Chemical studies of *Launaea nudicaulis* Hook f. extracts with Antioxidant and Urease Inhibitory Activities

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Summary: An activity guide isolation of *Launaea nudicaulis* Hook f, medicinal plant of Indo-Pak region has shown antioxidant potentials *via* its polar solvent soluble fractions while urease inhibition studies (*in vitro*) indicated compound **8** and **9** as a good urease inhibitors. Eight compounds have also been isolated for the first time from *Launaea nudicaulis* Hook f., namely, Scopoletin **1**, lupeol **2**, *β*-amyrin **3**, *β*-sitosterol 3-*O*-*β*-D-glucopyranoside **4**, stigmasterol 3-*O*-*β*-D-glucopyranoside **5**, 6-hydroxy flavone **6**, 7-methoxy flavone **7** and kaempferol **8**, respectively. Their structures were elucidated by EI-MS, FABMS, ^{1}H-NMR spectroscopic data.

Keywords: Asteraceae, *Launaea nudicaulis*, Urease inhibition activity, Antioxidant activity.

Introduction

Among the angiosperms Asteraceae is the largest plant family of Pakistan having 15 tribes of 650 species and the knowledge regarding this large and important family is far from completion [1]. *Launaea* belongs to (tribe Lactucaea) the same family, comprises of 40 species [1, 2] and have a decent folkloric history as a remedy in traditional medicinal system in Indo-Pak region due to their properties like tonic, diuretic, aperients, soporific, galactagouge, febrifuge, as a taraxacum substituent and to treat infected wounds [2-4]. Out of the 20 Pakistani species of the genus (*Launaea*), *Launaea nudicaulis* is the most studied one and regarded as a medicinal plant because of its utilization during constipation, bilious fever, children fever, skin itching, cuts, ulcers, swellings, conjunctivitis, suppuration of abscess, eczema, eruption, rheumatism and toothache [4-6]. *L. nudicaulis* has also shown the anti-fungal effects on Mungbeans [7] along with anti-bacterial, cytotoxic, insecticidal, hypoglycaemic and anti-inflammatory activities [8-12]. *L. nudicaulis* is a good source of triterpenes while other important natural compounds like coumarins, flavonoids, phenolics and essential oils are also reported [13-17]. The medicinal and chemical background of the *Launaea nudicaulis* prompted us to conduct bioassay guided isolation. *Launaea nudicaulis* (fractions and compounds) was firstly subjected to evaluate the urease inhibition and then antioxidant potentials were also tested.

Results and Discussion

The ethanolic extract of the whole plant of *Launaea nudicaulis* Hook f. was suspended in water and successively extracted with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water-soluble fractions. Firstly urease inhibition assay of these farctions gave the results as given in Table-1. The EtOAc fraction was found to be active and investigated. Right from this fraction a good urease inhibitors kaempferol **8** and cichoriin **9** was successfully purified having 60.4±0.72 and 181.4±1.46 IC_{50} value respectively compatible with (Thiourea) standard. Determination of antioxidant behaviors of *Launaea nudicaulis* revealed the water and methanol fractions of plant having significant antioxidant potential truly comparable with the standard antioxidant (propyl gallate) because of polar nature while moderate activities by other fractions against DPPH scavenging activity. While in case of metal chelating assay again polar parts, water and methanol fractions are slight better than the other inactive non-polar fractions as in Table-2. The methanol, chloroform and ethyl acetate-soluble fractions were subjected to column chromatographic techniques as described in the experimental to obtain nine known compounds where **1-7** are being reported for the first time from this species. The isolated chemical constituents are scopoletin **1**, lupeol **2**, *β*-amyrin **3**, *β*-sitosterol-3-*O*-*β*-D-glucopyranoside **4**, stigmasterol 3-*O*-*β*-D-glucopyranoside **5**, 6-hydroxy flavone **6**, 7-methoxy flavone **7**, kaempferol **8**, and Cichorin **9** respectively on the basis of their respective spectral data as in Fig. 1.

Table-1: Urease inhibition studies of *Launaea nudicaulis* (Fractions/compounds).

Sample (Fraction/Compound)	IC_{50} ±S.E.M
Water	**N.S.**
Methanolic extract	**N.S.**
EtOAc	**167.2±1.2**
$CHCl_3$	**N.S.**
***n*-Hexane**	**N.S.**
1	**N.S.**
2	**N.S.**
3	**N.S.**
4	**N.S.**
5	**N.S.**
6	**N.S.**
7	**N.S.**
8	**60.4±0.72**
9	**181.4±1.46**
***Thiourea**	**21 ± 0.01**

*Standard drug
S.E.M. = Standard error mean
N.S. = Non Significant

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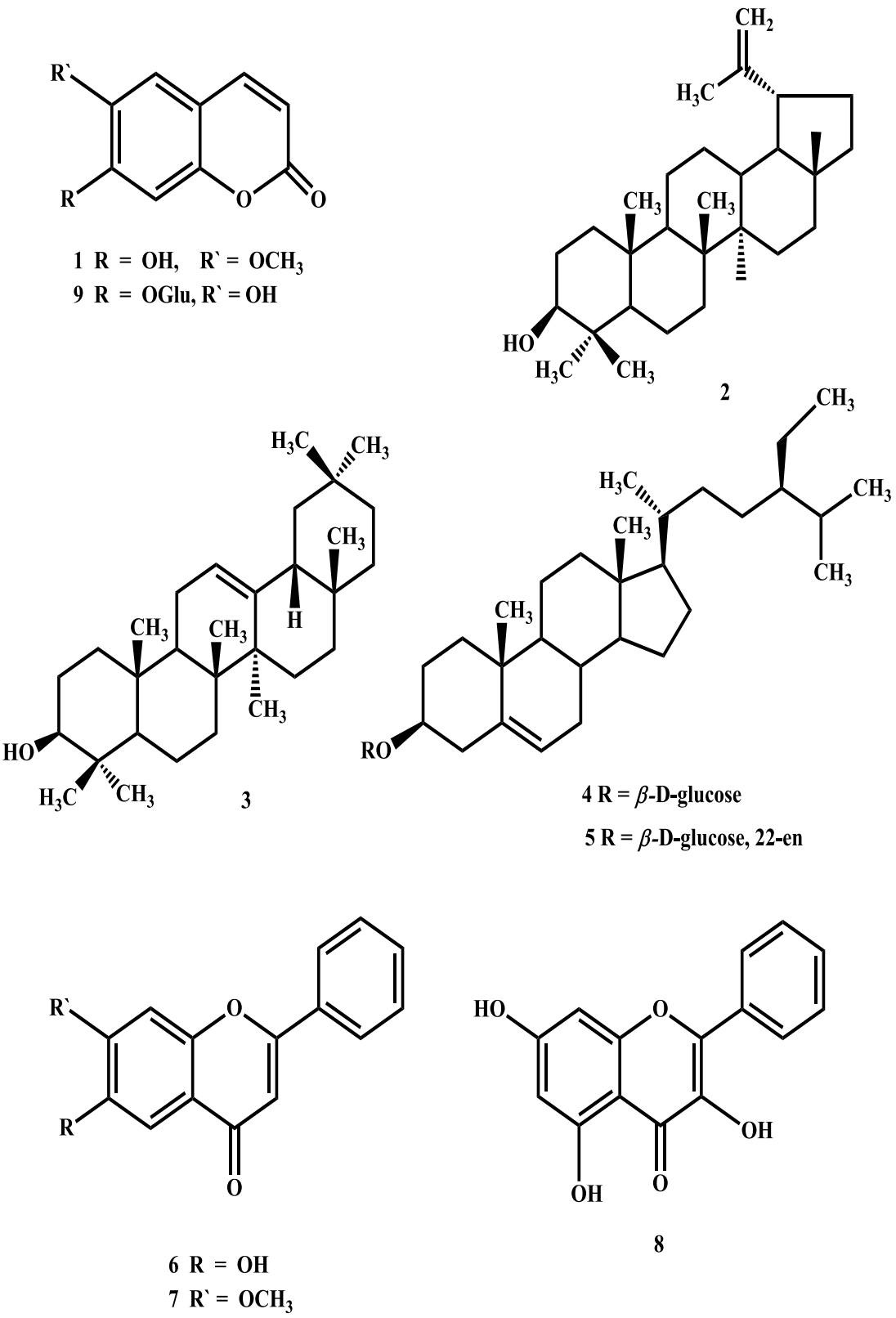

Fig. 1: Structures of compounds isolated from *Launaea nudicaulis.*

Table-2: Antioxidant activity of different fractions of *Launaea nudicaulis.*

Fractions	% RSA ± SEM* (250µg/mL)		
	DPPH Radical Scavenging	Superoxide Anion Scavenging	Metal Chelating
Water	89.65±2.75	81.45±1.82	62.57±1.21
Methanol	82.13±1.92	79.84±1.62	67.65±1.47
Hexane	58.28±1.08	25.61±2.87	25.68±3.78
Chloroform	61.88±2.01	31.29±3.15	36.98±2.40
Ethyl Acetate	78.06±1.45	57.45±1.09	34.59±1.78
Propyl gallate	93.01±0.27	91.24±1.60	-
EDTA	-	-	98.26±0.31

RSA= Radical Scavenging Activity,
*SEM = Standard Error of Mean, values are the mean ± standard error of mean, n = 3.

Experimental

General

Column chromatography was carried out using silica gel of 70-230 and 230-400 mesh. Aluminium sheets precoated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as spraying reagent (heating). Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The melting points were recorded on Gallenkamp apparatus and are uncorrected. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, HREIMS, FABMS and HRFABMS spectra were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H-NMR spectra were recorded on Bruker AMX-400 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants *(J*) are in Hz.

Plant Material

The whole plant of *Launaea nudicaulis* Hook f. was collected from Karachi (Malir cantt, Hazara gotth, Toll plaza super highway), Pakistan, in winter season of 2006. The plant was identified by Mr. S. Ehteshamul Haque, Associate professor; Department of Botany, University of Karachi and sample specimen was deposited in herbarium section of the mentioned department with voucher no. GHs 86243.

Extraction and Isolation

The freshly collected plant material (whole plant, 27 kg) of *Launaea nudicaulis* Hook f. was shade dried (8.73 kg), ground and extracted with methanol (3 x 15 Lit, each for 10 days). The combined methanolic extract was evaporated under reduced pressure at room temperature to yield a residue (512 g). The whole extract was suspended in water and successively extracted with *n*-hexane (143 g), chloroform (111 g), ethyl acetate (97 g), *n*-butanol (44 g) and water (89 g) soluble fractions. The chloroform soluble fraction was subjected to column chromatography over silica gel and eluted with *n*-hexane, *n*-hexane-chloroform, chloroform, chloroform-methanol and methanol in increasing order of polarity to get five sub-fractions. The sub-fraction obtained with *n*-hexane:$CHCl_3$ (7.0:3.0) was a semi pure compound, which was re-chromatographed over silica gel and eluted with *n*-hexane:EtOAc (6.5:3.5) solvent system to get compound **3**. The fraction obtained with *n*-hexane:$CHCl_3$ (3.0:7.0) was a mixture of three spots which was again re-chromatographed over silica by using *n*-hexane: $CHCl_3$ (2.3: 7.7, 2.1:7.9 and 1.8: 8.2) to afford compound **6, 7** and **8** respectively. The sub-fraction obtained with pure chloroform was a binary mixture, re-chromatographed over silica gel and successively eluted with $CHCl_3$: MeOH (9.5:0.5) and CH_2Cl_2: MeOH (8.3:1.7) to provide compounds **5** and **4**, respectively. The ethyl acetate soluble fraction was subjected to column chromatography over silica gel and eluted with *n*-hexane, *n*-hexane-ethylacetate, l and pure ethylacetate in increasing order of polarity to get five sub-fractions. The first sub-fraction obtained with *n*-hexane:EtOAc (7.5:2.5) was a binary mixture, combined it and re-chromatographed over silica eluting with *n*-hexane: EtOAc (5.8:4.2) yield the compounds **1** and **2** from the top and the tail, respectively. The fraction obtained with pure EtOAc was further purified with column chromatography using CH_2Cl_2: MeOH (8.6:1.4) to yield compound **9**.

Bioassays

To study the urease inhibition method mentioned in literature [18] was adopted. While the antioxidant evaluations namely, DPPH radical scavenging assay, superoxide anion scavenging assay, metal (Iron) chelating assay were made as reported [19-25].

Scopoletin **1**

Crystallized from acetone (11mg), M. P. 169-170°C;1R (KBr) ν_{max} cm^{-1}:3210, 2890, 1725, 1465; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.88 (1H, d, *J* = 9.5 Hz, H-4), 7.13 (1H, s, H-5), 6.79 (1H, s, H-8), 6.31 (1H, d, *J* = 9.5 Hz, H-3), 3.79 (3H, s, OMe); HR-FAB-MS $[M+1]^+$, *m/z* (rel. int.) 193 (90) (calcd. for $C_{10}H_9O_4$, 193.0131). The physical and spectral data showed complete agreement with those reported in the literature [14].

Lupeol **2**

White needles (26 mg), M. P. 215°C, $[\alpha]_D^{25}$: + 27° (*c* 0.025, MeOH), 1R (KBr) ν_{max} cm^{-1}: 3435 (OH), 3074, 1645 (C=C), ^{1}H-NMR ($CDCl_3$, 400

MHz) δ: 4.66, 4.54 (1H, each, br s, CH_2-29), 3.63 (1H, dd, *J* = 10.6, 4.2 Hz, H-3), 2.35 (1H, ddd, *J* = 5.7, 5.7, 5.4 Hz, H-19), 1.62 (3H, br s, Me-30), 1.07 (3H, s, Me-26), 0.98 (6H, s, Me-25, Me-27), 0.92 (3H, s, Me-24), 0.86 (3H, s, Me-28), 0.80 (3H, s, Me-23), HR-EI-MS: *m/z* 426.3855 (calcd. for $C_{30}H_{50}O$, 426.3862). The physical and spectral data were in complete agreement with those reported values for lupeol (**2**) [26, 27].

β-Amyrin **3**

White crystalline (MeOH) (18 mg), M. P. 197-198°C, $[\alpha]_D$ ^{25}: +100 (*c* 0.22, $CHCl_3$), IR (KBr) ν_{max} cm^{-1}: 3510 (OH), 1625 (C=C), ^{1}H-NMR ($CDCl_3$, 500 MHz) *δ* : 5.11 (1H, m, H-12), 3.21 (1H, dd, *J* =10.1, 4.7 Hz, H-3), 1.08, 1.03, 1.01, , 1.00, 0.99, 0.92, 0.91 and 0.87 (3H, each s, Me-30-23), HR-EI-MS: *m/z* 426.3825 (calcd for $C_{30}H_{50}O$, 426.3861) The physical and spectral data were similar to the reported data [28, 29].

*β-Sitosterol 3-O-β-*D*-glucopyranoside* **4**

White amorphous powder (20 mg), M. P. 198°C, $[\alpha]_D$ ^{25}: -14.5^o (*c* 0.02, MeOH), IR (KBr) υ_{max} cm^{-1}: 3452 (OH), 3040 , 1648 (C= C), ^{1}H-NMR (C_5D_5N, 300 MHz) δ: 5.34 (1H, d, *J* = 7.1 Hz. H-1`), 5.10 (1H, d, *J* = 5.3 Hz, H-6), 3.88 (1H, m, H-3), 4.47.3.74 (m, Glc-H), 1.04 (3H, s, Me-19), 0.96 (3H, d, *J* = 6.2 Hz, Me-21), 0.88 (3H, t, *J* = 7.2 Hz, Me-29), 0.86 (3H, d, *J* = 6.4 Hz, Me-26), 0.84 (3H, d, *J* = 6.6 Hz, Me-27) and 0.73 (3H, s, Me-18), HR-FAB-MS^{+ve}: *m/z* 577.4444 $[M+H]^+$ (calcd. for $C_{35}H_{61}O_6$, 577.4438). The physical and spectral data were in similar data reported for the *β*-sitosterol 3-*O*-*β*-D-glucopyranoside [30-32].

*Stigmasterol 3-O-β-*D*-glucopyranoside* **5**

White amorphous powder (27 mg), M. P. 285-289°C, $[\alpha]_D$ ^{25}: -51.5^o (*c* 0.21, C_5H_5N), UV (MeOH) nm (log ε): λ_{max} 201 (2.8), IR (KBr) υ_{max} cm^{-1}: 3452 (OH), 1644 (C= C), ^{1}H-NMR (C_5D_5N, 400 MHz) (δ): 5.22 (1H, br d, *J* = 5.4 Hz, H-6), 5.16 (1H, dd, *J* = 15.2, 8.4 Hz, H-22), 5.03 (1H, dd, *J* = 15.2, 8.5 Hz, H-23), 4.76 (1H, d, *J* = 7.4 Hz, H-1`), 3.85 (1H, m, H-3), 4.45-3.80 (m, Glc.-H), 1.04 (3H, s, Me-19), 0.96 (3H, d, *J* = 6.2 Hz, Me-21), 0.87 (3H, d, *J* = 7.0 Hz, Me-29), 0.85 (3H, d, *J* = 6.5 Hz, Me-27), 0.62 (3H, s, Me-18), HR-FAB-MS^{+ve}: *m/z* 575.4261 $[M+H]^+$ (calcd. for $C_{35}H_{59}O_6$, 575.4267). All the physical and spectral data were similar to the reported data for the compound **5** [30, 31].

6-Hydroxyflavone **6**

Light pink (15 mg), M. P. 235-237°C , UV (MeOH) nm (log ε): λ_{max} 259 (3.6), 301 (3.6), IR (KBr) υ_{max} cm^{-1}: 3426 (OH), 1678 (CO), 1622-1409 (aromatic moiety), ^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.21 (1H each, dd, *J* = 7.8, 2.1 Hz, H-2′, -6′), 7.39 (1H, d, *J* = 2.4 Hz, H-5), 7.59 (1H each, dd, *J* = 7.8, 7.4 Hz, H-3′, 5′), 7.57 (1H, dd, *J* = 7.4, 2.1 Hz, H-4′), 7.48 (1H, d, *J* = 8.2, Hz, H-8), 7.41 (1H, dd, *J* = 8.2, 2.4 Hz, H-7), 6.88 (1H, s, H-3), HR-EI-MS: *m/z* 238.0619 $[M]^+$ (calculated for $C_{15}H_{10}O_3$, 238.0629). The ^{13}C-NMR data were similar to the reported data for the compound **6** [33].

7-Methoxyflavone **7**

Colourless needles (15 mg), M. P. 108-109°C, UV (MeOH) nm (logε): λ_{max} 300 (2.8), 250 (3.2), 236 (2.6), IR (KBr) υ_{max} cm^{-1}: 3450(OH), 1658 (C=O), 1600-1400 (aromatic moieties) cm^{-1}. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.01 (1H each, dd, *J* = 8.0, 1.8 Hz, H-2′, -6′), 7.95 (1H, d, *J* = 7.5 Hz, H-5), 7.60 (1H each, dd, *J* = 8.0, 7.2 Hz, H-3′, 5′), 7.54 (1H, dd, *J* = 7.2, 1.8 Hz, H-4′), 7.22 (1H, d, *J* = 2.1 Hz, H-8), 6.91 (1H, s, H-3), 6.89 (dd, *J* = 7.5, 2.1 Hz, H-6), 3.91 (3H, s, OMe). HR-EI-MS: *m/z* 252.0780 $[M]^+$ (calculated for $C_{16}H_{12}O_3$, 252.0786). All the physical and spectral data were matched with the previously reported in literature for the compound **7** [34].

Kaempferol **8**

Pale yellow powder (15 mg), M. P. 276-278°C, UV (MeOH) nm (logε): λ_{max} 349 (3.7), 267 (2.9), 220 (1.9), IR (KBr) υ_{max} cm^{-1}: 3417 (OH), 1661 (C=O), 1611-1379 (aromatic moieties) cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 8.07 (1H each, d, *J* = 8.5 Hz, H-2′, -6′), 6.94 (1H each, d, *J* = 8.5 Hz, H-3′, 5′), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.17 (d, *J*= 2.0 Hz, H-6). HR-EI-MS: *m/z* 286.0474 $[M]^+$ (calculated for $C_{15}H_{10}O_6$, 286.0477). All the physical and spectral data were similar to the reported data for the compound **8** [35].

Cichorin **9**

Gum like material (11mg), $[\alpha]_D$^{25}: -104°(*c* = 0.039, dioxan), IR ν_{max} (nujol) cm^{-1}: 3434 (OH), 1805 (ester), 1600-1400 (aromatic moiety), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.81 (1H, d, *J* = 9.5 Hz, H-4), 7.20 (1H, s, H-5), 7.04 (1H, s, H-8), 6.27 (1H, d, *J* = 9.5 Hz, H-3), 4.97 (1H, d, *J* = 7.5Hz, H-1`), 3.94-3.39 (6H, m, Glc-H). HR-EI-MS: *m/z* 340.2115 (calcd. for $C_{15}H_{16}O_9$, 340.2094). Spectral data of (**9**) was close to similarities given for [14] also reported from the same genus.

Conclusion

Undoubtedly the current studies have shown the urease inhibition and antioxidant significance of *Launaea nudicaulis* along with previous records [4-6]

promotes that source of folkloric medicine. While the presence of identified one potent urease inhibitory compound along with eight newly described bioactive components are suggesting it to be investigated pharmacologically and chemically in details.

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References

1. S. I. Ali and M. Qaiser, "*Flora of Pakistan*", No. **207**, Department of Botany, University of Karachi and Missouri Botanical Press, Missouri, USA, Pp.1 (2002).
2. E. Nasir and S. I. Ali "*Flora of West Pakistan*", Fakhri Printing Press, Karachi, Pp 712 (1972).
3. Krishnamurthi, "The *Wealth of India*", Vol. **I**, Council of Scientific and Industrial Research, New Delhi, Pp.68 (1969).
4. S. R. Baquar, "*Medicinal and Poisonous Plants of Pakistan*", Printas Press, Karachi, Pp. 31 (1989).
5. V. K. Singh and A. M. Khan,"*Medicinal Plants and Folklores*", New Delhi, India, Pp. 46, 135, 147, 153 (1989).
6. M. M. Bhandari, "*Flora of Indian Deserts*", Mps Reports, Jodhpur, India. Pp.182 (1988).
7. F. Mansoor, V. Sultana and S. Ehtesham-ul-haque, *Pakistan Journal of Botany*, **39**, 2113 (2007).
8. S. Rashid, M. Ashraf, S. Bibi and R. Anjum, *Pakistan Journal of Biological Sciences*, **3**, 630 (2000).
9. S. Rashid, A. Mohammad, B. Shahida and A. Rubeen, *Pakistan Journal of Biological Sciences*, **3**, 808 (2000).
10. R. A. Khan, M. R. Khan, S. Sahreen, N. A. Shah, J. Bokhari, U. Rashid, S. Jan, *Journal of Medicinal Plants Research*, **6**, 1403 (2012).
11. M. M, Shabana, Y. W. Mirhom, A. A. Genenah, E. A. Aboutabl and H. A. Amer, *Archives for Experimentelle Veterinarmedizin*, **44**, 389 (1990).
12. M. Nivsarkar, M. Mukherjee, M. Patel and H. C. Bapu, *Indian Drugs*, **39**, 290 (2002).
13. M. Behari, R. Gupta and T. Matsumoto, *Indian Drugs*, **21**, 366 (1984).
14. A. E. Ashraf and N. M. A. Hamid, *Khimiya Rastitel'nogo Syr'ya*, **1**, 65 (2006).
15. D. R. Gupta and B. Ahmed, *Phytochemistry*, **24**, 873 (1985).
16. T. M. Sarg, A. A. Omar, M. A. Ateya and S. S. Hafiz, *Egyptian Journal of Pharmaceutical Sciences*, **25**, 35 (1986).
17. K. Bahadur and S. Sharma, *Proceedings of the National Academy of Sciences, India, Sect. A*, **44**, A, 264 (1974).
18. M. W. Weatherburn, *Analytical Chemistry*, **39**, 971 (1967).
19. T. Hotano, *Nature Medicine* (Tokyo), **49**, 357 (1995).
20. F. Liu and T. B. Ng, *Life Sciences*, **66**, 725 (2000).
21. F. Candan, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **18**, 59 (2003).
22. D. P. Scott and F. Irwin, *Archives of Biochemistry and Biophysics*, **228**, 155 (1988).
23. B. Halliweill, *American Journal of Medicine*, **91**(3), 14 (1991).
24. B. Donata, M. Michael, P. Werner, R. V. Petras and G. Dainora, *European Food Research Technology*, **214**, 143-147 (2002).
25. Z. Kexue, Z. Huiming and Q. Haifeng, *Process Biochemistry*, **41**, 1296 (2006).
26. V.U. Ahmed and Atta-ur-Rahman, "Handbook of Natural Products Data, Pentacyclic Triterpenoids", Vol. **2**, Pp. 686, The Netherlands, Elsevier Science B. V. (1994).
27. E. Wenkert. G. V. Baddeley, I. R. Burfitt and L. N. Moreno. *Organic Magnetic Resonance*, **11**, 337 (1978).
28. H. Budzikiewicz, J. M. Wilson and C. D. Jerassi, *Journal of American Chemical Society*, **85**, 3688 (1963).
29. M. Shamma, R. E. Glick and R. O. Mumma, *Journal of Organic Chemistry*, **27**, 4512 (1962).
30. A. A. Bernard and L. Tokes, *Journal of Organic Chemistry*, **42**, 725 (1977).
31. I. Rubinstein, L. J. Goad, A. D. H. Clague and L. J. Mulherim, *Phytochemistry*, **15**, 195 (1976).
32. H. L. Holland, P. R. P. Diakow and G. J. Taylor, *Canadian Journal of Chemistry*, **56**, 3121 (1978).
33. D. W. Aksnes, A. standnes and O. M. Anderson, *Magnetic Resonance in Chemistry*, **34**, 820 (1996).
34. P. W. Freeman, S. T. Murphy, J. E. Nemorin and W. C. Taylor, *Australian Journal of Chemistry*, **34**, 1779 (1981).
35. L.K. Omosa, J. O. Midiwo, S. Derese, A. Yenesew, M. G. Peter and M. Heydenreich *Phytochemistry Letter*, **3**, 217 (2010).