extraction with petroleum ether (b.r. 40-60^{o}C). The oil obtained is analyzed for physico-chemical characteristics as per standard AOCS procedures [4] **(Table 1).**

The seed oil examined by Halphen reagent[5,] picric acid and 2,4-DNP TLC test[6] in addition to other chromatographic [direct, reversed phase and silica gel silver nitrate TLC] techniques[7].

Infrared spectra recorded on liquid film using Perkin Elmer Moder-577 spectrophotometer. ^{1}H NMR spectra run in deuteriated chloroform on a Jeol JNM LA 300 FT-NMR system.

The methyl esters of the fatty acids were prepared by the method of jamieson and Reid[8] using sulphuric acid as a catalyst. In most of the cases the crude methyl esters were purified by silica gel column chromatography prior to GLC analysis on a Perkin Elmer Sigma unit with DEGS column.

Pharmacological screening

LD_{50} study

The LD_{50} study of seed oils was carried out by using three month old mice of either sex weighing between 15-22g by the method of Millar and Tanter [9]. The test compounds were emulsified and administered intra peritoneal to a group of six mice and the animals were observed fir 24 hours.

Anti-inflammatory activity

It was carried out by using albino mice of either sex by the method of Brown and Robson [10].Inflammation was produced by using xylol on left ear and right ear served as control. The thickness was measured by using a micrometer screw guage after a regular interval of time **(Table 2)**.

Antibacterial activity

The activity was carried out by employing paper disc diffusion method, which was modified from original Bowie and Goud and Czeskinsky methods. The agar plates were incubated with test organisms (E.Coli and S.Aureus) by spreading uniformly. One disc from each sample was placed in petridishes with sterile forceps. The dishes were incubated for 24 hours at 37ºC. After 24 hours the antibacterial activity of test compound was found by measuring the zone of inhibition and the values were compared with the standard drugs[11-12]. **(Table 3)**

RESULTS AND DISCUSSION

The seeds of *Khaya senegalensis* yielded 63% of oil. Analytical data are given in (**Table 5).**

The presence of unusual fatty acid was ruled out by Halphen test, picric acid and 2,4-DNP TLC tests. TLC of the methyl ester indicated well defined spots for saturates , monoenes and diene comparative to those of authentic esters run alongside. None of the oil and methyl ester contains either conjugation, trans unsaturated or any other functional groups as indicated by the examination of IR, NMR and UV spectra.

GLC analysis of the ester conclusively supported the finding of argentation TLC in addition to the quantization of individual fatty acids **(Table 4)**.

The major fatty acid of *Khaya Seneganlesis* seed oil is oleic, (viz.73.0%) along with minor percentages of palmitic (10.9%), stearic (7.8%) and linoleic (7.3%) acids.

Other species belonging to Khaya genus were analyzed by Gunstone et al [13-14] who observed a high content of oil in the seeds. Oleic acid in them was also significantly high (53.0 to 73.0%) **(Table5)**.Therefore, it is felt that species of this genus require further analysis in detail for exploring their potential as a source of oleic rich oil.

Pharmacological screening has led to the conclusion that the seed oil of *Khaya senegalensis* (meliaceae) have a anti-inflammatory and a partial antibacterial activity. LD50 tests showed the seed oil is nontoxic.

In conclusion, it may be said that this work may help in exploring the potential of these little known species which are rich in oil content and have very useful fatty acid composition. The sample analyzed in this screening programme would serve as a guide for further study of the oil for specific uses and developmental research work.

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Table 1: Characterization of the oil

Refractive index	1.4693
Acid value	3.20
Iodine value	110.6
Saponification value	193.38
Saponification equivalent	290.1
Unsaponifiable matter	1.2097
Halphen test	-ve
2.4. D.N.P test	-ve
Turbidity test	-ve

Table 2: Antiinflammatory activity of *Khaya senegalensis* seed oil

Animal No.	**Control (Xylol) left ear**					**Test (Xylol+Oil) right ear**				
	0min	**30min**	**60min**	**90min**	**120min**	**0.min**	**30min**	**60min**	**90min**	**120min**
1	0.18	0.35	0.32	0.28	0.28	0.19	0.17	0.16	0.15	0.15
2	0.16	0.23	0.21	0.20	0.19	0.16	0.15	0.14	0.121	0.12
3	0.13	0.45	0.28	0.25	0.25	0.13	0.21	0.20	0.19	0.18
4	0.23	0.31	0.28	0.25	0.24	0.24	0.20	0.15	0.14	0.14
5	0.20	0.32	0.27	0.25	0.25	0.21	0.19	0.18	0.16	0.15
6	0.16	0.28	0.25	0.24	0.24	0.16	0.20	0.18	0.17	0.17
Mean	-	0.32	0.26	0.24	0.24	-	0.18	0.16	0.15	0.15
ST	-	±0.030	±0.014	±0.010	±0.011	-	±0.009	±0.009	±0.009	±0.008
T.Value	-	-	-	-	-	-	4.4	6.4	6.6	6.6
Probablity	-	-	-	-	-	-	P<0.01*	P<0.001**	P<0.001**	P<0.001**
%Inhibition	-	-	-	-	-	-	43.75%	38.46%	37.5%	37.5%

Values are statistically significant at *P<0.05 & more significant at **P<0.01.
ns= not significant, SEM: Standard error mean

Table 3: Antibacterial activity of *Khaya senegalensis* seed oil

Serial number	Test samples	Antibacterial activity against	
		E.Coli	S.Aureus
1	Test oil	9mm	10mm
2	Ampicillin	40mm	32mm
3	Chloramphenicol	32mm	28mm

(Note: Test oil= Khaya senegalensis seed oil)

Table 4: **Fatty acid composition of *Khaya senegalensis* seed oil**

Fatty acids	Methyl ester composition by GLC (wt%)
Myristic acid	0.1
Palmitic acid	10.93
Stearic acid	7.77
Oleic acid	72.95
Linoleic acid	7.95

Table 5: Fatty Acid Composition of Other Species Belonging to Khaya Genus

Sl. No.	Source	Oil content, %	16:0	18:0	18:1	18:2
1	Khaya anthotheca[11]	82.0	15.0	9.0	53.0	22.0
2	Khaya grandifolia[11]	59.0	10.0	13.0	58.0	17.0
3	Khaya ivoresis[11]	62.0	8.0	5.0	67.0	20.0
4	Khaya nyasica[11]	72.0	11.0	12.0	63.0	14.0
5	Khaya senegalensis[12]	63.0	11.0	8.0	73.0	7.0

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