International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1558-1532, April-June 2010

Pentacyclic Triterpinoids from Betula utilis and Hyptis suaveolens

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Abstract: Himalayan Silver Birch/Bhurjapatra, named "*Betula utilis*", and Wilayati tulsi named "*Hyptis suaveolens*" were collected from local Himalayan region. From them, two triterpenoids (compounds 1 and 3) were isolated and structure of the compounds were determined via chemical reactions spectroscopic analysis and also compared with structures of authentic betulin and betulinic acid. From the results it was concluded that structure of triterpenoid obtained from bark of *Betula utilis* and root of *Hyptis suaveolens* were similar to betulin and betulinic acid.
Keywords: *Betula utilis*, *Hyptis suaveolens*, Betulin, Betulinic acid.

Introduction

According to literature betulin is a pentacyclic triterpene with the lupane skeleton whose existences in nature have been reported since 1788, isolated from birch bark as pure chemical substance by sublimation and elemental analysis of this natural constitutes was done in 1876 (Hayak et. al., 1989)[1]. Traditionally birch bark has been used as antiseptic (Batty et. al. 1973)[17], rheumatism, gout, malaria (Hager)[18] by human being. Betulin is a naturally occurring triterpene seems to be an ideal starting material for the synthesis of biologically active betulinic acid. Betulin can be isolated (Ender et. al. 2003)[2] from the outer layer of the bark of *Betula alba* with maximum yield. It can also be isolated from the Himalayan birch tree, *Betula utilis*, contains betulin up to 12% of its weight. Thus, betulin has been transformed into betulinic acid via *jone's oxidation method*. Betula bark also reported to contain karachic acid which is aromatic and has antiseptic properties according to previous literature (Nadakarni et. al 1976, Selvam et. al. 2008)[3,4]. Though, less exhaustive phytochemical studies have been conducted on the *Betula utilis*. Betulin and its derivatives however possess biological effects, e.g. Anti-inflammatory, antiviral, anti-HIV, hepato protective. (Dzubak et. al. 2006)[5]. Similarly, Betulinic acid is also a natural compound derived from betulin and also found plentiful in most trees of *Betula* species. It was first isolated from african bush *Ziziphus mauritiana* Lam. (Simonsen et. al. 1957)[6] and also from *Vauquelinia corymbosa* (Trumbull et. al. 1976)[7] also present in bark of *Platanus hispanica* (Urban 2004)[8]. Betulinic acid was studied more extensively then betulin, principally owing selective antitumor activity against human melanoma cell culture and anti-HIV activity. Some of derivatives of betulinic acid also show high anti-HIV and antiviral activities (Dzubak et. al. 2006)[5]. A previous study shows that the plant root of *H. suaveolens* contains 3-β-hydroxylup-20(29)-en-27-oic acid (Triguna et. al. 1983)[9]. Previously it was reported to contain a natural HIV-integrase inhibitor, urosolic acid (Chatterjee et. al. 1997)[10]. Later, a trypsin

inhibitor was purified and characterized from seeds of *H. suaveolens* (Aguirre et. al. 2004)[11]. It has therapeutic qualities as well as insecticidal properties. Literature designates that leaf extracts are used to cure swellings, abscesses and haemorrhoids. Virtually each and every part of *H. suaveolens* is found to have some use in traditional medicine to treat various diseases. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases (WOI)[13]. *Hyptis suaveolens* is traditionally used for the treatment of lung infections, colds, pain, fever, cramps and skin diseases. *Hyptis suaveolens* has been also reported to active against *Candida albicans* and selected Gram-positive and Gram-negative bacteria (Iwu et al. 1990, Rojas et al. 1992)[14,15]. Roots decoction of *Hyptis suaveolens* is vastly known for its appetizing property (Chatterjee 1997)[10]. Essential oils isolated from aerial parts of this plant have showed, antifungal (Singh 1992)[16]. In the present paper we reported the isolation and identification of triterpinoids, betulin and betulinic acid from stem bark extracts of *Betula utilis* and roots of *Hyptis suaveolens* respectively in an attempt to provide scientific justification of presence of these bioactive compounds and its use in medicine.

Materials and Methods

General

Melting points were taken in a capillary method and uncorrected. Thin layer chromatography (TLC) was carried out on precoated silica gel TLC plates and the spots were detected under UV spectrophotometer. IR spectra were recorded as KBr discs on a Shimadzu FT-IR 8400S spectrometer. MS spectra were obtained under electron impact in Joel SX-102. Nuclear Magnetic Resonance spectra were measured on a Brucker DRX-300 NMR spectrophotometer. Chemical shifts (δ) are expressed in ppm from tetramethylsilane (TMS) as an internal standard. Authentic chemicals (betulin and betulinic acid) used are of analytical grade.

Plants Material

Hyptis suaveolens Poit. (Lamiaceae) is highly-fragranced basil to 1.5 m tall with quadrate bushy stems and ovate leaves about 4.5 cm long and 3.5 cm wide, the sidelines serrulate, lower surface compactly filled with hairs; petioles are upto 3 cm long. Flowers in small cymes along branch ends with reduced leaves. Flowers consist of calyx about 5 mm long and 10 mm long in fruit, ribbed. Corolla is blue, Nutlets about 1.2-1.5 mm long, slightly notched at the end (Stone, 1970)[12]. *Betula utilis* D. Don is a moderate-sized tree that grows up to 20m in height. The bark is smooth, shining, reddish white or white, with white horizontal lenticels belonging to the family *Betulaceae*. It is widely distributed in northern India and is generally known for its therapeutic values in human medicine and in resource-poor rural and urban households.

Triterpenes from *Betula utilis*

Barks of Himalayan silver birch was hydrolyzed with 10% potassium hydroxide solution, and the subsequent unsaponifiable neutral material (500 g) was mixed with sufficient ethyl alcohol. The mixture was refluxed till 3-4 h using soxhlet apparatus and afterwards filtered while hot. The filtrate was concentrated to dryness in vacuum and the filtered residue was again passed through column using methyl alcohol (250 ml). This practice was repeated for twice. Then each time four fractions were collected. The last portion of each was recrystallized from acetone to give 10-12 % of colorless solid (compound 1), later identified as betulin.

Triterpenes from *Hyptis suaveolens*

Dried root of *Hyptis suaveolens* "Wilayati tulsi" was washed in 10% potassium hydroxide solution, dried and crushed. Then the subsequent 500 g unsaponifiable dried roots were moistened by 400 ml of benzene. The mixture was refluxed using soxhlet apparatus for 4-5 h till the solvent in syphon becomes colorless and filtered while hot. The filtrate was concentrated to dryness in vacuum. Then, the filtered residue was again successively eluted with petroleum ether, benzene and ethyl acetate on a column chromatograph. The ethyl acetate fraction was recrystallized with aqueous methanol this yielded 0.07-0.09% of colorless needles (Compound 3), which was identified as betulinic acid when compared to marketed product. Unfortunately the yield of colorless needles was very less.

Conversion of compound 1 to compound 3

Furthermore the Compound 1 can be converted to betulinic acid in two steps by oxidation with Jones' Reagent at 0°C for 2-3 h and then selective reduction of formed betulonic acid with appropriate agent in tetrahydrofuran for 8-10 h at 0-4°C. Addition of HCl stops the reaction resulting in production of a mixture of α and β betulinic acid (Compound 3). β- Betulinic acid was separated in a quantitative yield by recrystallizing the mixture with methanol in appropriate ratio. Successful formation of compound 1 to compound 3 also confirms the presence of betulin in bark of *Betula utilis*.

Result and Discussion

FT-IR data of the compounds 1, isolated from Betula utilis assist the principal IR absorbance band found at 3433, 1725, 1648, 1560, 1230 and 881ν_{max} cm^{-1}. The range of betulin (authentic) is subjected by broad

bands at 3430, 1716, 1641, 1600, 1581, 1291 and 884 cm^{-1}. Comparison of FT-IR data of Compound 1 and betulin (authentic) reveals the significant resemblance as well as alteration in position of bands. However ideally betulin is the major component of *Betula utilis* there must be some other constituent also present along with betulin, in the bark which causes the interference in infrared spectra. A sharp variation was observed in spectra when betulin converted to betulinic acid via jones' oxidation method. However the bands of resultant compound 3 (3550 for –OH, 1672 for C=O, 1240, 1028 cm^{-1}) were approximately similar to that of Betulinic acid (authentic). On the other hand IR data of Compound 3 derivative of Compound 1 and Compound 2 (3450 for –OH, 1670 for C=O, 1210, 1024 cm^{-1}) isolated from *Hyptis suaveolens* was also similar to authentic betulinic acid (3508 for –OH bond, 1690 for C=O band, 1290 cm^{-1}). The mass spectrum of betulin and compound 1 were also comparable having molecular ion at 442 and 440 separately. Similarly, compound 2, 3 and betulinic acid showed molecular ion peaks at 459, 526 and 456 respectively. According to the mass spectra peaks present in range of *m/z* 208-206, 163-210 and *m/z* at 189 confirms the presence of lupane-type structure of the compounds and these data were constant with various previously published data. The mass spectra with a splinter peak at 411 intensity (M^+ -CH_2OH) recalls that the structure of compound 1 analogous betulin with 2^O –OH group distinguishable to betulinic acid. The IR and Mass spectral data of Betulin, Betulinic Acid, Compound 1, Compound 2 and Compound 3 has been demonstrated in **Table 2** given below. In ^{1}H-NMR spectral analysis of compound 1 and 2 isolated from *Betula utilis* and *Hyptis suaveolens* correspondingly showed many methyl signals at high field and the absence of aromatic protons suggested a terpenoidal structure. The ^{13}C NMR spectrum and the molecular mass of Compound 1 were identical to betulin while 2 and 3 were indistinguishable to those of betulinic acid. ^{13}C-NMR data of compound 1 showed signals at 28.9, 16.5, 16.2, 16.5, 15.0, 60.3, 19.3 and 109.3 for C_{23-27}. While a hydroxyl group containing carbon (C_3) displayed signal at δ_C=76.8. Signals assigned in proton and carbon NMR studies for Compound 1 and 3 were also found to be comparable with authentic betulin and betulinic acid. The rest of the signals of the spectra were also analogous. The NMR data of Betulin, Betulinic Acid, Compound 1, Compound 2 and Compound 3 has been demonstrated in **Table 1a, b** given below.

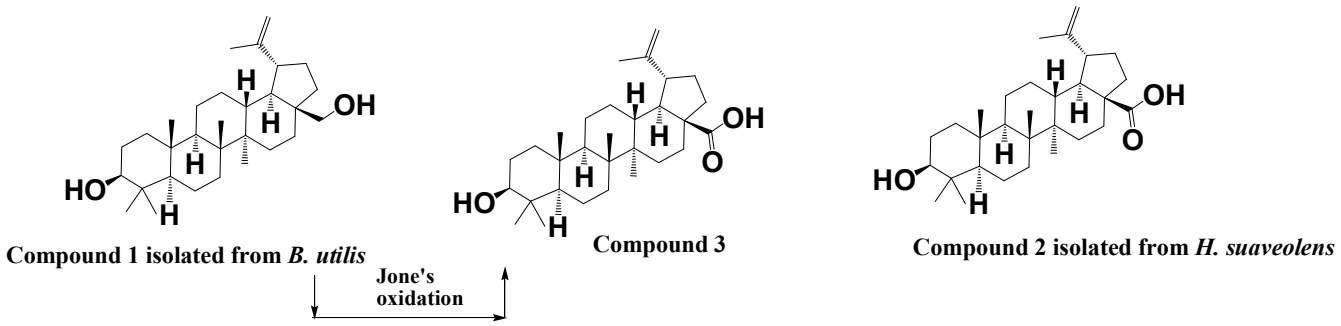

Figure 1: Representation of Structure of compounds.

Table 1a: ^{13}C-NMR data of compounds

^{13}C	BETULIN (Authentic)	COMP. 1	BETULINIC ACID (Authentic)	COMP. 3	COMP. 2
	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm
1	38.6	38.5	39.0	38.7	39.2
2	18.7	18.9	27.6	18.2	20.4
3	79.8	76.8	78.2	81.3	79.1
4	38.6	38.3	39.1	38.5	38.1
5	55.0	54.2	55.5	55.2	55.0
6	18.1	20.0	18.4	21.0	18.0
7	34.2	32.9	34.5	34.7	33.2
8	41.3	40.3	40.8	41.3	40.0
9	50.2	50.5	50.7	50.5	49.7
10	37.0	37.2	37.3	37.2	36.8
11	20.6	21.0	21.0	21.6	21.2
12	25.4	24.9	25.7	25.7	25.1
13	37.1	38.5	38.1	38.3	38.1
14	42.3	41.9	42.5	42.4	41.9
15	29.8	30.2	30.2	31.7	30.9

16	30.1	29.9	32.9	32.0	31.8
17	47.6	48.1	47.1	47.6	47.6
18	47.2	49.4	48.1	49.4	50.1
19	47.8	46.9	49.2	49.7	49.7
20	149.9	151.1	150.1	150.3	149.1
21	29.9	30.6	30.6	30.8	31.1
22	34.0	34.6	37.0	37.4	37.1
23	28.6	28.9	27.9	28.6	28.8
24	15.9	16.5	15.5	15.7	15.7
25	16.2	16.2	16.4	16.6	16.4
26	16.1	16.5	16.7	16.4	15.9
27	15.0	15.0	15.0	15.0	15.0
28	60.7	60.3	180.3	182.3	181.1
29	20.1	19.3	108.9	109.3	108.9
30	109.4	109.3	19.6	19.4	19.2

Table 1b: ^{1}H-NMR data of compounds

^{1}H	**BETULIN (Authentic)**	**COMP. 1**	**BETULINIC ACID (Authentic)**	**COMP. 3**	**COMP. 2**
	δ_H ppm	δ_H ppm	δ_H ppm	δ_H ppm	δ_H ppm
3α	3.10	3.15	3.30	3.28	2.99
19	2.89	2.90	2.30	2.29	2.27
23	0.90	0.92	0.93	0.91	0.89
24	0.80	0.77	0.75	0.79	0.75
25	0.82	0.85	0.82	0.84	0.81
26	0.91	0.99	0.96	0.98	0.92
27	1.01	1.00	0.96	0.99	0.98
28	3.22	3.30	3.25	3.23	3.15
28'	3.90	3.70	-----	-----	----
29	4.60	4.72	4.70	4.65	4.62
29'	4.80	4.88	4.80	4.78	4.76
30	2.00	1.69	1.64	1.70	1.67

Table 2: IR cm^{-1}, MS data of compounds

S.No.	**m.p. ^oC**	**UV λ_{max}**	**IR ν_{max} cm^{-1}**	**MS m/z, (intensity %):**
Betulin	256–257	208	3430, 1716, 1641, 1600, 1581, 1291, 881	442(M^+, 38), 411(5, M^+-CH_2OH), 207(76), 189 (100).
Compound 1	238-240	215	3433, 1725, 1648, 1560, 1230, 884.	440(M^+, 38), 411(5, M^+-CH_2OH), 208(76), 189 (100).
Betulinic Acid	316-318	208	3508,1710, 1690,1641, 1600,1580, 1290	456 (5, M^+), 440 (10, M^+, -CH_3), 438 (20, M^+, -H_2O), 426 (10), 415 (25, M^+, -C_3H_5), 208 (10), 206 (8), 163 (80), 135 (63), 107 (60), 105 (40), 79 (53), 41 (100).
Compound 3	285-290	210	3550, 1730, 1672, 1610, 1600, 1240, 1028	526(0.5, M^+), 466(5, M), 440 (10, M^+, -CH_3), 415 (20, M^+, -H_2O), 426 (25, M^+, -C_3H_5), 208 (10), 41 (100).
Compound 2	320-322	218	3450, 1715, 1670, 1590, 1210, 1024	459 (0.5, M^+), 446(5, M), 440 (10, M^+, -CH_3), 410 (20, M^+, -H_2O), 422 (25, M^+, -C_3H_5), 210 (10), 41 (100).

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