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Original Research Article

Isolation and phytochemical studies of sterols from seeds of *Moringa oleifera* Lam.

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Abstract

In the present work, petroleum ether extract from the seeds of *Moringa oleifera* were analyzed phytochemically for the presence of sterols. Phytochemical studies revealed the presence of sterols, by using chromatographic and spectroscopic techniques two sterols namely stigmasterol and β-sitosterol , were identified in the petroleum extract.

Key words: *Moringa oleifera*, petroleum extract, sterols, stigmasterol, β-sitosterol, chromatographic and spectroscopic techniques

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1.0 Introduction

Since ancient times, humans have dependent on plants because of their useful medicinal properties. Plants have been one of the major development sources of medicines. *Moringa oleifera* Lam. is a member of the Moringaceae family known as Sahjana,sainjna in Hindi, drumstick tree in English. For nearly 5000 years, it is an widely as integral part of food. It is reported to possess various medicinal values such as antipyretic[1], antiurolithiatic and diuretic [2], hepatoprotective [3], anaphylactic [4], anti-hhyperglycemic[5], anti-tumour[6], cardioprotective ,antithyroid [7] activities.

Phytosterols which are one of the important phytochemicals are also called plant sterols are a group of steroiod alcohols, which are present in number of plants. Phytosterols are taken as food additives to lower cholesterol.

By considering the pharmacological significance of phytochemicals and medicinal value of Moringa oleifera, in the present work an attempt has been done to detect their possible presence of sterols in the titled plant.

2.0 Materials and methods

The seeds of *Moringa oleifera* were collected from Agra. The seeds were air dried under shade for twelve days. The seeds were powdered and subjected to hot extraction with petroleum ether in a Soxhlet apparatus for 72 hours. The extract was filtered and then concentrated by distillation on vaccum.The extract was subjected to silica gel column chromatography using petroleum ether-EtOAc (10:1) and the elutes were subjected to various spectroscopic studies.

^{1}HNMR and ^{13}CNMR were recorded on a Bruker advance 400 MHz spectrometer. The EI- mass spectrum was recorded on Shimadju QP2000 mass spectrometer, UV-spectra was recorded on Shimadju UV-160 spectrophotometer

3.0 Results and discussion

In the present work an attempt has been made to isolate sterols from petroleum ether- EtoAc fractions of seeds of *Moringa oleifera*. The fractions were found to contain two compounds and these compounds were subjected to ^{1}HNMR, ^{13}CNMR and mass spectroscopy analysi. Compound-1 was obtained as white crystalline solid with melting point of 166-167^{o}C , while compound was a white powder with melting point of 136^{0}C. Data of spectroscopic studies of the separated compounds is as follows.

Compound-1: ^{1}H NMR(400 MHz, $CDCl_3$) δ (ppm) :3.51 (1H, *tdd*,H-3),5.36(1H,m, H-6), 4.98 (1H,m,H-20),5.12(1H,m,H-21) 0.71 (3H,s,Me-28), 1.03(3H, s, Me- 29), 0.80(3H ,d, J=6.6Hz,Me-27) 0.82 (3H,d, J=6.6Hz,Me-26), 0.91 (3H,d, J=6.2Hz, Me-19), 0.83 (3H,t, J=7.1Hz, Me24) ^{13}C NMR (DMSO,100MHz,): δ (ppm) = 37.5 (C-1),31.8 (C-2), 72.2 (C-3), 42.3 (C-4),140.9(C-5), 121.7 (C-6),31.8 (C-7), 31.8 (C-8), 50.1 (C-9), 36.5(C-10),21.2 (C-11), 39.8 (C-12),42.3(C-13), 56.9 (C-14),24.3(C-15),28.9(C-16),56.0(C-17),12.2(C-18),19.5(C-19),138.6(C-20),129.5(C-

21),46.2(C-22),25.4(C-23),12.2(C-24),29.4(C-25),20.3(C-26),19.7 (C-27),18.8(C-28),12.1(C-29)

Compound-2: ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 5.38 (1H,*dd*, J=5.2Hz,H-6),3.54(1H,t,J=4.08Hz,H-3),2.34(1H,*ddd*,J=13.0;5.0;2.0Hz,H-4a),0.69 (3H,s,Me-28), 1.01 (3H,s,Me-29), 0.80 (3H,d, J=6.4Hz, Me-27),0.82 (3H,d,J=6.4Hz,Me-26), 0.93 (3H,d, J=6.5Hz, Me-19), 0.84 (3H,t,J=7.1Hz,Me-24),^{13}CNMR ($CDCl_3$, 100MHz,): δ (ppm) =37.3 (C-1),31.6 (C-2), 71.6 (C-3), 42.4 (C-4),140.8(C-5), 121.7 (C-6),31.9 (C-7), 31.8 (C-8), 50.2 (C-9), 36.5(C-10),21.0 (C-11), 39.8 (C-12),42.2(C-13), 56.6(C-14),24.3 (C-15), 28.3(C-16),56.1(C-17),11.9(C-18), 19.5(C-19),36.0(C-20),18.8(C-21), 33.9(C-22),26.2(C-23),45.7(C-24),29.0(C-25),19.9(C-26),19.0 (C-27), 22.9(C-28),12.0(C-29)

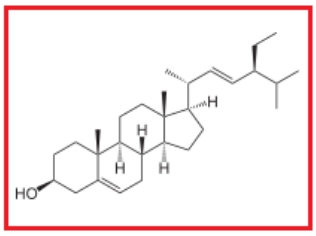

Figure-1: Stigmasterol (Compound-1)

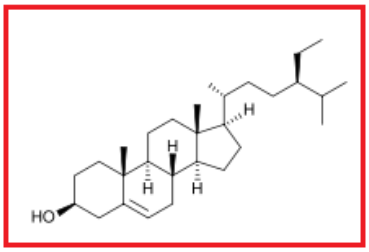

Figure-2: β-Sitosterol (Compound-2)

The EI-mass spectrum of compound-1 showed the molecular ion at m/z 412[M^{+}]. The UV maximum was appeared at 257nm. ^{1}H NMR spectra showed the presence of two methyl singlets at δ 0.71 and δ 1.03, three methyl doublets at δ 0.80, δ 0.82, δ 0.91 and methyl triplet at δ 0.83. Olefenic proton appeared at δ 4.98, δ 5.12 and δ 5.36. The proton corresponding to H-3 of a sterol moiety was appeared as triplet of doublet of doublet at δ 3.51. ^{13}CNMR showed 29 carbon signals including six methyl, nine methylenes, 11methane and three quarternary carbons.The alkene carbons appeared at C-5 and C-6 and at C-20 and C-21.

The EI-mass spectrum of compound-2 showed the molecular ion at m/z 414[M^{+}]. The UV maximum was appeared at 203nm. In the ^{1}HNMRspectra of compound 2, one proton signal appeared at δ5.38, corresponding to H-6 olefenic proton and H-3 proton appeared at 3.54. Singlets at δ 0.69 and δ 1.01, three methyl doublets at δ0.80, δ0.82, δ0.93 and methyl triplet at 0.84. ^{13}CNMR showed 29 carbon signals including six methyl groups. The alkenes carbon appeared at C-5 and C-6. All other signals are corresponding to sterol nucleus.

There are previous reports of presence of sterols in *Moringa oleifera.* To further confirm the presence of sterols, the spectroscopic data of compounds-1 and 2 were compared with Stigmosterol and β-Sitosterol. The outcome indicate that the isolated compounds duing the study were

found to be Stigmasterol and Sitosterol. This is in accordance with earlier reports in which sterols were reported in many plants [8,9]. The physical and spectral data showed complete agreement with the literature [10,11]..

4.0 Conclusion

Medicinal plants are a valuable gift of nature to the suffering of humanity. The usefulness of medicinal plants for various diseases can be found even in ancient literature. With the advancement science, it was confirmed that the medicinal properties of the plants was due to the presence of various classes of phytochemicals. *Moringa oleifera* is reported to possess wide range of medicinal properties. There are many reports in which many Phytochemicals are isolated from various parts of Moringa oleifera. In the present work also an attempt has been done to isolate phytochemicals like sterols from the seeds of the titled plant. Fractionation of petroleum ether extracts yielded two compounds. Data from physical, chemical and spectral characteristics, the isolated compounds were concluded as stigmasterol (Figure-1) and β-Sitosterol(Figure-2). Stigmasterol possess, antioxidant, hypoglycemic and thyroid inhibiting properties [12], it is also effective against cardiovascular disease[13] and also used as anti-inflammatory and analgesic agents[14]. β-sitosterol is reported to possess antioxidant and anti- diabetic properties. Human liver microsome studies revealed that β- sitosterol inhibits the cholesterol absorption, reduces the symptoms of benign prostatic hyperplasia [15], and also possess anti-inflammatory and anti-pyretic activities. The isolation of Stigmasterol and β- sitosterol from *Moringa oleifera* indicates that the possible reason behind the medicinal properties of plant. However, further scientific evaluation is required to establish therapeutic efficacy of isolated compounds.

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