

# Physical and Spectral Characterization of Acetone extract of root of *Launaea Asplenifolia* Hook

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## **Abstract:**

*Launaea Asplenifolia* (Willd.) Hook. f. belongs to Asteraceae (Compositae) family and the genus *Launaea*. The members belonging to this family are found everywhere on the surface of the earth (cosmopolitan) in each possible type of habitat. The plants may be herbs, shrubs and very rarely trees and climbers. The species is considered as weed found in all types of land and frequently occurs in rice fields of south and Southeast Asia.

Many biological activities are associated to these plants including bactericide, fungicide, insectidal and diuretic. Several Triterpenes, Sesquiterpene lactones, Coumarins, flavonoids, steroids have been isolated from plants of this genus.

**Keywords:** *Launaea Asplenifolia* (Willd.) Hook, phytochemicals, Flavonoids, Steroids.

## **Introduction:**

*Launaea Asplenifolia* (Willd.) Hook. f. belongs to Asteraceae (Compositae) family and the genus *Launaea*. The Asteraceae is the largest family of the angiosperms containing about 950 genera and probably 20,000 species. The members belonging to this family are found everywhere on the surface of the earth (cosmopolitan) in each possible type of habitat. Most of them are herbaceous but from tropical regions the trees have also been reported. The plants may be herbs, shrubs and very rarely trees and climbers. Many plants possess a milky juice while in the other members the juice is watery, resinous and bitter [1].

The genus *Launaea* comprises about 40 species. This genus shows its importance in folk medicine. The genus *Launaea* presents interesting phytochemical features such as the

Occurrence of Terpenoids, phenolics, flavones and Coumarins. Coumarins from the genus have shown antibacterial activities. One of the species of this genus *L. arborescens* is used as livestock mainly for camel and is medicinally important for its anti-diarrhoeic and antispasmodic activity. Milk of the plant is used in the treatment of skin diseases [2].

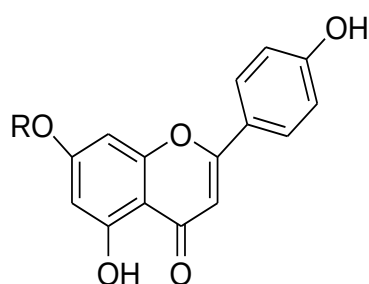
*Launaea Asplenifolia* Hook belongs to family Asteraceae. The Plant is annual herb 12- 25 cm tall, stem glabrous, simple or branched at the top. The species frequently found on sandy banks of river. Flowering and fruit season of the plant is from November to February. In Marathwada generally it is distributed in Aurangabad, Nanded and Osmanabad districts. Leaves of the plant are used as a vegetable and also in Ayurvedic medicine system [3]. Traditionally the kol tribes of Vindhyan region of Uttar Pradesh use the

plant paste of the species in the treatment of leukoderma a skin disease [4]. The species is considered as weed found in all types of land and frequently occurs in rice fields of south and Southeast Asia [5]. Other observation of the species as weed in wheat crop was done by M.K. Sidhu [6]. Antifungal activity of water extract of the plant was observed against the pathogen *Rhizoctonia solani* [7]. Frequent on sandy banks of river. Leaves are used as vegetable and in Ayurvedic Medicines.

### Review of Literature:

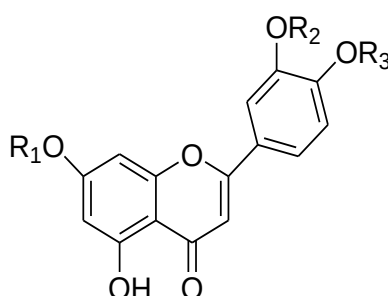
Asteraceae is a vast family of flowering plants, distributed in all parts of the world. Most of the plants are annuals or perennial herbs. Many plants of this genus are used as ornamental plants. Many biological activities are associated to these plants including bactericide, fungicide, insectidal and diuretic. Several Triterpenes, Sesquiterpene lactones, Coumarins, flavonoids, steroids have been isolated from plants of this genus. The present section aims at a brief review on the natural occurrence of compounds from this genus. The phytochemical studies on species of this genus dates back to 1969 with the isolation of taraxasterol and taraxasterol acetate from the leaves and roots of *Launaea pinnatifida* by K.R. prabhu and V. Venkateswarlu [8]. In 1974 K. Bahadur and S. Sharma reported palmitic, stearic, oleic and linoleic acids from the roots of *Launaea nudicaulis* [9].

Isolation of flavone glycosides was reported by Ragga et al [10] from *Launaea nudicaulis*. These flavonoids were identified as apigenin – 7 – o – glucoside (a), luteolin – 7 – o – glucoside (b), luteolin – 7 – o – rutinoside (c).



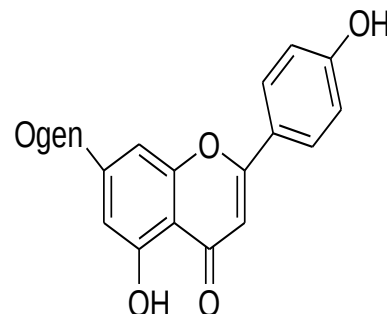
(a)(b)

(a) R = Glc



(c)

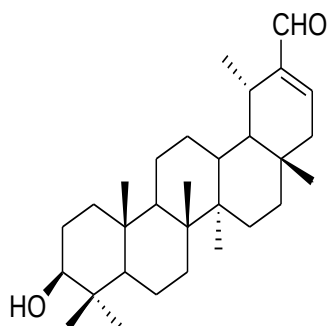
(b) R<sub>1</sub> = Glc R<sub>2</sub> = H R<sub>3</sub> = H



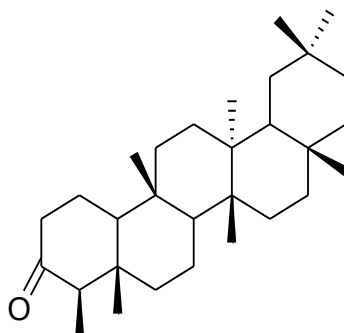
(c) R<sub>1</sub> = Rut R<sub>2</sub> = H R<sub>3</sub> = H

Apigenin – 7 – o – glucoside (a) and luteolin – 7 – o – glucoside (b) has also been reported in *Launaea Asplenifolia* Hook by Gupta et al [11]. Compounds (a) and (b) have also been reported in *Launaea tenuiloba* (Boiss) by Abdel – Salem et al [12].

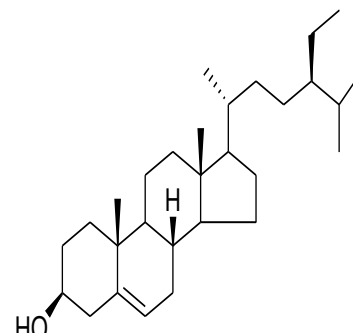
Ψ – taraxasterol 30 – aldehyde (d) and friedelin (e) along with β – sitosterol (f) and stigmasterol have been isolated from *Launaea spinosa* (Forssk) by Sokkar et al [13].



(d)



(e)



(f)

Several sterols have been isolated from *Launaea Nudicaulis* Hook F. by Behari et al [14]. These are cholesterol, brassica sterol, campesterol,  $\beta$  – sitosterol, stigmasterol, fucosterol,  $24\beta$  -  $\Delta^7$  – ergosten –  $3\beta$  – ol, stigmasta – 7, 24 (28) – diene –  $3\beta$  – ol,  $\Delta^7$  – stigmastenol and  $\Delta^7$  – cholesterol. Coumarins isolated from this genus are esculetin and cichoriin in *Launaea Nudicaulis*, *Launaea Residifolia* and *Launaea Tenuiloba* (Boiss) respectively by Sarg et al [15] Gherraf et al [16] and Abdel Saleh [12]. Scopoletin and isoscopoletin were also reported by Gherraf et al [16].

Among the sesquiterpenes reported from this genus are crepidiaside A, guaianolide lactucin, the corresponding di hydro derivative  $11\beta$  –  $13$  – dihyrolactucin, 8 – acetyl -  $11\beta$  –  $13$ –dihydrolactucin by Sarg et al [17] and 8–deoxylactucin, jaquinelin ( $11\beta$ – $13$ – dihyrolactucin), lactucin from *Launaea Spinosa*, *Launaea Mucronata* (Forssk) and *Launaea Tenuiloba*. Aliphatic acid such as octacosanic acid [18], alcohols hexacosanol, octacosanol [12] are reported from this genus. Ferulic acid and Methyl Caffeoate has been isolated from *Launaea arborescens* by R.M. Giner et al [19].

## Experimental:

### Preparation of Acetone Extract:

*Launaea Asplenifolia* Hook was collected from the fields and the plant material was identified by Department of Botany, Shrikrishna Mahavidyalaya, Gunjoti. The plant was deposited in the Herbarium of the Botany department.

The root and shoot of *Launaea Asplenifolia* Hook were shade dried and powered separately. The root powder (500 gm.) of *Launaea Asplenifolia* Hook sequentially extracted with acetone and methanol in a Soxhlet apparatus for 48 hours. The resulting acetone extract was then concentrated under reduced pressure by using rotary vacuum evaporator (Model Superfit India) to obtain a residue (20 gm.). The acetone extract showed eight prominent spots on thin layer chromatography (TLC). Solvent system used for TLC was Chloroform: Methanol system (5:5).

### Chromatographic Separation of the Acetone Extract:

The acetone extract (20 gm.) was chromatographed on a silica gel column (60 – 100 mesh), (150 × 15 cm) and eluted with a step wise gradient of Chloroform: Methanol (100, 90:10, 85:15, 80:20, 75:25, 70: 30, 100 by volume). A total of 109 fractions of 10 ml each were collected, and the similar ones were poured to eight groups. The grouping was done according to their composition determined by thin layer chromatography. Spots were detected by using various detecting techniques such as UV chamber, Iodine Chamber, spraying with 10% methanol-sulphuric acid spray reagent and then heating on a hot plate.

**Group I:** The fractions 1–8 was concentrated under reduced pressure and a yellowish semisolid was obtained (60 mg.). This compound was designated as compound A.

**Group II:** The fractions 9–15 was concentrated under reduced pressure and a greenish solid was obtained (250 mg.). This solid was designated as compound B.

**Group III:** The fractions 16–25 obtained by the elution of chloroform: methanol (90:10) showed one prominent spot-on TLC. These fractions were mixed together and allowed to concentrate under reduced pressure to obtain a creamy white solid (50 mg.). This compound was designated as compound C.

**Group IV:** The fractions 30–45 was concentrated in a vacuum to obtain a yellow solid (140 mg.). This compound was designated as compound D.

**Group V:** Fractions 46–60 was concentrated under reduced pressure that showed one prominent spot-on thin layer chromatography gave a reddish solid (50 mg.) and designated as compound E.

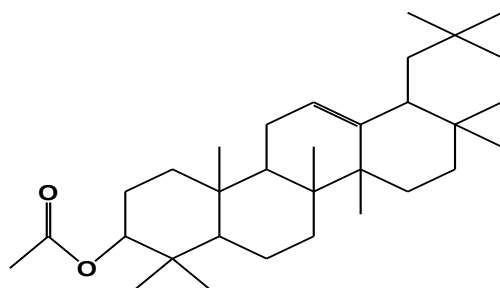
**Physical and Spectral Characterization of the compounds isolated from acetone extract of root of Launaea Asplenifolia Hook:****Compound A.****Physical State:** Solid **Yield:** 60 mg. **MP:** 242 °C**UV  $\lambda_{\max}$  (CH<sub>3</sub>OH):** 205**IR (KBr)  $\nu_{\max}$ :** 2982, 2850, 2362, 1734, 1460, 1383, 1261, 1174, 1099, 1020, 879, 802, 729 cm<sup>-1</sup>.**<sup>1</sup>H NMR (400 MHZ, CDCl<sub>3</sub>)  $\delta$ :** 0.77 (1H, S), 0.78 (1H, S), 0.96(1H, S), 0.99(1H, S), 1.02(1H, S), 0.84(1H, S), 0.85(1H, S), 0.89(1H, S), 2.02(3H, S), 4.60(1H, dd, J=8.04, 5.9Hz), 5.40 (1H, t, J=3.5 Hz).**ESI-MS m/z:** 468**Compound B.****Physical State:** Amorphous Solid **Yield:** 50 mg. **MP:** 218 °C**UV  $\lambda_{\max}$  (CH<sub>3</sub>OH):** 205 nm**IR (KBr)  $\nu_{\max}$ :** 3400, 2935, 2875, 1680, 1620, 1430, 1380, 1050, 995 cm<sup>-1</sup>.**<sup>1</sup>H NMR (400 MHZ, CDCl<sub>3</sub>)  $\delta$ :** 0.75 (3H, S), 0.78 (3H, S), 0.83 (3H, S), 0.94 (3H, S), 0.96 (3H, S), 1.02 (3H, S), and 1.66(3H, S), 4.57 (1H, S), 4.67 (1H, S), 3.22 (dd, J= 11.5 Hz) , 2.36 ( ddd, J = 11.2 Hz, J = 10.8 HZ, J = 6.1 HZ).**ESI-MS m/z:** 426**Compound C.****Physical State:** White Solid **Yield:** 15 mg. **MP:** 215 °C**UV  $\lambda_{\max}$  (CH<sub>3</sub>OH):** 209 nm**IR (KBr)  $\nu_{\max}$ :** 2962, 2850, 1734, 1460, 1380, 1174, 995 cm<sup>-1</sup>**<sup>1</sup>H NMR (400 MHZ, CDCl<sub>3</sub>)  $\delta$ :** 4.68 (1H, S), 4.58 (1H, S), 4.48 (1H, dd, J=4.4, 12.9 HZ), 2.06(3H, S), 1.70 (3H, S), 1.02 (3H, S), 0.92 (3H, S), 0.86 (3H, S), 0.82 (3H, S), 0.80 (3H, S), 0.78 (3H, S).**ESI-MS m/z:** 468**Compound D.****Physical State:** Crystalline Solid **Yield:** 140 mg. **MP:** 255 °C**UV  $\lambda_{\max}$  (CH<sub>3</sub>OH):** 195 nm, 203 nm, 224 nm.**IR (KBr)  $\nu_{\max}$ :** 2924, 2852, 1708, 1641, 1460, 1383, 1261, 1190, 1084, 1039, 920, 877 cm<sup>-1</sup>.**<sup>1</sup>H NMR (400 MHZ, CDCl<sub>3</sub>)  $\delta$ :** 0.88 (3H, d, J = 6.8 HZ), 0.70. 0.86, 0.94, 0.99 (6 H), 1.03, 1.16, (3H, S), 2.37 (2 H, m), 2.26 (1H, q, J = 6.6 HZ).**ESI-MS m/z:** 426**Compound E.****Physical State:** White solid **Yield:** 15 mg. **MP:** 148 °C**UV  $\lambda_{\max}$  (CH<sub>3</sub>OH):** 209 nm, 240 nm**IR (KBr)  $\nu_{\max}$ :** 3437,2926,2854, 2380,1599, 1518, 1460,1415, 1271, 1226, 1157, 1116, 1074, 823, 702 cm<sup>-1</sup>.**<sup>1</sup>H NMR (400 MHZ, DMSO)  $\delta$ :** 0.72 (3H, S), 0.98 (3H, S), 1.01 (3H, d, J=6.6Hz), 0.84 (3H, d, J=6.2 Hz), 0.78 (3H, d, J=6.5 Hz), 0.78 (3H, d, J=7.5), 5.02 (1H, dd, J=15.6Hz, J=8.5Hz), 5.22 (1H, dd, J=16.0 Hz, J=8.5 Hz), 5.31 (1H, S), 3.17 (1H, dd, J=12.2 Hz, J=6.1Hz).**ESI-MS m/z:** 412**Result and Discussion:****Structure of Compound A:**

The sub-fraction obtained from acetone soluble fraction of the plant (20 gm.), was subjected to column

chromatography. The column was first eluted with chloroform and with increasing polarities of chloroform-methanol mixtures. Elution of the column with pure chloroform yielded a solid compound (60 mg) designated as Compound A with melting point 242 °C. The ESI-MS spectrum of Compound A showed the  $[M^+]$  ion at  $m/z$  468, which was in agreement with the molecular formula  $C_{32}H_{52}O_2$  consistent with six degrees of unsaturation. The Mass spectrum of Compound A also showed characteristic fragmentation of amyrin type of skeleton with a double bond at the C-12 [20].

The UV spectrum of Compound A showed characteristic absorption at 205 nm, 276 nm indicating the presence of ester group in the compound. The IR spectrum displayed absorptions at  $1734\text{ cm}^{-1}$  (C=O) stretching in ester,  $1099\text{ cm}^{-1}$ ,  $1174\text{ cm}^{-1}$  and  $802\text{ cm}^{-1}$  for (C-O) and (C=C) stretching respectively. The  $^1\text{H-NMR}$  spectrum of Compound A ( $\text{CDCl}_3$ , 400 MHz) showed singlet for eight tertiary methyl's at  $\delta$  0.77, 0.78, 0.84, 0.85, 0.89, 0.96, 0.99 and 1.02 each. The signal at  $\delta$  2.02 indicates methyl protons linked to carbonyl moiety in ester group. An olefinic proton resonated as a triplet at  $\delta$  5.40 ( $J=3.5\text{ Hz}$ ) ascribed to the C-12 proton. In the spectrum, a double doublet of one proton integration at  $\delta$  2.30 ( $J=10.3, 4.4\text{ Hz}$ ) was due to the C-3 $\alpha$  axial proton.

The  $^{13}\text{C-NMR}$  spectrum of Compound A revealed the presence of nine methyl, ten methylene, five methine and eight quaternary carbons. The chemical shifts of these signals, GC-MS spectrum and other physical data suggests that the compound is 12-Oleanen-3-yl-acetate (3- $\alpha$ ) isolated first time from the roots of *Launaea Asplenifolia* Hook and is a new natural source.



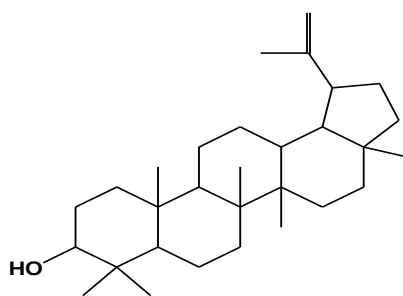
12- Oleanen- 3-yl-acetate (3- $\alpha$ ) ( 1 )

### Structure of Compound B:

Compound B was isolated as a white powder with a melting of 216-217 °C. The molecular formula of the compound was deduced as  $C_{30}H_{50}O$  from the EI-MS spectrum which showed significant mass peak at 426. The other fragments were at  $m/z$  411, 218 and 207 which shows the pentacyclic triterpene skeleton with an isopropenyl group [21, 22]. The IR absorption band at  $3400\text{ cm}^{-1}$  indicates the presence of hydroxyl group in the compound. The bands at  $1680, 1620\text{ cm}^{-1}$  is indicative of C=C stretching for olefin. The C-H stretching in methyl group exhibited the absorption at  $1380\text{ cm}^{-1}$ .

The  $^1\text{H-NMR}$  spectrum of compound B showed signal for seven 3H singlet at  $\delta$  0.75, 0.78, 0.83, 0.94, 0.96, 1.02 and 1.66 for the tertiary methyl protons. The presence of terminal isopropenyl group for 1H each was confirmed at  $\delta$  4.57 and 4.67 multiplets. The above data indicates that the compound B belongs to the lupane series of triterpenoids. Compound B showed a double doublet signal at  $\delta$  3.21 ( $J=11.6, J=5.2\text{ Hz}$ ) indicating the presence of proton germinal to a hydroxyl group. The signal at  $\delta$  2.36 ( $J=11.2\text{ Hz}, J=10.8\text{ Hz}, J=6.1\text{ Hz}$ ) was due to a single proton doublet of triplet.

The  $^{13}\text{C-NMR}$  spectra of Compound B revealed the presence of seven methyl, eleven methylene and five methine carbon atoms. From the above data and EI-MS of compound B, the compound was characterized as lupeol (2) which is previously reported from the same plant by Gupta et al [23].



Lupeol ( 2 )

### Conclusion:

The physical and spectral characterization of compound A of acetone extract of root of *Launaea Asplenifolia* Hook showed the presence of 12-Oleanen-3-yl-acetate (3-  $\alpha$ ) isolated first time from the roots of *Launaea Asplenifolia* Hook and is a new natural source. The spectral characterization of compound B showed that the compound as lupeol which is previously reported from the same plant from the shoot of the plant.

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