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A Triterpene and A Phytosterol from *Rhynchosia Minima*

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ABSTRACT

The Chemical examination of the n-hexane extract of *Rhynchosia minima* results the separation of two compounds. To separate these compounds, the dried powder of *Rhynchosia minima* was succumbed to hot extraction with n-hexane and submitted to chromatography.

Mass spectra for the separated compounds appeared their molecular formula which was $C_{30}H_{50}O$ for compound 1 and $C_{29}H_{48}O$ for compound 2. In the ^{1}H-NMR range of compound 1, H-3 proton revealed as a triplet of a double doublet (tdd) at δ 3.13, H-29 protons give two multiplets at *δ* 4.50 and δ 4.62 and seven methyl protons showed up at *δ* 27.46, 15.34, 16.09, 15.99, 14.55, 18 and 19.31. In the ^{1}H-NMR range of compound 2, H-3 proton revealed as a triplet of a double doublet (tdd) at *δ* 3.45, olefinic proton H-6 revealed as a multiplet at δ 5.28 and six methyl protons showed up at δ 12.18, 19.3, 18.97, 21.02, 21.09 and 12.04. From the physical, chemical and spectral characteristics, compound 1 and 2 were distinguished as Lupeol and Stigmasterol.

Keywords: *Rhynchosia minima*, triterpene, sterol, Structure elucidation.

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I. Introduction

The legume family (Leguminosae) is one of the foremost effective ancestries of blooming plants. With ca. 751 genera and ca. 19,500 species [1]. It is considered the third-largest angiosperm family. It encompasses a worldwide dissemination traversing all major biomes and shaping biologically imperative constituents of temperature, Mediterranean, tropical, arid, seasonally dry, rain, woodland, and savanna ecosystems [2]. The legume speaks to one of the foremost marvelous illustrations of control and utilization of a plant family by human societies around the world. This has included the domestication of a set of globally important food crops, such as soybean, culinary beans, groundnut, lentil, chickpea and pea, as well as imperative temperature and tropical scavenge crops such as alfalfa, clovers and leucaena.

The Leguminosae is ordinarily partitioned into three subfamilies, but there are those who proceed to recognize three partitioned families. The subfamilies are typically characterized as being simple to recognize based on morphological characters, but the Caesalpinioideae isn't a monophyletic group. With the legume family being so imperative environmentally and financially, it has been the extraordinary center of taxonomists since the time of Candolle [3]. The economically and ecologically important family Leguminosae [1] or Fabaceae has been the center of various later phylogenetic examinations at the subfamily, tribe and generic-group level [4]. These, as well aphylogenies of the family demonstrate that the currently accepted classification of the family into the three well-known, long-recognized and broadly acknowledged subfamilies, Caesalpinioideae DC., Mimosoideae DC., and PapilionoideaeDC is obsolete and does not reflect our current information of phylogenetic connections within the family, with near to 770 genera and over 19,500 species. [1], [4].

The Leguminosae is the third biggest angiosperm family after Asteraceae and Orchidaceae. Financially, Leguminosae is second in importance to Poaceae. It is assessed, for illustration, that add up to world sends out of beats (i.e., legume crops were collected for their dry seeds) have more than multiplied between1990 and 2012, was extended from 6.6 to 13.4 million tons, and in 2012 the esteem of beat trades was assessed at US$ 9.5 billion (Food and Agriculture Organization [FAO].

Among the plants of Fabaceae family, which is utilized in conventional medication, *Rhynchosia* species which have possessed a conspicuous part. *Rhynchosia* genus comprises of around 300 species circulated all through the tropical and subtropical zones around the world, out of which twenty-two species happen in India [5]-[6].

The species having a place to the genus *Rhynchosia* (Fabaceae) are herbs, twining or erect bushes. Past phytochemical examinations on a few species of Rhynchosia appeared that the genus is select to abundant generation of C-glycosyl flavonoids [7]. A few of the

separated compounds of *Rhynchosia* genus and their plant extract show curiously biological activities, counting antioxidant, anti-inflammatory, antimycobacterial and antiproliferative [8]-[10]. Therefore, the improvement of drug agents from common root is of extraordinary significance, based on the conventional and pharmacological uses of the genus *Rhynchosia*.

A. Rhynchosia minima

Rhynchosia minima DC (Fabaceae) commonly known asTurvel, is a twining or a trailing yearly dispersed all through India, Sri Lanka, and United States [11]. The plant is detailed as poisonous to fish and utilized by fisherman. The seeds are bitter and noxious and seed extract shows particular agglutinating activity on human RBC [12].

In the folkloric system of medicine leaves ofthe plant are reported as abortifacient. Decoction prepared from leaves is used by tribal of North Maharashtra region as an abortifacient [13]. The tribals of Sikkim are using leaves of the plant in the treatment of wounds, helminthic infections and as an abortifacient [14]. The phytochemical studies on theplant revealed the presence of steroidal glycoside, ergosterolperoxide, stigmasterol and lupeol [15]. It was also reported to contain C-glycosylflavones, sitostrol, gallic acid, protocatechuic acid [16] and hydroquinone diacetate [17].

The plant has tremendous potential to be used in forage, pharmaceutical and other agricultural products, and more importantly, there are no known major threats to this species; however, the plant has negligible uses worldwide, including in Pakistan. Traditionally, *R. minima* is applied to alleviate boils, colds, respiratory infections, diarrhea, dysentery and joint pains, and has been used for causing abortion and as an ecbolic and for general healing. It is also used as a food (in sweets), and its seeds are used as repellents and have antimicrobial potential. Furthermore, a number of important compounds that could fight cancer and may reduce carcinogenesis have been isolated from the seeds [18].

II. Materials and Methods

A. General experimental procedure

NMR spectra were recorded on spectrometer in $CDCl_3$ at 500 MHz for ^{1}HNMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was carried outby utilizing silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic supported plates coated with silica gel and the plates were visualized by using vanillin/H_2SO_4 as a sprayer arrangement taken after by warming.

B. Plant material and collection

Aerial parts develop *Rhynchosia minima* plants were collected from the desert region encompassing the Aswan university campus at Sahari City (Aswan) in 2016. The species was distinguished as R. minima by M. El-Sayed and was at that point air- dried in a cool shady place. A voucher specimen has been deposited at the Herbarium of the Workforce of Science, Aswan, Egypt.

C. Plant Materials extraction

The aerial parts of R. minima were converted to powder. The material (600 g) was exchanged to a container and soaked in 2.5 L analytical graded n-hexane for 72 hours, then evaporate the eluate to dryness on a water bath to obtain crude extract.

D. Separation of compound from n-Hexane Extract

Column chromatography of *n*-hexane extract (1.61 g) was loaded utilizing silica gel which was packed utilizing wet packing method in *n*-hexane. The column was eluted utilizing solvent systems with increasing polarity starting with *n*-hexane (100%) then *n*-hexane: methylene chloride in shifting proportions by utilizing gradient elution method and the fractions were obtained and concentrated. Two compounds were obtained from the fraction hexane: methylene chloride (6:4).

Lupeol (1)

Brown powder, **^{1}H NMR** (500 MHz, $CDCl_3$): δ_H 0.90 (s, H_3-23), 0.69 (s, H_3-24), 0.76 (s, H_3-25), 0.97 (s, H_3-26), 0.88 (s, H_3-27), 0.72 (s, H_3-28), 1.61 (s, H_3-30), 3.13 (1H, dd, H-3), 4.62 (1H, s, H-29a), 4.50 (1H, s, H-29b).

^{13}C NMR ($CDCl_3$): δ_C 37.46 (CH_2, C-1), 29.1 (CH_2, C-2), 79.02 (CH, C-3), 38.75 (C, C-4), 55.36 (CH, C-5), 18.33 (CH_2, C-6), 34.33 (CH_2, C-7), 40.88 (C, C-8), 50.50 (CH, C-9), 37.21 (C, C-10), 20.96 (CH_2, C-11), 25.21 (CH_2, C-12), 38.11 (CH, C-13), 42.86 (C, C-14), 27.98 (CH_2, C-15), 35.61 (CH_2, C-16), 43.01 (C, C-17), 48.36 (CH, C-18), 47.99 (CH, C-19), 150.93 (C, C-20), 29.89 (CH_2, C-21), 40.01 (CH_2, C-22), 27.46 (CH_3, C-23), 15.34 (CH_3, C-24), 16.09 (CH_3, C-25), 15.99 (CH_3, C-26), 14.55 (CH_3, C-27), 18 (CH_3, C-28), 109.29 (CH_2, C-29), 19.31 (CH_3, C-30).

Stigmasterol (2)

White powder, **^{1}H NMR** (500 MHz, $CDCl_3$): δ_H 0.63 (s, CH_3-18), 0.94 (s, CH_3-19), 0.90 (s, CH_3-21), 0.72 (s, CH_3-26), 0.76 (s, CH_3-27), 0.78 (s, CH_3-29), 3.45 (m, CH-3), 5.28 (dd, *J*= 1.8, 4.9 Hz, CH-6), 4.99 (dd, *J*= 8.5, 15.0 Hz, CH-22), 5.12 (dd, *J*=8.4, 15.0 Hz, CH-23).

^{13}C NMR ($CDCl_3$): δ_C 29.66 (CH_2, C-1), 36.53 (CH_2, C-2), 71.81 (CH, C-3), 40.41 (CH_2, C-4), 140.79 (C, C-5), 121.68 (CH, C-6), 29.32 (CH_2, C-7), 31.7(CH, C-8), 50.2 (CH, C-9), 37.29 (C, C-10), 21.19 (CH_2, C11), 39.71 (CH_2, C-12), 42.34 (C, C-13), 56.80 (CH, C-14), 28.86 (CH_2, C-15), 25.36 (CH_2, C-16), 56.03 (CH, C-17), 12.18 (CH_3, C-18), 19.37 (CH_3, C-19), 42.24 (CH, C-20), 18.97 (CH_3, C-21), 129.35 (CH, C-22), 138.27 (CH, C-23), 51.24 (CH, C-24), 31.9 (CH, C-25), 21.02 (CH_3,C-26), 21.09 (CH_3, C-27), 24.35 (CH_2, C-28), 12.04 (CH_3, C-29).

III. Results and Discussion

*A. Compound **1***

Was obtained as a brown powder, its molecular formula was assigned as $C_{30}H_{50}O$ (Fig. 1).

^{1}H-NMR spectrum in $CDCl_3$ of compound 1, showed two doublets signals at δ_H 4.62 and 4.5 for the exomethylene protons H-29a and H-29b respectively and a triplet signal at δ_H 3.13 for the hydroxylated proton, H-3. Also, it indicated the presence of seven singlet signals characteristic for the seven methyl groups. Moreover, it

showed several multiplet signals for the other remaining protons.

^{13}C-NMR spectrum in $CDCl_3$ of compound **1** suggested the presence of thirty carbons, which were classified by DEPT as follows:

Seven methyl carbon signals at [δc 27.46 (C-23), 15.34 (C-24), 16.09 (C-25), 15.99 (C-26), 14.55 (C-27), 18 (C-28), and 19.31 (C-30)], eleven methylene carbon signals at [δc 37.46 (C-1), 29.1 (C-2), 18.33 (C-6), 34.33 (C-7), 20.96 (C-11), 25.21 (C-12), 27.98 (C-15), 35.61(C-16), 29.89 (C-21), 40.01 (C-22), 109.29 (C-29)], six methine carbon signals at [δc 79.02 (C-3), 55.36 (C-5), 50.50 (C-9), 38.11 (C-13), 48.36 (C-18), 47.99 (C-19)], and six quaternary carbon signals at [δc 38.75(C-4), 40.88 (C-8), 37.21 (C-10), 42.86 (C-14), 43.01 (C-17), 150.93 (C-20)].

Confirmation of compound **1** was given by the HMBC analysis, the most imported correlations were observed between: H-24 (δ_H 0.69, s) and C-23(δ_C 27.46), C-4 (δ_C 38.75), C-5 (δ_C 55.36), and C-3 (δ_C 79.02); H-28 (δ_H 0.72, s) and C-16 (δ_C 35.61), C-22 (δ_C 40.01), C-17 (δ_C 43.01) and C-18(δ_C 48.36); H-25 (δ_H 0.76, s) and C-4 (δ_C 38.75), C-9 (δ_C 50.5), C-5 (δ_C55.36); H-27 (δ_H 0.88,s) and C-15 (δ_C 27.98); H-23 (δ_H 0.90,s) and C-5 (δ_C 55.36), C-24 (δ_C 15.34), C-3 (δ_C 79.02); H-26 (δ_H 0.97, s) and C-7 (δ_C 34.3), C-9 (δ_C 50.5); H-9 (δ_H 1.26, t) and C-7 (δ_C 34.3); H-30 (δ_H 1.61, s) and C-19 (δ_C 47.99), C-20 (δ_C 150.93).

*B. Compound **2***

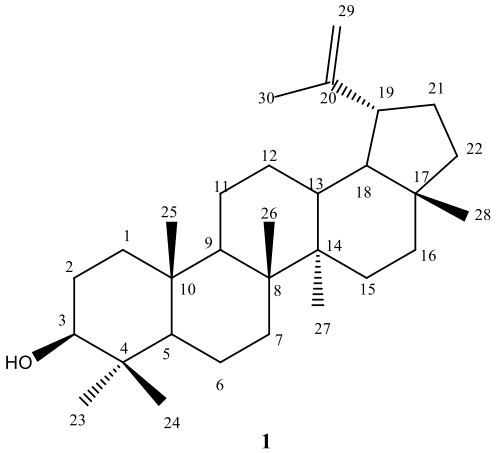
Was obtained as a white powder, its molecular formula was assigned as $C_{29}H_{48}O$ Fig. 1.

^{1}H-NMR spectrum in $CDCl_3$ of compound **2**, showed two methyl singlets at δ_H 0.63 and 0.94 for the protons H-18 and H-19 respectively, and three methyl doublets that appeared at δ_H 0.72, 0.76 and 0.90 for the protons H-26, H-27 and H-21 respectively, and one methyl triplet at δ_H 0.78 for the proton H-29. Also, I it showed protons at δ_H 4.99, 5.12 and 5.28 for the proton H-22, H-23 and H-6 respectively.

Compound **2** indicated the presence of six singlet signals characteristic for the six methyl groups. Moreover, it showed several multiplet signals for the other remaining protons.

^{13}C-NMR spectrum in $CDCl_3$ of compound **2** suggested the presence of twenty nine carbons, which were classified as follows: six methyl carbon signals at [δc 12.18 (C-18),19.3(C-19), 18.97 (C-21), 21.02 (C-26), 21.09 (C-27) and 12.04 (C-29]. nine methylene carbon signals at [δc 29.66 (C-1), 36.53 (C-2), 40.41 (C-4), 29.32 (C-7), 21.19 (C-11), 39.71 (C-12), 28.86 (C-15), 25.36(C-16) and 24.35 (C-28], eleven methine carbon signals at [δc 71.81 (C-3), 121.68 (C-6), 31.7 (C-8), 50.2 (C-9), 56.80 (C-14), 56.03(C-17), 42.24 (C-20), 129.35 (C-22), 138.27 (C-23), 51.24 (C-24) and 31.9 (C-25)], and three quaternary carbon signals at [δc 140.79 (C-5), 37.29 (C-10) and 42.34 (C-13)].

Confirmation of compound **2** was given by the HMBC analysis, the most imported correlations were observed between: H-18 (δ_H 0.63, s) and C-20 (δ_C 42.2), C-13 (δ_C 42.34), C-14 (δ_C 56.80), H-26 (δ_H 0.72, s) and C-25 (δ_C 31.91), H-27 (δ_H o.76, s) and C-24 (δ_C 51.24), C-25 (δ_C 31.9), H-19 (δ_H0.94, s) and C-9 (δ_C 50.2), C-2 (δ_C 36.53), C-5 (δ_C 140.79).



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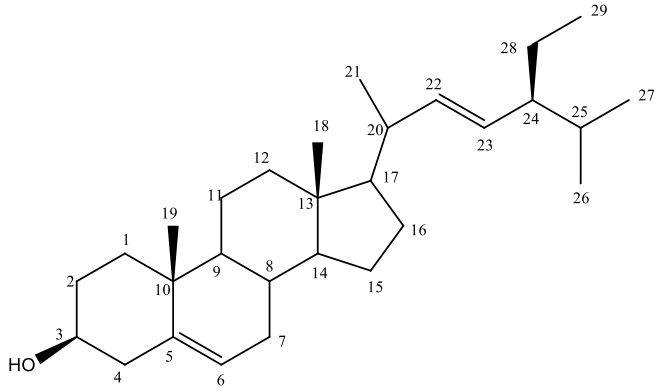

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Fig. 1. Structures of Lupeol (1) and Stigmasterol (2).

IV. Conclusion

From the above physical, chemical and spectral characteristics, two compounds were separated from *n*-hexane extract of *Rhynchosia minima*. The structures of the isolated compounds were distinguished as Lupeol (1) and Stigmasterol (2). The complete ^{1}H and ^{13}C NMR spectral assignments of two separated compounds were made based on COSY, HSQC, HMBC and MS spectroscopic data.

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