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DEMETHYL LEPIDINE, A NEW ALKALOID FROM *LEPIDIUM SATIVUM* SEEDS

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ABSTRACT

Two imidazole alkaloids (I & II) have been isolated from the seeds of Lepidium sativum L. Compound I O-demethyllepidine was isolated for the first time and its structure was elucidated on the basis of spectral analysis, while compound II was identified as lepidine and its identification was confirmed by X-ray crystallography.

INTRODUCTION

As a continuation of our phytochemical investigation on Egyptian plants of medicinal and biological interest, the present work deals with *Lepidium sativum* L. known as Hubba-tur-Rashad[1],

Lepidium sativam L. (Family Cruciferae) is an annual herb widely distributed throughout the Mediterranean Sea Region[1].

In folkloric medicine, it was reported that Lepidium was used in inflammatory conditions, to relieve pain, as tooth pain, joints and sciatica[2]. It is used also for treatment of some skin diseases as scabis, leukoderma and also prevents hair falling[2]. Also some *Lepidium* species proved to have antimalarial activity[3] and antimicrobial activity against mycobacterium tuberculosis[4]. Reviewing the available literature on different *Lepidium* species, several volatile components

which resemble the result of enzymatic decomposition of glucosinolates were reported from L. sativum L.[5,6] Several authors detected flavonoids in different L. species[7], while alkaloids detected in L. latifolium were without complete study[8].

Recently four compounds were reported from the seeds of L. sativum, namely: ethyl sinapinate, N-N dibenzyl thiourea N-N dibenzyl urea and a new alkaloid termed lepidine[9]. Here, we describe the isolation and structure determination of a new alkaloid I, named O-demethyl lepidine together with lepidine. The confirmation of structure of lepidine was also performed by X-ray structure analysis.

EXPERIMENTAL

Lepidium sativum L. seeds were collected in May 1985 from plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut University. The plant was identified and authenticated by Dr. Ibrahim Hassan, Prof. of Fluoriculature and Horticulture , Faculty of Agriculture, Assiut University.

^{1}H-NMR and ^{13}C-NMR were recorded at 90 MHz (WH 90, Bruker) and 400 MHz (WH 400 Bruker), using CD_3OD-d_4 as solvent and TMS as internal standard. Mass spectra were run on MS 30 and MS 50 (70 ev.300 UA) with data system DS 50 instrument (A.E.I.). IR spectra were recorded on a Perkin-Elmer integrated ratio using KBr. UV were measured on a cory 17 (Varian) spectrophotometer. Sephadex LH-20 (Pharmacia), silica gel (Mallinckrodt Serva) and alumina (Prolabo) were used for column chromatography.

X-ray analysis of II : All measurements were made using "Bindungslangen (pm) Und Bindungswinkel (Grad). PU/Ae:2203.66 CM/AE:1.5 .

Extraction and Isolation of Alkaloids : One kg of the air dried ripe seeds was crushed. defatted with pet.ether then exhaustively extracted with methanol by stirring at 40°C. TLC of the methanolic extract using aluminium oxide G and $CHCl_3$-MeOH (9:1) as a developer, revealed at least five Dragendorff's positive spots.

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Fifty g. of the concentrated methanolic extract was chromatographed on alumina column (6 x 130 cm, 2kg). Elution was performed using $CHCl_3$- MeOH (8:2) and fraction 50 ml each were collected; similar fractions were concentrated, where two groups are abtained, A(20-35) and B(40-45). Fraction A was evaporated (3 g.)and chromatographed over sephadex L. H-20 column (5 x 160 cm, 120 g.), eluted with methanol and the eluate was collected in 30 ml fractions. Fractions 10-25 containing single Dragendorff's positive spot, were combined, evaporated to dryness, recrystallized from methanol to give 300 mg of compound II Fraction B was evaporated and the residue (1 g) was chromatographed over silica gel column (3x120 cm 50 g). Elution was carried out using $CHCl_3$-MeOH (8:2) and fractions 50 ml each were collected. Fractions 13-17 showed single Dragendorff's positive spot. Crystallization was carried out from methanol to give 40 mg of compound I.

The remaining column chromatographic fractions containing mixed minor components, have been conserved for further future investigation.

O-demethyl Lepidine (I): Colourless needles (MeOH), m.p.214-215°C, $[\alpha]_D^{20}$ =0(MeOH; C=1]. IR (KBr) ν cm^{-1} 3700-2400,1610 and 1580, UV λ_{max}^{MeOH} : 272 and 278 nm. MS m/z(rel. int.) 346.1433(100) [($C_{20}H_{18}N_4O_2$) required 346.14301, 345(M^+-H), 329(M^+-OH) (15), 173 ($C_{10}H_9N_2O$)(80), 172($C_{10}H_8N_2O$)(12), 158 ($C_{10}H_{10}N_2$) (12), 156 ($C_{10}H_8N_2$) (10), 149($C_6H_3N_3O_2$) (12) and 81(25), ^{1}H-NMR:(400 MHz, CD_3OD) δ: 3.91(S,-CH_2-), 3.98(S,-CH_2-) 6.62(ddd,H-4,J=8, 1.5,0.9Hz), 6.66(dd,H-6, J=7,5,1.5Hz) , 6.77(br,s. H-2) 6.83 (dd, H-9, J=8, 1.5Hz), 6.86(S,H-19, H-20), 6.90(dd, H-11, J=8 , 1.5 Hz), 6.94 (S,H-17, H-18), 7.03(dd,H-5, J=7.5, 8Hz), 7.13(dd H-10 , J=7.5,8Hz) The spectrum of (1) in DMSO-d6 showed in addition to the previous signals, the exchangeable protons of OH and 2 NH at δ 9.5,11.7 and 11.82 respectively.

Lepidine(II), colourless cubes (MeOH) ,m.p. 204-205°C (lit., 208-210°C)[9] [α]$_D^{20}$ =0 (MeOH ; C=1]. IR(KBr) ν cm^{-1}, 3700-2600, 2300, 1610 and 1580 UV λ_{max}^{MeOH} : 268, 278 nm. MS m/z (rel.int.) 360.1587 (32) ($C_{21}H_{20}N_4O_2$ requires 360.1586), 359(M^+-H)(5), 342 ($C_{21}H_{18}N_4O$) (8), 188($C_{11}H_{12}N_2O$), 187 ($C_{11}H_{11}N_2O$) (100), 174 ($C_{10}H_{10}N_2O$)(5), 172($C_{10}H_8N_2O$) (14), 156(4), 143(4) and 81(13), ^{1}H-NMR: (400 MHz, CD_3OD) δ:3.67 (S,OCH_3), 3.92(S, CH_2-), 3.96 (S,CH_2-) 6.56(dd,H-4, J=8, 1.5Hz) ,6.63 (br.s, H-2) ,6.77(dd, H-6, J=8, 1.5Hz), 6.82 (dd,H-9, J=8, 1.5Hz), 6.84 (S,H-19, H-20), 6.92(S,H-17, H-18), 6.98 (dd, H-11, J=8, 1.5Hz), 7.13 (Pseudo t,H-5, J=8Hz), 7.18 (pseudo t, H-10, J=8Hz).

RESULTS AND DISCUSSION

Compound I, $C_{20}H_{18}N_4O_2$(HRMS), $[\alpha]_D^{20}$ 0(MeOH,C=1), m.p. 214-215°C, has UV spectrum λ_{max}^{MeOH} at 272 and 278 nm, which is closely similar to that of lepidine[9]. The IR showed strong hydroxyl, amino and aromatic absorptions at 3700-2400, 1610 cm^{-1} respectively. The ^{1}H-and ^{13}C-NMR spectra revealed similar patterns to those of lepidine[9] except the absence of the signal corresponding to the methoxyl group. The ^{13}C-NMR showed 18 signals for 20 carbons, 7 signals sttributed to 7 quaternary carbons, 9 signals for 11 C-H groups and two signals for methylenic carbons.

The HRMS of I, provides some information which could facilitate the systemic identification of an alkaloid of the lepidine series[9]. The spectrum showed a base peak at m/z 346 in addition to significant peaks at 173($C_{10}H_9N_2O$) and 172 ($C_{10}H_8N_2O$).

Compound II, was identified as lepidine by comparing its UV, IR, MS, ^{1}H and ^{13}C-NMR with previously published for lepidine[9]. X ray crystallography of II (Table I), revealed the relative configuration (Fig. 1) The close correspondance of the distribution of the protons in ^{1}H-NMR to those in the configuration proved the structure of lepidine.

Table 1: Intramolecular bond angles of compound II.

$N_{11}-C_{10}-N_{14}$	111.2
$C_{10}-N_{11}-C_{12}$	106.4
$N_{11}-C_{12}-C_{13}$	107.9
$C_{12}-C_{13}-N_{14}$	108.1
$C_{10}-N_{14}-C_{13}$	106.4
$N_{24}-C_{23}-N_{27}$	110.7
$C_{23}-N_{24}-C_{25}$	106.1
$N_{24}-C_{25}-C_{26}$	108.7
$C_{25}-C_{26}-N_{27}$	108.0
$C_{23}-N_{27}-C_{26}$	106.6

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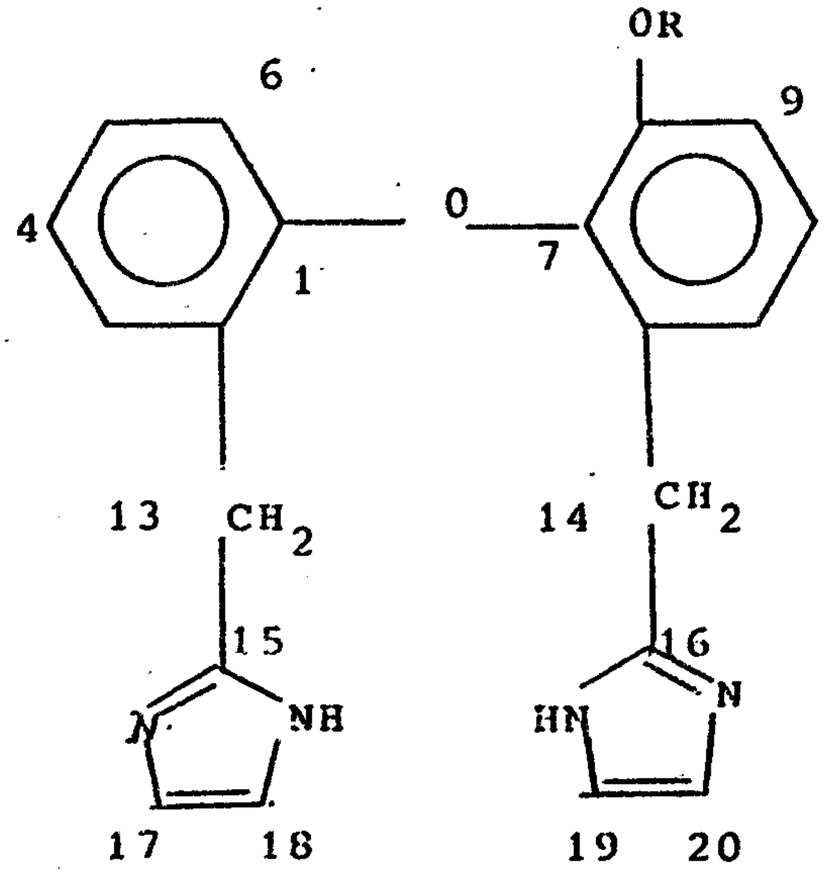
Table 2 : ^{13}C-NMR* Spectral Data of Alkaloids I & II

Carbon No.	Alkaloid I	Alkaloid II
1	159.4	158.1
2	117	115.4
3	140.7	139.4
4	127.0	126.2
5	130.5	129.8
6	113.9	111.9
7	148.1	147.0
8	151.5	152.7
9	116.4	113.1
10	++ 121.9	122.4
11	++ 122.8	122.7
12	133.5	132.4
13	35.0	34.6
14	29.9	29.3
15	147.2	146.2
16	141.2	141.2
17	+ 122.3	121.6
18	+ 122.5	121.7
19	+ 122.3	121.6
20	+ 122.5	121.7
OCH_3	--	56.1

\+ Values may be interchangeable

* Solvent CD_3OD-d_4



(I) R = H 0-demethyl lepidine

(II) R = CH_3 lepidine.

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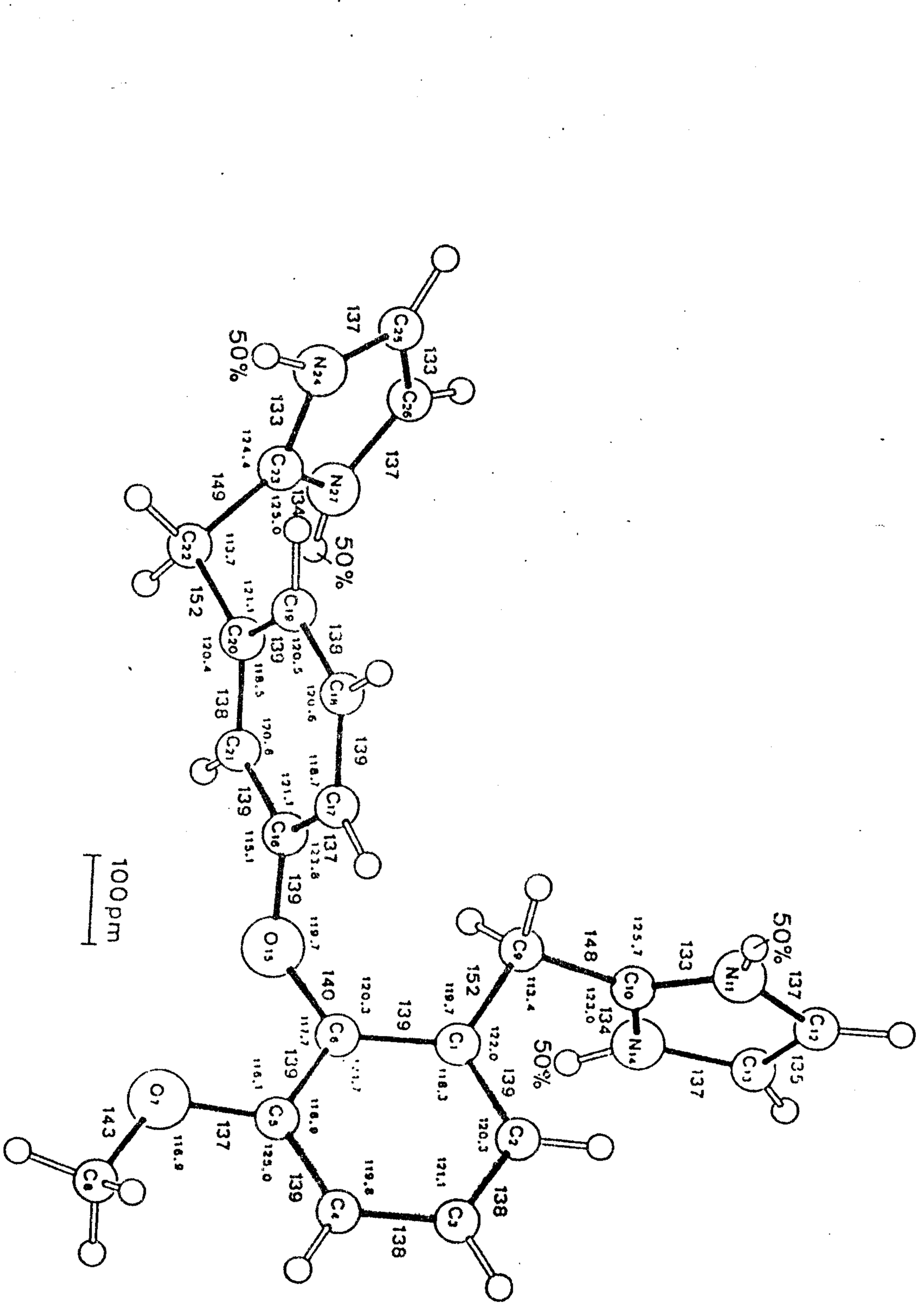

Fig (1) Stereoscopic view of the molecule with hydrogens as spheres

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دى ميثيل ليبيدين ،قلوانى جديد من بذور الليبديم ساتيڤم (حب الرشاد)

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يعتبر عشب الليبديم ساتيڤم من النباتات متعددة الفائدة فيستخدم لعلاج آلام الاسنان والمفاصل ولعلاج الملاريا وله تأثير ضد الميكروبات .

وبذوره المعروفه باسم حب الرشاد تستخدم فى علاج كثير من الامـــــــراض الجلديـــــة .وفى محاولة لفصل المواد الفعاله لهذا النبات لدراستها والتعرف عليها ، تم فصل قلوانيان من البذور وتم دراستهماطبيعيا وفيزيائيا وطيفيـــــا (طيف الاشعة فوق الحمراء ـ وتحت البنفسجية والرنين المغناطيـــــسى لأنويـــة الهيدروجين والكربون ـ ومطياف الكتلة عالى التحليل) وتم التعرف على :

١ ـ الليبيڤين والتاكد من التركيب البللورى له باستخدام تحليل أشعة اكس .

٢ ـ أو ـ دى ميثيل ليبديـــن .

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