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Phytochemical and Antimicrobial Analysis of Root Extract of *Aspargus adscendens*

REKHA TRIPATHI[1]* and ASHUTOSH SHARMA[2]

[1]Department of Applied Sciences, Maharaja Surajmal Institute of Technology, New Delhi-110058, India.
[2]Principal, St. Xaviers PG College, Phagi-303005, Jaipur, India.
*Corresponding author E-mail: rekhatripathi@msit.in

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ABSTRACT

The aim of this study was to characterize the phytochemicals and to investigate the antimicrobial efficacy of the shade dried roots of the *Asparagus adscendens*. The powder roots were extracted with chloroform and after removal of solvent under reduced pressure was subjected for chromatographic separation. After column chromatography and purification five compounds i.e. Hexacosanyltriacontanoate, hexatriacontanone, 2,3,5,7-tetramethoxydi hydrophenanthrene, β-sitosterol and stigmasterol were characterized with the help of physical and spectral analysis (IR, ^{1}HNMR, ^{13}CNMR and mass). The crude extract was tested against the selected bacteria and fungi using disc diffusion method. The root extract exhibited significant antibacterial activity with maximum efficacy against *E. coli* (activity index 0.82 at 1000 μg/disc and 0.73 at 500 μg/disc).

Keywords: *Asparagus adscendens* roots, Spectral analysis, Antibacterial activity, Antifungal activity.

INTRODUCTION

Asparagus genus belongs to Family liliaceae is a perennial plant. The young shoots are used as vegetable or salad. This genus possess various types of biological properties e.g. antioxidants, anti-inflammatory, anti-hepatotoxic anti-oxytocic, immunostimulant, antibacterial and reproductive agents[1-2]. The genus asparagus includes about 300 species around the world. The tuberous roots of asparagus are main source as the drug shatavar or shatavari. The drug in crude form is used in increase secretion of milk and also improves appetite in lactating women. Tuberous roots of *Asparagus currillus* after mixing with honey is given in case of diarrhea, diabetes and dysentery. One of the important species is *Asparagus adscendens* known as yellow musli is mainly grown in Asian countries and also in Garhwal valley. Traditionally it is recommended as nerve tonic and also used for memory impairment. In ayurvedic system of medicine it is helpful in treatment of female disorders[3-6]. Asparagus polysaccharides also exhibited health benefits against tumor cells[7]. In India *Asparagus adscendens* is mainly distributed in Garhwal (Himalayan) hills, Punjab, Madhya

Pradesh, Uttar Pradesh, Himachal Pradesh, Bihar, West Bengal, Orissa, Jammu and Kashmir and lower Himalayan Hills. It is a shrub with tall, sub erect, smooth stem having ascending branches and white tuberous roots. *Asparagus ascendens* is a rich source of nutritious starch with low calorie, sodium values and good source of vitamins [8-10].

Asparagus adscendens is mostly used as indigenous medicine. On the basis of literature survey the plant contains 3-β-O-{β-O-glucopyranosyl}-stigmasterol, 3-β-O-{β-O-glucopyranosyl (1-2)-β-L-arabinophyranosyl}-stigmasterol[11], 3-heptadecanone, 8-hexadecanoic acid, tritriacontane, palmitic, stearic acid[12], oligofurastanosides, spirostanosides13, β-sitosterol-β-D-glucoside, spirostanol glycosides and furostanol glycosides[14,15]. The plant is known for its antioxidant, antiamnesic activities, used in diarrhea, dysentery, leucorrhoea, nutritive values, tonic, beneficial in stress management, inflammatory conditions and general debility[16-18].

EXPERIMENTAL

Roots of *Asparagus adscendens* were collected from Tehri Garhwal, Uttarakhand, India in the month of September 2020 and confirmed from the herbarium of Botany Dept of H.N.B. Central University Srinagar, Garhwal (U.K.).

Shade dried and powdered roots (2 kg) were extracted with chloroform on a steam bath for 36 h then the extract was filtered hot and concentrated under reduced pressure.

Isolation and Identification of Phytochemicals

110 g crude extract of roots of *Asparagus adscendens* was eluted with the solvents of increasing polarity over a column of silica gel. On elution five compounds were isolated, purified and characterized by co-TLC, mixed m.p. and spectral analysis (IR, ^{1}HNMR, ^{13}CNMR and mass spectral data).

Compound 1: Hexacosanyl triacontanoate

On elution with petroleum ether white powdered compound hexacosanyl triaconanoate, m.p. 68ºC was obtained. The IR spectrum exhibited characteristic absorption at 2900, 2835, 1735, 1705 (C=O), 1260, 725, 710 cm^{-1}. The mass spectrum (m/z) showed M^+ at 816, 447, 435, 381 etc.

Compound 2: Hexatriacontanone

Further Elution with petroleum ether-chloroform (9:1) yielded hexatriacontanone, crystallized from acetone as a white powder, m.p. 76ºC. The spectral data were obtained as; IR(KBr) 2910, 2850, 1720 (C=O), 1130, 720, 715 cm^{-1}, MS; 520(M^+).

Compound 3: 2,3,5,7-tetramethoxyphenanthrene

Eluting the column with Chloroform yielded 2,3,5,7-tetramethoxyphemanthrene as white powder, m.p. 122-23ºC. Spectral data observed as IR(KBr) 1760, 1590, 1460, 1470, 1372, 1280 cm^{-1} etc. MS (m/z) 301$(M+H)^+$, 300$(M)^+$, 286, 285, 258, 228, 226, 181, 150, etc.

Compound 4: Stigmasterol

Petroleum ether-chloroform (1:1) yielded stigmasterol as colorless solid powder, m.p. 167-68ºC. Its spectral data are as: IR (KBr) 3410-3220 (OH), 1467 (C=C bonding), 1380, 1362, 1260, 1055, 965 etc. MS m/z 412 (M^+), 399, 384, 370, 369, 314, 302, 273 etc.

Compound 5: β-sitosterol

Further elution of column with chloroform-ethylacetate (80:20) yielded β-sitosterol after crystallization with methanol as white powder, m.p. 134-135ºC. The spectral data areas: IR(KBr) 3505, 3420, 2960, 2920, 1595, 1462, 1380, 1050 etc, MS(m/z) 414(M^+), 397, 396, 383, 369, 255, 213 etc.

Antimicrobial analysis

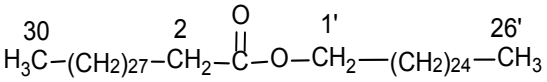
The crude chloroform extract of roots of *Asparagus adscendens* was screened against *Escherichia coli, Staphylococcus aureus*, *Proctus vulgaris* and *Salmonella paratyphi B*. for bactericidal efficacy and against *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* and *Rhizoctonia bataticola* for fungicidal activity by using *Disc diffusion* method[19].

RESULT AND DISCUSSION

Phytochemical Analysis

Compound (1) was isolated as white powder. The mass spectrum exhibited [M^+] at 816 corresponding to molecular formula $C_{56}H_{112}O_2$. IR spectrum (KBr) of the compound 1 indicated the presence of ester group by showing characteristic absorption at 1735 cm^{-1}. The other characteristic absorption were located at 2900, 2835 cm^{-1} for

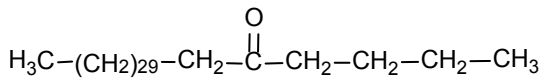
C-H stretching and 1260 cm^{-1} for C-O stretching of the ester group. ^{1}HNMR spectrum showed two triplets for two protons each at δ 3.90(C-1') and δ2.16(C-2) corresponding to the methylene group attached to ester oxygen and ester carbonyl group. The terminal methyl group was observed at 0.85 for six protons.



Hexacosanyltriacontanoate (1)

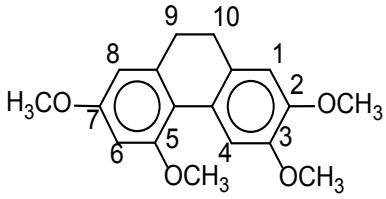
In ^{13}CNMR spectrum of compound 1 the carbonyl carbon of ester (—C(=O)—O) group appeared at δ174.4. Thus compound 1 was characterized as hexacosanyl triacontanoate[20,21].

Compound 2 was obtained as white powder and the molecular formula was determined as $C_{36}H_{72}O$ by mass spectroscopy. The molecular ion observed at m/z 520.4(M^+) with other important ions at m/z 464, 463, 435, 85 etc. In the proton NMR spectrum (δ ppm, $CDCl_3$) four protons appeared at 2.27 (J=6.5Hz) as a triplet for the two methylene group attached to carbonyl (C=O) group. A triplet for six protons was observed at δ0.83 for methyl groups. A broad singlet at δ1.25 showed the presence of remaining sixty two protons. In ^{13}CNMR spectrum absorbance at δ210 confirmed the presence of carbonyl carbon. IR spectrum (KBr) indicated the presence of carbonyl group at 1720 cm^{-1}. Thus the compound 2 was identified as hexatriacontanone[22].



Hexatriacontanone (2)

Compound 3 (white powder) was determined as $C_{18}H_{20}O_4$ with 301($M+H)^+$, 300(M^+). Other prominent ions were observed at m/z 286, 285, 258, 228, 227, 226, 182, 181, 150, 105 etc. In IR spectrum (KBr) showed characteristic absorptions at 1760, 1590, 1450, 1470, 1370, 1285, 1225 etc. In ^{1}HNMR spectrum (δppm, $CDCl_3$) of compound (3) exhibited four aromatic protons as singlet at δ7.90(1H), 6.70(1H) and 6.42(2H). Complete assignment of ^{1}HNMR and ^{13}CNMR is given in Table 1, thus compound (3) was identified as 2,3,5,7-tetramethoxydihydrophenanthrene[23-25].



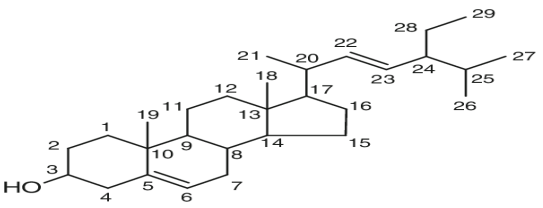
2,3,5,7-tetramethoxy-9,10-dihydrophenonthrene

Table 1: Spectral data of 2,3,5,7-tetramethoxydihydrophenanthrene

H/C	^{1}H	^{13}C
1	6.70 (s)	111.0
2	-	146.8
3	-	146.0
4	7.90 (s)	112.0
4a	-	125.2
4b	-	116.2
5		157.6
6	6.42(s)	98.0
7	-	158.4
8	6.42(s)	104.8
8a	-	139.6
9	2.70(m, 4H)	31.0
10	2.70(m,4H)	28.6
10a	-	129.8
2-OCH_3	3.90(s, 6H)	56.0
3- OCH_3	3.80(s, 6H)	55.8
5- OCH_3	3.85(s, 3H)	55.4
7- OCH_3	3.80(s, 3H)	55.0

Compound (4) was obtained as colorless solid after crystallization from methanol. On the basis of spectral data the molecular formula was assigned as $C_{29}H_{48}O$. The molecular ion was observed at m/z 412(M^+) with the other prominent ion at 399, 384, 369, 302, 273, 255 etc in the mass spectrum. The IR (KBr) spectrum displayed characteristic absorptions at 3410-3220 cm^{-1} indicating the presence of O–H stretching and 1467 cm^{-1} for C=C bending vibrations. Absorptions at 1380, 1362, 1055, 955 etc. are characteristic for steroids.

In ^{1}HNMR spectrum ($CDCl_3$, δppm) showed a pair of double doublets at 5.10(J=15.3Hz) and 5.18 (J=15.3Hz) for olefinic protons at C-22 and C-23. The large J values indicated the trans geometry of protons. A broad triplet at 5.30 was accounted for the proton present at C-6 position. A multiplet observed at 3.45 for one proton accounted for C-3 methine.

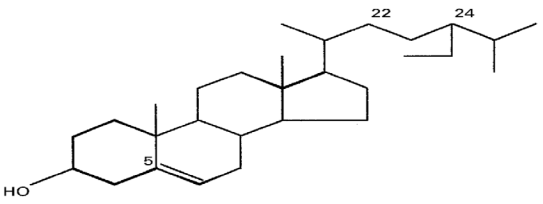


Stigmasterol

The presence of six methyl groups in compound (5) was observed and their positions has been given in parenthesis i.e. 0.71(s,3H,C-18), 0.84(t,3H,C-29), 0.94(s,3H,C-19), 1.00(d,3H,C-21) and 1.15(d,6H,C-26 and C-27). Remaining 26 protons here observed from 1.24 to 2.26 and characterized as stigmasterol[26,27].

Compound (5) after crystallization with ethanol yielded white powder. On the basis of mass spectrum and ^{1}HNMR, molecular formula for compound (5) was established as $C_{29}H_{50}O$, m/z 414(M^+). The other prominent fragments were located at m/z 397, 383, 369, 255 etc. In IR spectrum (KBr) the O-H stretching were observed at 3550-3420 cm^{-1}. The presence of carbon–carbon double bond was confirmed by the absorption at 1595 cm^{-1}. Absorption at 1060 was located which confirms the C-O stretching.

In ^{1}HNMR ($CDCl_3$, δppm) spectrum the methyl groups were observed at 0.65(s,3H, C-18), 0.97(s,3H,C-19), 1.23(d,3H,C-21), 0.83(d,3H, C-26), 0.90(d,3H,C-27), 0.94(t,3H,C-29).



β-sitosterol

Confirming the presence of six methyl groups. A multiplet at 3.48 for one proton confirmed the presence of hydroxyl group at C-3 position. A triplet for one proton at 5.20 with coupling constant J-2.8Hz was due to presence of –OH group at C-3 position. On basis of above data compound (5) was characterized as β-sitosterol[28].

Antimicrobial analysis

The test extract exhibited anti bacterial activity against all the test bacteria, the maximum activity was observed against *E. coli* (activity index 0.82 at 1000 μg/disc and 0.73 at 500 μg/disc). The root extract also showed significant activity against *S. aureus* (activity index 0.77 at 1000 μg/disc and 0.56 at 500 μg/disc) In case of antifungal activity only *F. moniliforme* and *R. bataticola* exhibited some activity.

Table 2: Antibacterial and Antifungal activity of Root extract of *Asparagus adscendens*

		Test Bacteria				Test Fungi			
Dose		*E. coli*	*S. aureus*	*P. vulgaris*	*S. paratyphi B*	*A. flavus*	*A. niger*	*F. moniliforme*	*R. bataticola*
1000 mg/disc	IZ	18.0	14.0	6.0	8.0	±	±	11	9
	AI	0.82	0.77	0.30	0.42			0.55	0.43
500 mg/disc	IZ	16.0	10.0	-	±	±	±	8	6
	AI	0.73	0.56					0.40	0.29

IZ-inhibition zone (in mm) including the diameter of disc (6mm)
AI-activity index (inhibition zone of sample/ inhibition zone of standard)
Standard-Amikacin=10 mg/mL (bacteria); Mycostatin=100 units/disc (fungi)
(±) Trace activity: (-) No activity

CONCLUSION

Five phytochemicals i.e. hexacosanyl-triacontanoate, hexatriacontanone, 2,3,5,7 -tetramethoxydi hydrophenanthrene, β-sitosterol and stigmasterol were isolated and characterized with the help of spectral studies from the root extract of *Asparagus adscendens*. Chloroform extract of roots of *A. adscendens* possess active compounds which exhibited significant anti bacterial activity against *E. coli* and *S. aureus*. It can be natural and harmless alternate of antibiotics for the treatment of many bacterial infections.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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