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ORIGINAL ARTICLE

The Potential Aphrodisiac Effect of *Ferula drudeana* Korovin Extracts and Isolated Sesquiterpene Coumarins in Male Rats

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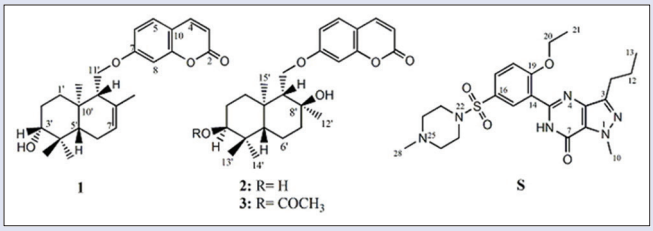
ABSTRACT

Background: Many members of the genus *Ferula* are used in traditional medicine as aphrodisiac. **Objectives:** The aim of this study is to confirm the aphrodisiac potential of *Ferula drudeana* Korovin as listed in the Turkish traditional medicine and to isolate the active metabolites using male rats. **Materials and Methods:** The $CHCl_3$ soluble fraction showed promising activity. Chromatographic purification resulted in the isolation of three sesquiterpene coumarins. Isolated compounds structures were determined as feselol (1), samarcandin (2), and 3′-O-acetyl samarcandin (3) based on the physical and spectral characters. Single doses of *F. drudeana* $CHCl_3$ soluble fraction, aqueous fraction (200 mg/kg BW), 1 and 2 (10 mg/kg BW), and sildenafil citrate (10 mg/kg BW) were orally administered to male Wistar albino rats by gavages. Mount latency, mount frequency (MF), intromission latency, intromission frequency (IF), ejaculation latency, and postejaculatory interval (PEI) were studied. In addition, copulatory efficiency and intercopulatory efficiency were calculated. **Results:** Oral administration of *F. drudeana* roots extracts, 1 and 2 significantly increased MF and IF. The latencies of mount and intromission were reduced significantly and ELs were prolonged. Treatment with the extracts, 1 and 2 resulted in the reduction of the PEI. The highest aphrodisiac activity in male rats was exhibited by 2. **Conclusion:** The present findings provide experimental evidence that *F. drudeana* roots, 1 and 2 possess aphrodisiac activities by enhancing the sexual behavior of male rats. The obtained results supported the traditional claims about the use of *Ferula* species for male sexual dysfunction.

Key words: Aphrodisiac, *Ferula drudeana*, intromission latency, mount frequency, sesquiterpene coumarins

SUMMARY

- Biologically guided purification of *Ferula drudeana* resulted in the identification of feselol, samarcandin, and 3′-O-acetyl samarcandin
- The extract, fractions, and isolated compounds were tested for their aphrodisiac activity
- Samarcandin showed stronger aphrodisiac effect than feselol.



Abbreviations used: BW: Body weight; ML: Mount latency; MF: Mount frequency; IL: Intromission latency; IF: Intromission frequency; EL: Ejaculation latency; PEI: Postejaculatory interval; CE: Copulatory efficiency; ICE: Intercopulatory Efficiency; SC: Sildenafil citrate; g: Gram; mg: Milligram; m.p.: Melting point; id: Internal diameter; TLC: Thin layer chromatography; ppm: Part per million; UV: Ultraviolet; EIMS: Electron impact mass spectroscopy; GC: Gas chromatography; H: Protons; ^{13}C: Carbon 13; NMR: Nuclear magnetic resonance; DEPT: Distortionless enhancement by polarization transfer; COSY: Correlated spectroscopy; HSQC: Heteronuclear single quantum correlation; HMBC: Heteronuclear multiple bond correlation; NOESY: Nuclear overhauser effect spectroscopy.

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INTRODUCTION

Sexual relationship is recognized worldwide among the most important components of a normal, healthy lifestyle, and general well-being. Erectile dysfunction beside other sexual dysfunction represent serious public health problem as indicated from epidemiological data. Nowadays, male sexual dysfunction is known to affect the sexual life of millions of men globally.[1] This fact led to the development of some available aphrodisiacs. These drugs can excite libido or arouse sexual instinct. According to their mode of action, aphrodisiacs are classified into three groups as follows: drugs increasing libido (i.e., sexual desire), drugs increasing potency (i.e., effectiveness of erection), and drugs increasing sexual pleasure.[2] The available treatment options include phosphodiesterase inhibitors such as sildenafil, tadalafil or vardenafil, α2-adrenergic antagonists such as yohimbine and apomorphine dopamine-receptor agonists.[1] If these oral therapies are ineffective, intracavernous and intraurethral injections or penile prostheses are indicated. However, these alternatives beside their high cost suffer from serious side effects. Medicinal plants continue to provide valuable therapeutic agents, both in the modern and in traditional medicine.[3] Several medicinal plants have been reported in a number of studies to enhance sexual functions in experimental animals.[4]

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Genus *Ferula* L. is one of the largest genera of the family Apiaceae includes many popular species in traditional medicine and proved to be promising source of biologically active entities. The genus is distributed from the Canary Islands through the Mediterranean region, Middle East, Central Asia, Western China to east, and northern India.[5,6] The genus *Ferula*, the third-largest genus in the family, is composed of about 180 species, nine of which are endemic to Turkey.[7] The genus is recognized as one of the most important genera in Turkey where it is represented by 25 taxa[8] locally known as "Çakşır," "Çakşır otu," or "Çaşır."[9]

Many biological activities were reported for the extracts or the essential oils of the genus, including anticancer, anthelmintic, anticonvulsant, aphicidal, antioxidant, anti-spasmodic, anti-nociceptive, antidepressant, antiprotozoal, antiulcer, antispasmodic, and antinociceptive activities.[10] In traditional medicine, the oleo-gum resin of the most important species of the genus *F. assa-foetida* L. is reported to have aphrodisiac activity.[10] In Brazilian traditional medicine, the hot water extract prepared from the dried leaves and stems is used orally to treat erectile dysfunction and as an aphrodisiac.[11] In USA, extract of the resin is taken orally have been used as aphrodisiac.[12] Different parts of *Ferula gummosa* are also used as aphrodisiac.[13-16] Shirsh-el-Zallouh, the local name of *Ferula hermonis* has been long utilized for the treatment of impotence and frigidity.[17-19] According to ethnobotanical studies, some plants of *Ferula* species are listed in Turkish traditional medicine as aphrodisiacs.[20] The roots of many *Ferula* species are used as aphrodisiac in Eastern Turkey.[8]

Phytochemical studies of the genus *Ferula* resulted in the identification of bioactive sulfur compounds, triterpenes, sesquiterpene esters, sesquiterpene lactones, isocarotane esters, daucane esters, and sesquiterpene coumarins.[10]

Essential oil, extracts of the fruits and roots of *Ferula drudeana* as well as Samarcandin and conferol were tested for antimicrobial and anti-oxidant activities.[21] We previously reported on the anti-oxidant and antihyperglycemic effects of the plant root extract.[22]

As the root parts of *Ferula* species are used in Turkish traditional medicine as aphrodisiacs, therefore, the present work was undertaken to confirm the possible aphrodisiac effect of *F. drudeana* extract and its major components in male rats.

MATERIALS AND METHODS

General

Melting points measurements were performed on open capillary tubes Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus and were uncorrected. Ultraviolet (UV) absorption was measured by a Unicum Helios UV-Visible spectrophotometer. Optical rotations of the isolated were recorded using Jasco P-2000 Polarimeter. One-dimensional and two-dimensional-nuclear magnetic resonance (NMR) experiments were measured on UltraShield Plus 500 MHz (Bruker) (NMR Unite at the College of Pharmacy, Prince Sattam Bin Abdulaziz University) spectrometer operating at 500 MHz for ^{1}H and 125 MHz for Carbon 13 (^{13}C) atoms, respectively. The chemical shift values are presented as δ (ppm) relative to the solvent peak resulted from the residual undeuterated atoms. The coupling constants (J) of ^{1}H are reported in Hertz (Hz). Electron impact mass spectroscopy using Shimadzu-gas chromatography/mass spectrometry were recorded for the isolate compounds. Silica gel 60/230–400 mesh (EM Science) and silica gel 60 F_{254} (Merck) were used for column chromatography and thin-layer chromatography, respectively.

Plant material

The roots of *F. drudeana* Korovin were collected from Kayseri, Yahyali, Çamlica village, 1529 m during the Summer of 2013. Taxonomic identification was determined by Dr. Sura Baykan. A voucher specimen (#5520) was deposited at the Herbarium of Faculty of Pharmacy (IZEF), Ege University, Izmir, Turkey.

Extraction and purification

The dried powdered plant material (1400 g) was extracted by percolation with methanol till exhaustion. The methanol extract was concentrated under reduced pressure to give 230.2 g of the total methanol extract. The total extract was fractionated by liquid-liquid extraction between aqueous methanol (2:1) and $CHCl_3$ to yield 156.05 g $CHCl_3$ soluble fraction and 74.15 g aqueous fraction.

Part of the $CHCl_3$ fraction (10 g) were chromatographed over silica gel column chromatography (250 g, 5 cm id) eluted with light petroleum followed by light petroleum, ethyl acetate mixtures with gradual increasing the percentage of ethyl acetate. Fractions eluted with 15% ethyl acetate in light petroleum (1.2 g) were subjected to another silica gel column chromatography (50 g, 2 cm id) eluting with $CHCl_3$ to afford 225 mg of 1.

Fractions eluted with 20% ethyl acetate in light petroleum (2.3 g) were further purified on silica gel column chromatography (150 g, 3 cm id) using $CHCl_3$, followed by $CHCl_3$, methanol in a gradient system. Fractions eluted with 0.5% methanol (715 mg) were subjected to RP18 silica gel column (30 g, 2 cm id) using 30% water in methanol as eluting system to afford 326 mg of 2 and 15 mg of 3.

Isolated compounds identification

Feselol (1)

$C_{24}H_{30}O_4$: White amorphous solid, melting point (m.p.) 115°C–116°C, $[\alpha]_D$ = −70 (c = 0.08, MeOH), UV λ_{max} (MeOH) 246, 293, 324 nm. ^{1}H and ^{13}C NMR (C_6D_6) [Tables 1 and 2]. ^{1}H NMR (CD_3OD): δ_H 7.52 (d, *J* = 9.4 Hz, H-4), 7.24 (d, *J* = 8.6 Hz, H-5), 6.70 (overlapped, H-6), 6.68 (bs, H-8), 6.12 (d, *J* = 9.4 Hz, H-3), 2.08 (t, *J* = 3.5 Hz, H-1'), 1.36 (dd, *J* = 10, 8.5 Hz, H-1'), 3.22 (dd, *J* = 11.0, 4.5 Hz, H-3'), 5.54 (d, *J* = 1.4 Hz, H-7'). EI-MS (%) *m/z*: 382 (M+, 23).

Samarcandin (2)

$C_{24}H_{32}O_5$: White crystalline solid, m.p. 176°C–177°C, $[\alpha]_D$ = 43 (c = 0.06, MeOH), UV λ_{max} (MeOH) 244, 291, 325 nm. ^{1}H and ^{13}C NMR (C_6D_6) [Tables 1 and 2]. EI-MS (%) *m/z*: 400 (7), 383 (M^+-OH, 100).

Acetlyation of (2)

About 10 mg of 2 were dissolved in 0.5 ml of pyridine then few drops of acetic anhydride were added. The mixture allowed to react for 24 h in dark and then dried under nitrogen. The left solid was chromatographically homogeneous and resulted in formation of 3.

3'-O-Acetyl Samarcandin (3)

$C_{26}H_{34}O_6$: White crystalline solid, m.p. 131°C–132°C, $[\alpha]_D$ = 34 (c = 0.05, MeOH), UV λ_{max} (MeOH) 246, 292, 327 nm. ^{1}H and ^{13}C NMR (CD_3OD) [Tables 1 and 2]. EI-MS (%) *m/z*: 442 (11), 425 (M^+-OH, 35).

Sexual behavior testing protocol

Animals

Sexually active Wistar strain male (220–250 g) and female (150–160 g) albino rats were used for the present study. Rats were obtained from the Lab Animal Care Unit at the College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA. Each animal was kept in separate cages under a reversed 12 h light/dark cycle, controlled temperature (21°C ± 1°C), and relative humidity (55 ± 10%). Standard rodent food and tap water were available *ad libitum*.

Table 1: ^{1}H nuclear magnetic resonance data (δ ppm, J in parenthese in Hz) for compounds 1–3*

Pos	1	2	3**
3	5.91 (d, *J*=9.5 Hz)	6.22 (d, *J*=9.3 Hz)	6.21 (d, *J*=9.4 Hz)
4	6.64 (d, *J*=9 Hz)	7.93 (d, *J*=9.3 Hz)	7.94 (d, *J*=9.4 Hz)
5	6.64 (d, *J*=9 Hz)	7.49 (d, *J*=8.3 Hz)	7.48 (d, *J*=8.3 Hz)
6	6.54 (dd, *J*=8.5, 2.1 Hz)	6.92 (dd, *J*=8.3, 2.1 Hz)	6.92 (dd, *J*=8.3, 2.0 Hz)
8	6.57 (d, *J*=2.1 Hz)	6.96 (d, *J*=2.1 Hz)	6.96 (d, *J*=2.0 Hz)
1’	1.61 (dt, *J*=13.2, 3.3 Hz) α, 0.94 (m) β	1.79 (overlapped) α, 1.36 (m) β	1.77 (overlapped), 1.34 (m)
2’	1.48 (m) α, 1.44 (m) β	1.79 (overlapped) α, 1.57 (overlapped) β	1.77 (overlapped), 1.56 (overlapped)
3’	3.01 (dd, *J*=11.3, 4.4 Hz) β	3.32 (bs) α	4.62 s
5’	1.06 (t, *J*=8.3 Hz) β	1.57 (overlapped) β	1.56 (overlapped)
6’	1.88 m	1.46 (d, *J*=13.4 Hz) β, 1.15 (overlapped) α	1.47 (d, *J*=13.5 Hz), 1.12 (overlapped)
7’	5.47 (bs)	1.86 (bs) α, 1.64 (dd, *J*=13.1, 3.2 Hz) β	1.85 bs, 1.62 (dd, *J*=13.2, 3.4 Hz)
9’	1.96 (bs) β	1.90 (dd, *J*=6.5, 4.3 Hz) β	1.89 (dd, *J*=6.3, 4.1 Hz)
11’	3.73 (dd, *J*=9.7, 3.1 Hz) β	4.40 (dd, *J*=9.8, 1.6 Hz) β	4.42 (dd, *J*=9.6, 1.8 Hz) β
	3.61 (dd, *J*=9.7, 5.8 Hz) α	3.98 (dd, *J*=9.8, 6.5 Hz) α	3.98 (dd, *J*=9.6, 6.3 Hz) α
12’	1.69 s	1.14 s	1.17 s
13’	0.97 s	0.99 s	0.98 s
14’	0.87 s	0.72 s	0.73 s
15’	0.77 s	0.77 s	0.78 s

*Assignments based on COSY, HSQC, HMBC, NOESY and comparison with literature; **$COCH_3$: 2.09 s. COSY: Correlated spectroscopy; HSQC: Heteronuclear single quantum correlation; HMBC: Heteronuclear multiple bond correlation; NOESY: Nuclear overhauser effect spectroscopy

Table 2: ^{13}C nuclear magnetic resonance data (δ ppm) for compounds 1-3*

Pos	1	2	3**
2	159.92	161.30	161.40
3	113.08	112.30	111.99
4	142.31	143.31	143.51
5	128.40	128.55	128.37
6	112.84	113.11	113.15
7	161.67	162.20	162.38
8	100.78	101.39	100.99
9	156.29	155.94	155.89
10	112.30	112.18	112.43
1’	37.49	32.74	32.69
2’	27.42	25.17	22.24
3’	78.13	75.23	78.26
4’	38.53	37.21	37.29
5’	49.25	48.24	48.46
6’	23.24	19.98	19.77
7’	123.67	44.05	43.97
8’	132.12	71.97	71.83
9’	53.71	59.57	59.48
10’	35.58	37.84	37.77
11’	66.56	66.23	66.46
12’	21.46	24.15	23.98
13’	27.94	28.39	28.14
14’	15.21	21.94	21.88
15’	14.59	15.72	15.29

*Assignments based on COSY, HSQC, HMBC, NOESY and comparison with literature; **$COCH_3$: 177.24, 19.97. COSY: Correlated spectroscopy; HSQC: Heteronuclear single quantum correlation; HMBC: Heteronuclear multiple bond correlation; NOESY: Nuclear overhauser effect spectroscopy

Chemicals and drugs

Sildenafil citrate (SC) (Viagra) (Pfizer Inc., USA), hydroxyprogesterone (Bayer Pharma AG, Germany), and estradiol benzoate (Misr Co., for Pharm. Ind., Egypt) were used. Extracts and pure materials were prepared with 2% of Tween 80 (Sigma-Aldrich, USA) in water.

Preparation of male rats

Male rats were trained by pairing with mature females, three times for 4 days. Males, which did not demonstrate any sexual attention during the experimental period was considered as a sluggish male and dismissed from the experiment.

Preparation of female rats

Female rats were prepared following the reported method by Estrada-Reyes *et al.*[23] In brief, female receptivity was induced by the subcutaneous injection of estradiol benzoate (8 mg/rat), followed by hydroxyprogesterone (2 mg/rat), 48 h later. The sexual activity of the female rats was confirmed before the experiment by pairing them with males, other than the normal control, reference and experimental male rats. The most sexually active female rats were selected for the experiment.

Experimental procedure

The experiment was conducted 4 h after hydroxyprogesterone administration in a calm laboratory under faint red light in transparent cages of 50 cm × 30 cm × 30 cm dimensions as described by Al-Shdefat *et al.*[24] Eight groups of sexually experienced male rats that were showing reactive sexual activity ($n = 6$) were selected for the experiment and kept singly in separate cages. Group I served as a normal control and were given the vehicle 2%v/v Tween 20. Group II (Reference): received SC at a dose of 10 mg/kg. The III–VI groups were treated with 200 mg/kg of $CHCl_3$ soluble fraction and the aqueous fraction, respectively. In addition, rats of the VII and VIII groups were treated with 10 mg/kg of 2 and 1, respectively. The vehicle, SC, extracts, and sesquiterpene coumarins were administered orally as single doses through an orogastric tube.

Sexual behavior analysis

After 30 min, female rats were housed in the male cages with one female-to-one male ratio and the sexual behavior of the male animals was immediately begin and continued for first two mating series. The measured parameters followed the reported one: [25]

1. The time from the introducing of a female rat into the cage of the male until the first mount (mount latency [ML])
2. The time from the introducing of a female rat into the cage until the first intromission by the male (intromission latency [IL])
3. The number of mounts before ejaculation (mount frequency [MF])
4. The number of intromissions before ejaculation (intromission frequency [IF])
5. The time from the first intromission of a series until the ejaculation (ejaculation latency [EL])
6. The time from ejaculation until the first intromission of the following series (postejaculatory interval [PEI]).

The test was considered to be negative if the intromission and ejaculatory latency was >20 min.[1]

In the second mating series, only the EL was estimated. Depending on the previously mentioned parameters, the followings can be computed: copulatory efficiency (CE) = (IF/MF) × 100 and intercopulatory efficiency (ICE) = (IF/[MF + IF]) × 100.[25]

Statistical analysis

The statistical significance of difference between the means was determined using one-way analysis of variance with *post hoc t*-test. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Chromatographic purification of the $CHCl_3$ fraction resulted in the isolation of three compounds. The UV spectrum of of each compound showed three maxima pointed out to coumarin derivatives.

The EI-MS of compound 1 showed an M^+ 382 at *m/z* consistent with the molecular formula $C_{24}H_{30}O_4$. ^{13}C NMR [Table 2] and distortionless enhancement by polarization transfer (DEPT) 135 experiments showed 9 signals for 7-oxygenated coumarin skeleton. The two quaternary carbon signals at δ_c 159.92 and 161.67 ppm were assigned to α-pyrone carbonyl and the oxygenated C-7, respectively. The ^1H NMR spectrum [Table 1] of 1 showed 5 aromatic methine proton signals forming two spine systems. The two doublets at δ_H 6.12 (d, J = 9.4 Hz) and 7.52 (d, J = 9.4 Hz) (CD_3OD spectrum) were assigned to C-3 and C-4 protons, respectively. The other three methines form an ABX spine system at δ_H 7.24 (d, J = 8.6 Hz, H-5), 6.70 (overlapped, H-6), 6.68 (bs, H-8) (CD_3OD spectrum). The H-6 and H-8 signals were better resolved in the C_6D_6 spectrum at δ_H 6.54 (dd, J = 8.5, 2.1 Hz) and 6.57 (d, J = 2.1 Hz), respectively.

In addition, the ^{13}C NMR showed 15 carbon signals sorted by DEPT135 and HMQC experiments into $4XCH_3$, $4XCH_2$, 4XCH, and 3 quaternary carbons. The singlet at δ_H 1.69 and δ_c 21.46 ppm were assigned to methyl group on an olefinic carbon. The broad singlet at δ_H 5.47 (5.54, d, J = 1.4 Hz, CD_3OD spectrum) correlated to carbon signal at δ_c 123.67 ppm were assigned by the help of heteronuclear multiple bond correlation (HMBC) experiment to the second olefinic carbon. Analyses of COSY and HMBC experiments allowed the assignment of the oefinic carbons to positions C-7' and C-8'. Such skeleton for 1 is similar to feselol and its 3'-epimer mogoltacin.[26-28] The identification of 1 as feselol [Figure 1] was achieved by studying the well resolved splitting pattern of the proton signals as well as nuclear overhauser effect spectroscopy (NOESY) experiment correlation. The assignment of H-3' orientation as β was possible from the upfield shift of H-1', H-3', and H-5' compared with mogoltacin.[26-28] The C-14' methyl signal chemical shift at δ_c 15.21 ppm was in support of β-oriented H-3'. The $J_{2'ax, 3'ax}$ value = 11.3 Hz further support the β-orientation of H-3'. Final prove for the suggested relative stereochemistry of 1 resulted from the NOESY experiment where H-3' at δ_H 3.01 showed correlation with the β-oriented H-5' at δ_H 1.06, H-9' at δ_H 1.96, and C-13' methyl at δ_H 0.97 ppm.

The EI-MS of compound 2 showed an M^+ 400 at *m/z* consistent with the molecular formula $C_{24}H_{32}O_5$. The major difference between 2 and 1 is the disappearance of the olefinic signals of 1 and instead CH_2 signal at δ_H 1.86 bs, 1.64 (dd, J = 13.1, 3.2 Hz), δ_c 44.05 ppm assigned for C-7' and quaternary carbon signal at δ_c 71.97 ppm assigned for oxygenated C-8'. The chemical shift of C-12' methyl shifted to δ_H 1.14 and δ_c 24.15 ppm compared with 1. The data of 2 indicate a samarcandin-type sesquiterpene coumarin.[29-31] The relative configuration of 2 was assigned based on NOESY experiment. Cross peaks were observed between the α-oriented H-3' at δ_H 3.32, H-12' at δ_H 1.19, H-14' at δ_H 0.85, and H-15' at δ_H 0.96 ppm. The β-oriented H-13' at δ_H 0.99 showed correlations with the overlapped H-2' and H-5' at δ_H 1.57 and H-6' at δ_H 1.46 ppm. The above provided evidences allowed the identification of 2 as samarcandin [Figure 1].[29-31]

Spectral data indicated that 3 is the 3'-acetyl derivative of 2 [Figure 1]. The H-3' proton signal appeared at δ_H 4.62 ppm, while the acetyl signals were observed at δ_H 2.09, δ_C 19.97 and 177.24 ppm. Acetylating of 2 resulted in the formation of 3 supporting its structural elucidation.

The extracts of *F. drudeana* and the major sesquiterpene coumarins 1 and 2 were tested for their potential aphrodisiac activity in male rats using SC as the reference drug. The observations of the sexual behavior study are presented in Tables 3 and 4. The present results reveal that *F. drudeana* extracts, 1 and 2 improve male sexual behavior in decreasing order of 2 > 1 > $CHCl_3$ soluble fraction > aqueous fraction. The results showed that SC, 2 and 1 significantly reduced the mounting latency (41.2 ± 2.85, 49.6 ± 3.42, and 52.1 ± 2.98 s, respectively) and IL (67.6 ± 4.85, 81.7 ± 5.95, and 90.7 ± 5.65 s, respectively) as compared to the normal control group (98.7 ± 4.28 and 168.5 ± 7.52 s, respectively). They induced significant increases in the MF (25.6 ± 1.41, 22.7 ± 0.89 and 20.3 ± 0.72, respectively) and IF (17.4 ± 0.81, 14.8 ± 0.95 and 12.1 ± 0.61, respectively) of male animals as compared to the normal rats (11.4 ± 0.46 and 5.4 ± 0.37, respectively). The MF and the ML reflect sexual interest or libido, whereas the IF and IL are useful indices of the sexual excitement and the efficiency of erection.[32] Therefore, the reduced durations of ML and IL as well as the increased values of MF and IF recorded in SC, 2 and 1-treated rats suggests enhanced sexual interest, libido, and erection.[33]

In addition, SC, 2 and 1 prolonged ejaculation latency in the 1st series (431.7 ± 8.72, 412.6 ± 10.80 and 404.7 ± 8.19 s, respectively) and in the 2nd series (481.0 ± 9.25, 453.6 ± 9.38 and 434.9 ± 10.40 s, respectively) of male animals as compared to the normal rats (344.6 ± 8.59 and 385.7 ± 6.52 s, respectively). SC, 2 and 1 reduced PEI (327.2 ± 14.28, 344.4 ± 12.95 and 370.7 ± 11.42 s, respectively) as compared to the normal control group (518.5 ± 17.25 s). The significant increase in the duration of ejaculation latency in the 1st and 2nd series (EL-1 and EL-2, respectively)

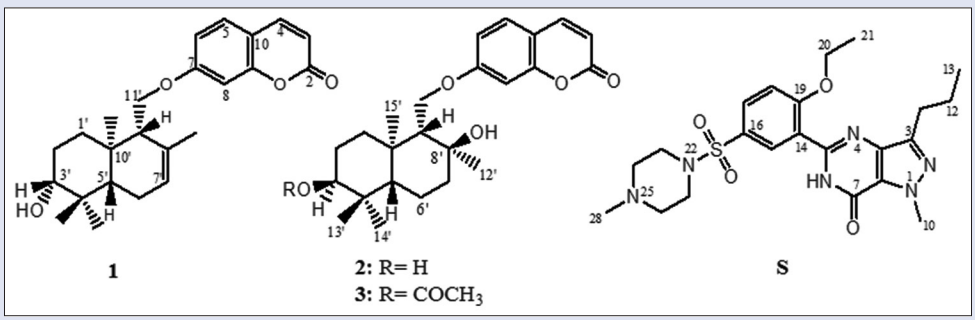

Figure 1: Chemical structures of compounds 1–3 and S

as well as the decrease in the duration of the refractory period between the 1st and 2nd series of mating (PEI), confirm that SC, 2 and 1 have the potential to improve copulatory performance of male rats.

The percentages of copulatory and the intercopulatory efficiencies were the highest in the group exposed to SC, 2 and 1 compared with the normal control group [Figure 2]. The present results revealed that the highest aphrodisiac activity in male rats was exhibited by 2. ML, MF, IL, IF, EL, and PEI of male rats exposed to 2 are comparable to those of SC-treated rats. The higher effect of 2 compared with 1 can be explained from the three-dimensional plots of 1, 2, and SC [Figure 3]. The configuration of 2 in space is much similar to that of SC rather than 1. Structure of 1 also lacks the C-8' hydroxyl group present in 2 which is most likely equivalent to C-19 oxygenation in SC [Figure 1]. This investigation established experimental data supporting the use of the roots of *F. drudeana* as potent stimulator of sexual behavior.

CONCLUSION

The results demonstrated that *F. drudeana* roots and their Sesquiterpene coumarins significantly improve sexual behavior of male rats. These results establish the traditional claim for the roots of *F. drudeana* as aphrodisiacs. Both Sesquiterpene coumarins were more effective than *F. drudeana* extracts. Accordingly, the results proposed that 2 and 1 might

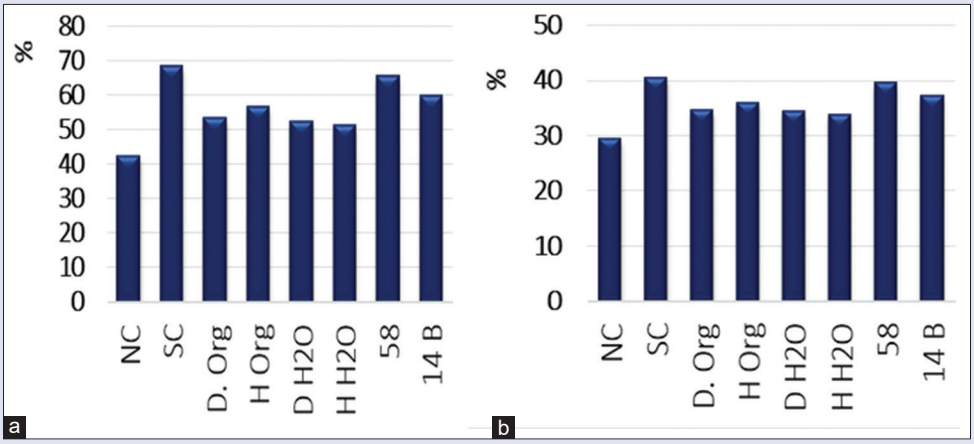

Figure 2: Effect of (sildenafil citrate), *Ferula drudeana* $CHCl_3$ soluble fraction, aqueous fraction, 1 and 2 on the copulatory efficiency (a) and intercopulatory efficiency (b) of male rats

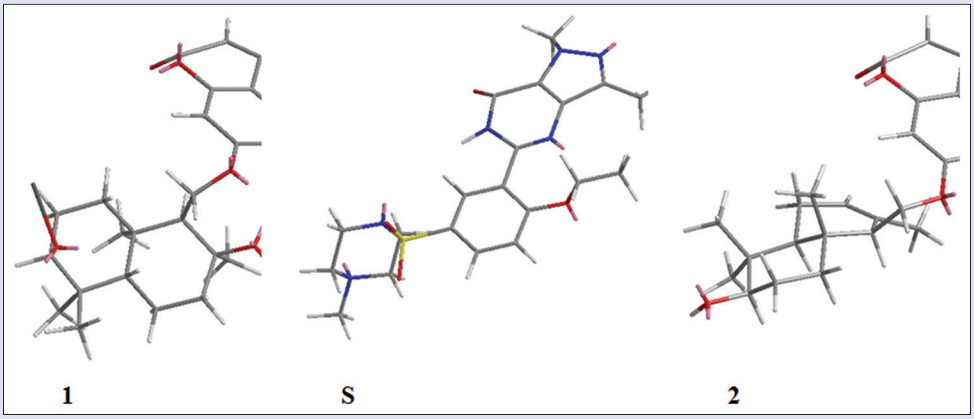

Figure 3: Three-dimensional plot of 1, Sildenafil (S) and 2

Table 3: Effect of (sildenafil citrate), *Ferula drudeana* $CHCl_3$ soluble fraction, aqueous fraction, 1 and 2 on the mount latency, mount frequency, intromission latency and intromission frequency and copulatory efficiency of male rats

Groups	ML (s)	MF	IL (s)	IF	CE (%)
NC	98.7±4.28*	11.4±0.46*	168.5±7.52*	5.4±0.37*	41.9±2.92*
SC	41.2±2.85^{i}	25.6±1.41^{i}	67.6±4.85^{i}	17.4±0.81^{i}	68.0±2.85^{i}
$CHCl_3$	84.3±3.04^{i,*}	14.1±0.61^{i,*}	138.2±6.84^{i,*}	7.5±0.52^{i,*}	53.2±1.67^{i,*}
Aqueous	86.5±3.16^{i,*}	13.4±0.51^{i,*}	142.1±6.61^{i,*}	7.0±0.39^{i,*}	52.2±1.65^{i,*}
1	52.1±2.98^{i,*}	20.3±0.72^{i,*}	90.7±5.65^{i,*}	12.1±0.61^{i,*}	59.6±2.14^{i,*}
2	49.6±3.42^{i}	22.7±0.89^{i}	81.7±5.95^{i}	14.8±0.95^{i}	65.2±2.47^{i}

Values are expressed as mean±SEM, n=6 rats/group. ^{i}Significance compared to NC group at P<0.05; *Significance compared to SC group at P<0.05. ML: Mount latency; MF: Mount frequency; IL: Intromission latency; IF: Intromission frequency; CE: Copulatory efficiency; SC: Sildenafil citrate; SEM: Standard error of mean; NC: Normal control

Table 4: Effect of (sildenafil citrate), *Ferula drudeana* $CHCl_3$ soluble fraction, aqueous fraction, 1 and 2 on the ejaculation latency in 1st series, post-ejaculatory interval and ejaculation latency in 2nd series of male rats

Groups	EL-1 (s)	PEI (s)	EL-2 (s)	ICE
NC	344.6±8.59*	518.5±17.25*	385.7±6.52*	29.5±0.56*
SC	431.7±8.72[i]	327.2±14.28[i]	481.0±9.25[i]	40.5±1.25[i]
$CHCl_3$	374.4±9.14[i,*]	395.3±12.58[i,*]	417.1±8.18[i,*]	34.7±0.67[i,*]
Aqueous	371.8±8.25[i,*]	457.2±14.60[i,*]	410.9±7.62[i,*]	34.3±0.72[i,*]
1	404.7±8.19[i,*]	370.7±11.42[i,*]	434.9±10.40[i,*]	37.3±0.58[i,*]
2	412.6±10.80[i]	344.4±12.95[i]	453.6±9.38[i]	39.5±1.23[i]

Values are expressed as mean±SEM, *n*=6 rats/group. [i]Significance compared to NC group at $P<0.05$; *Significance compared to SC group at $P<0.05$. EL-1: Ejaculation latency in 1st series; PEI: Postejaculatory interval; EL-2: Ejaculation latency in 2nd series; CE: Copulatory efficiency; ICE: Inter CE; SC: Sildenafil citrate; SEM: Standard error of mean; NC: Normal control

represent new prototype agents of potential aphrodisiac activity in the treatment of male sexual dysfunction. More studies are desired including the revealing of their mechanisms responsible for sexual behavior enhancement.

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Conflicts of interest

There are no conflicts of interest.

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