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Isolation and Characterization of Steroids of Nutraceutical Value in *Physalis minima*

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Abstract

There is great demand of nutraceutical secondary metabolites in the world so as to feed the population by improving agricultural production with new varieties of food crops but what is still more important is to add nutrition into the food. *Physalis minima*, plant of *Solanaceae* family, contain certain secondary metabolites which enhance the over all metabolic functions of the body. Withasteroids are one of such phytochemicals that are generally regarded as safe. These chemicals are almost monopoly of the plants of *Solanaceae*. The leaves, stem and roots of *P. minima* have been examined to yield several steroids, identification of which has been discussed in this paper. These withasteroids contribute to the potential nutraceutical and health function value since incorporation of withanolides in the diet may prevent or decrease the growth of tumors in humans.

Key words: *Physalis minima*, *Solanaceae*, withanolide, withasteroids, nutraceutical

INTRODUCTION

Physalis minima L. (*Solanaceaus*) is an annual herb growing wildly in India, Ceylon, Sri Lanka and tropical region of Africa and is also a common weed of vegetable farms in Singapore and Malaysia (1). The plant has a bitter taste and Indian popular medicine recommends it, *Inter alia*, as a tonic, diuretic, purgative, laxative and in the treatment of inflammations of the spleen (2-4). The leaves, stem and to a lesser extent the roots have been found to exhibit cholinesterase activity as indicated by the neostigmine inhibited hydrolysis of acetyl thiocholine iodide (5). In India leaves are used in treatment of in factious hepatitis and gonorrhea, the roots are used as a vermifuge, febrifuge and for diabetes while the fruits reported to be edible are used preserves, against dropsy, urinary diseases and gout. *Physalis minima* have been widely used in the folk medicine (6-8). The plant possesses the secondary metabolites C_{28}-steroidal lactones based on the ergostane frame work are exclusive to the *Solanceae* (9).

There is presence of several steroidal molecules from the whole plant but the detailed chemical constituents of different parts of the plant is lacking. Our work on the flowers and fruits yielded several components (10). However, on extending our studies, on leaves, stem and roots has yielded several steroids whose identification is discussed presently.

MATERIALS AND METHODS

Instrumentation

Melting points were taken on Fisher-Johns melting apparatus. The ^1H and ^{13}C NMR spectra were recorded $CDCl_3$ using TMS as internal standard on Bruker AV-300 FTNMR spectrometer at 300 and 75 MHz, respectively. 2D NMR spectra were also recorded on the same instrument. EI Mass spectra were recorded on a Jeol D 300 spectrometer while FAB Mass spectra were recorded on JEOL SX 102/DA-6000 Mass Spectrometer. FT IR spectra were recorded on a Perkins Elmer 1710 B instrument. Column chromatography was performed on silica gel (60~120 mesh, Merck). Thin layer chromatography (TLC) was performed on silica gel G (10 to 40 mesh, Merck) in a solvent system ($CHCl_3$:EtOAc :MeOH:C_6H_6=70:2:4:24). Spots were visualized by spraying anisaldehyde-sulphuric acid reagent and heating in the oven at 110°C.

Collection of plant material

The plant materials (leaves, stem and roots) of *Physalis minima* used for this study were collected in September 2004 from Lucknow, India. The weights of fresh plant materials were as follows: 1.014 kg leaves, 2.815 kg stem and 213.0 g roots.

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Extraction and chromatographic separation of the constituents of chloroform extracts

Extraction and isolation of compounds from leaves: The fresh leaves (1.014 kg) were ground in the presence of liquid nitrogen and extracted with 25% methanol three times (2.0 L first time and 1.0 L each for second and third time) in percolator by keeping at room temperature overnight. The extracts were concentrated on rotavapour at 50°C under reduced pressure. The concentrated aqueous extract was defatted with n-hexane three times (600 mL×3) and was further partitioned with chloroform three times (500 mL). The chloroform extract was treated with anhydrous sodium sulphate and dried completely on rotavapour at 40°C under reduced pressure. The chloroform extract (2.0 g) thus obtained, was adsorbed on silica gel (2.5 g) and chromatographed over a glass column of silica gel (110×2.5 cm) using 275 g silica gel as stationary phase and n-hexane as a mobile phase. The polarity was increased sequentially by adding 10, 20, 30, 40, 50, 60, 70, 80, 90% ethyl acetate in n-hexane, then pure ethyl acetate and finally 2.5, 5, 10 and 25% methanol was added in ethyl acetate. Several small fractions were collected and pooled into nine major fractions based on their TLC pattern. Fraction 1 and 2 gave nothing of interest while fraction 3 and 4 yielded stigmasterol (R_f 0.30, ethyl acetate:n-hexane=1:4, 16.0 mg) and β-sitosterol (R_f 0.25, EtOAc:n-hexane=1:4, 12.0 mg). Fraction 5 on crystallization in ethyl acetate yielded withanone (R_f 0.60, 12.0 mg) while fraction 6 after further purification gave withanone (6.0 mg) and withaferin A (R_f 0.52, 14.0 mg). Fraction 7 after separations afforded mainly withaphysalin A (R_f 0.50, 17.0 mg) while fraction 8 after further purification by column chromatography yielded withaphysalin A (4.0 mg) and withaphysalin B (R_f 0.45, 10.0 mg). Fraction 9 was a complex mixture.

Extraction and isolation of compounds from stem: The fresh stems (2.815 kg) were ground in the presence of liquid nitrogen and extracted with 25% methanol in water three times (3.0 L first time and 2.0 L each for second and third time) by keeping at room temperature overnight. The extract was concentrated on rotavapour at 50°C and reduced pressure. The concentrated aqueous extract was defatted with n-hexane (800 mL×3) and was further partitioned with chloroform three times (600 mL). The chloroform extract was treated with anhydrous sodium sulphate and dried completely on rotavapour at 40°C temperature and reduced pressure. The chloroform extract (8.0 g) thus obtained, was adsorbed on silica gel (6.0 g) and chromatographed over a glass column of a silica gel (140×3.0 cm) using 390 g silica gel as stationary phase and n-hexane as mobile phase. The polarity was increased sequentially by adding 10, 20, 30, 40, 50, 60, 70, 80, 90% ethyl acetate in n-hexane, then pure ethyl acetate and finally 10 and 25% methanol was added in ethyl acetate. Several small fractions were collected and pooled into 7 fractions based on their TLC pattern. Fraction 1 and 2 gave nothing of interest while fraction 3 yielded withaphysalin A (R_f 0.52, 19.0 mg). Fraction 4 after purification gave withaphysalin A (4.0 mg) and withaphysalin B (R_f 0.45, 21.0 mg) while fraction 5 after separations afforded mainly withaphysalin B (5.5 mg). Fraction 6 and 7 were complex mixtures.

Extraction and isolation of compounds from roots: The fresh roots (213 g) were ground in the presence of liquid nitrogen and extracted with 25% MeOH three times (500 mL first time and 300 mL each for second and third time) in percolator by keeping at room temperature overnight. The aqueous extract was concentrated on rotavapour at 50°C and reduced pressure and was defatted with n-hexane (200 mL×3). The defatted extract was further partitioned with chloroform three times (200 mL), treated with anhydrous sodium sulphate and dried completely on rotavapour at 40°C under reduced pressure. The chloroform extract (1.50 g) thus obtained, was adsorbed on silica gel (2.0 g) and chromatographed over silica gel using n-hexane as the mobile phase. The polarity was increased sequentially by adding 25, 40, 50, 60, 70, 80, 90% ethyl acetate in n-hexane, then pure ethyl acetate and finally 5 and 25% methanol was added in ethyl acetate. Several small fractions were collected and pooled into 5 fractions based on their TLC pattern. Fraction 1 gave nothing of interest while fraction 2 yielded withanolide A (R_f 0.64, 15.0 mg). Fraction 3 after further purification by preparative TLC gave withanolide A (3.5 mg) and withanone (R_f 0.60, 9.0 mg) while fraction 4 after crystallization in ethyl acetate afforded withaferin A (R_f 0.52, 13.0 mg). Fraction 5 was a complex mixture.

RESULTS AND DISCUSSION

Spectral data of the isolated compounds

The compounds identified from *Physalis minima* L. (Solanaceaus) were stigmasterol, β-sitosterol, withaphysalin A, withaphysalin B, withanolide A, withanone, withaferin A. Their spectral data were shown in Table 1. Stigmasterol and β-sitosterol were crystalline compounds, mp 167°C and mp 139°C, respectively. Withaphysalin A, withaphysalin B, withanolide A and withanone were shining crystals (EtOAc, EtOAc, $CHCl_3$ and EtOAc, respectively); mp 222~223°C, 261~262°C, 282~284°C and 283°C, respectively. For acetylation of withaphysalin B and withaferin A, 5 mg of the samples was acetylated with acetic anhydride (1.5 mL) in the presence of pyri-

Table 1. Spectral data of the compounds isolated from *Physalis minima* L.

Chemical name	mp	IR (KBr, cm^{-1})	MS *m/z* (relative intensity)
Stigmasterol	167°C	3450-3300 (OH), 2930, 1665, 1460, 1375, 1145, 1050, 825	412 $[M]^+$ ($C_{29}H_{48}O$) (32), 397 $[M-CH_3]^+$ (52), 394 $[M-H_2O]^+$ ($C_{29}H_{46}$) (10), 384 (30), 290 (20), 274 (30), 255 (100), 213 (14), 57 (30), 55 (25), 43 (45)
β-Sitosterol	139°C	3418 (OH), 2971, 2865, 1645, 1456, 1378, 1616 1145, 1050, 1020	414 $[M]^+$ ($C_{29}H_{50}O$) (16), 399 $[M-CH_3]^+$ (45), 396 $[M-H_2O]^+$ ($C_{29}H_{48}$) (32), 386 (35), 276 (32), 255 (65), 213 (20), 145 (60), 119 (33), 95 (40), 43 (100)
Withaphysalin A	222~223°C	3350, 2950, 2900, 1755, 1695, 1640	466 $[M]^+$, 430 $[M-2H_2O]^+$ (4), 415 $[430-Me]^+$ (2), 348 (5), 273 (10), 262 (100), 185 (10), 77 (20), 43 (25)
Withaphysalin B	261~262°C	3350, 2950, 2900, 1700, 1650, 1640	468 $[M]^+$, 432 $[M-2H_2O]^+$ (4), 417 $[432-Me]^+$ (2), 348 (5), 273 (10), 262 (100), 185 (10), 77 (20), 43 (25)
Withanolide A	282~284°C	3450, 2990, 2920, 2880, 1715, 1685, 1460, 1375, 1290, 1210, 1150, 1120, 1020, 900, 810, 620	470 $[M^+]$ (2.5), 452 $[M^+-H_2O]$ (2.5), 434 $[M^+-2H_2O]$ (5), 344 (65), 326 (25), 292 (5), 280 (5), 261 (12), 169 (100), 125 (35)
Withanone	283°C	3465, 2990, 2950, 2900, 1690, 1680, 1470, 1410, 1325, 1250, 1215, 1185, 1130, 1045, 1020, 800, 620	434 $[M-2H_2O]^+$ (4); 419 $[434-Me]^+$ (2); 348 (5); 273 (10); 262 (100); 185 (10); 77 (20); 43 (25)
Withaferin A	272°C	3430, 2970, 2940, 2880, 1710, 1690, 1450, 1410, 1320, 1225, 1200, 1185, 1125, 1030, 1025	470 $[M]^+$ ($C_{28}H_{38}O_6$) (11), 452 $[M-H_2O]^+$ (6), 434 $[452-H_2O]^+$ (4), 416 $[434-H_2O]^+$ (3), 347 (24), 329 (5), 311 (5), 285 (22), 267 (20), 241 (34), 197 (21), 141 (80), 124 (100), 123 (60), 105 (38)

Withaphysalin A, withaphysalin B and withanone were measured by EIMS, and withanolide A and withaferin A were carried out by FABMS.

dine (0.5 mL) after usual workup. It afforded the acetate (5 mg) that gave a single spot on TLC, R_f 0.63 and R_f 0.68, respectively. The ^1H and ^{13}C NMR data of six compounds and the ^1H NMR data of three compounds were shown in Table 2, and the chemical structures of seven compounds and two acetylated compounds isolated from the leaves, stem and roots of *Physalis minima* were shown in Fig. 1.

Identification of these compounds isolated from the leaves, stem and roots of *P. minima*

The leaves, stem and roots of *P. minima* were investigated individually for their major constituents. The leaves afforded β-sitosterol, stigmasterol, withanone, withaferin A, withaphysalin A and withaphysalin B while the stem contained withaphysalin A and withaphysalin B. The roots gave withanone, withaferin A and withanolide A. The structures of withanone, withaferin A and withanolide A were elucidated by spectroscopic methods and isolated first time from the plant parts while β-sitosterol, stigmasterol, withaphysalin A and withaphysalin B (3,4,10-12) by comparing their spectroscopic values with those as reported in the literature.

Withanolide A : The IR spectrum showed absorption bands due to hydroxyl group (3450 cm^{-1}), lactone (1715 cm^{-1}), ketone (1685 cm^{-1}) and epoxide (1120 cm^{-1}) along with other typical bands of CH stretching and bending vibrations. Strong UV absorption at 228 nm supported the presence of α,β-unsaturated ketone. Its mass spectrum showed mass ion $[M]^+$ at *m/z* 470 in FAB MS corresponding to $C_{28}H_{38}O_6$. The ^1H NMR spectrum showed two angular methyl singlets at δ 0.96, 1.31 for H-18, H-19 along with two olefinic methyl singlets at δ 1.89 and 1.95 for H-27 and H-28, respectively. While a methyl singlet at δ 1.18 was assigned to H-21 methyl, which suggested that C-20 is oxygenated. The typical double doublet at δ 4.21 (1H, J=12.0, 4.0 Hz) for H-22 clearly indicated that typical E ring lactone is present. Upfield shifting of H-22 (~0.15 ppm) also indicated that C-20 is oxygenated. The vinylic signals at δ 5.85 (1H, d, J=10.0 Hz) and 6.59 (1H, m) were attributed to H-2 and H-3, respectively, in a steroidal 2-en-1-one system. The upfield shifting of H-2 and H-3 suggested that C-4 in A ring is not oxygenated which was evident by the multiplet for H-3 and a double doublet (J=10.0, 4.5 Hz) at δ 2.80 and 2.46 for H-4. The doublet (J=3.2 Hz) at δ 3.04 for H-6 and a double doublet (J=3.2, 2.1 Hz) at δ 3.16 for H-7 along with a broad singlet at δ 3.32 for OH at C-5 were assigned for vicinal protons of α epoxide group clearly establishing that 5α-hydroxy-6,7α-epoxy-2-en-1-one unit exist in the molecule (13,14).

The ^{13}C NMR and DEPT spectra also supported the withanolidal structure. The assignment of each carbon is presented in Table 2. This compound resisted acety-

Table 2. ^{1}H and ^{13}C NMR data of isolated compounds

Name of the compounds	^{1}H NMR	^{13}C NMR
Stigmasterol	δ 0.66 (s, 3H, C_{18}-H), 0.96 (s, 3H, C_{19}-H), 0.75-0.92 (br s, 12H, 4xCH_3), 3.52 (m, 1H, C_3-H), 5.32 (dd, 1H, J=5.0, 1.5 Hz, C_6-H), 5.14 (dd, 1H, J=9.0, 14.0 Hz, C_{22}-H), 4.98 (dd, 1H, J=9.0, 14.0 Hz, C_{23}-H)	δ 37.8 (C-1), 30.4 (C-2), 71.7 (C-3), 41.5 (C-4), 140.6 (C-5), 121.6 (C-6), 30.3 (C-7), 30.4 (C-8), 51.6 (C-9), 37.0 (C-10), 21.0 (C-11), 39.0 (C-12), 42.7 (C-13), 55.4 (C-14), 23.0 (C-15), 28.1 (C-16), 55.6 (C-17), 12.0 (C-18), 18.4 (C-19), 40.4 (C-20), 19.6 (C-21), 138.2 (C-22), 129.9 (C-23), 48.6 (C-24), 32.0 (C-25), 19.9 (C-26), 21.0 (C-27), 23.2 (C-28), 12.1 (C-29)
β-Sitosterol	δ 0.65 (s, 3H, C_{18}-H), 0.99 (s, 3H, C_{19}-H), 0.70-0.94 (br s, 12H, 4xCH_3), 3.50 (m, 1H, C_3-H), 5.32 (dd, 1H, J=5.0, 1.5 Hz, C_6-H)	δ 37.3 (C-1), 28.0 (C-2), 71.7 (C-3), 36.8 (C-4), 140.6 (C-5), 121.6 (C-6), 32.3 (C-7), 30.0 (C-8), 49.2 (C-9), 37.4 (C-10), 21.1 (C-11), 39.7 (C-12), 42.1 (C-13), 55.9 (C-14), 23.6 (C-15), 27.7 (C-16), 55.9 (C-17), 12.1 (C-18), 19.3 (C-19), 40.6 (C-20), 19.3 (C-21), 36.2 (C-22), 29.9 (C-23), 48.6 (C-24), 27.1 (C-25), 19.9 (C-26), 20.5 (C-27), 23.3 (C-28), 12.9 (C-29)
Withaphysalin A	δ 5.85 (d,1H, J=1.0 Hz, C_2-H), 6.81 (m, 1H, C_3-H), 5.60 (m, C_6-H), 1.29 (s, 3H, C_{19}-CH_3), 1.50 (s, C_{21}-CH_3), 4.55 and 4.2, C_{22}-H), 2.56 (d, 2H, J=5.5 Hz, C_{23}-H), 1.88 (s, 3H, C_{27}-CH_3), 1.94 (s, 3H, C_{28}-CH_3)	δ 204.3 (s, C-1), 127.6 (d, C-2), 146.0 (d, C-3), 33.3 (t, C-4), 135.1 (s, C-5), 124.6 (d, C-6), 26.2 (t, C-7), 39.6 (d, C-8), 37.8 (d, C-9), 51.4 (s, C-10), 23.3 (t, C-11), 34.4 (t, C-12), 59.6 (s, C-13), 83.2 (d, C-14), 34.9 (t, C-15), 24.7 (t, (C-16), 55.2 (d, C-17), 177.4 (s, C-18), 18.4 (q, C-19), 83.5 (s, C-20), 26.3 (q, (C-21), 78.8 (d, C-22), 31.7 (t, C-23), 148.5 (s, C-24), 122.3 (s, C-25), 167.8 (s C-26), 12.4 (q, C-27), 20.5 (q, C-28)
Withanolide A	δ 5.85 (d, 1H, J=10.0 Hz, C_2-H), 6.58 (m, 1H, C_3-H), 2.80 and 2.46 (dd, 2H, J=10.0, 4.5 Hz, C_4-H), 3.32 (br s, 1H, C_5-OH), 3.04 (d, 1H, J=3.2 Hz, C_6-H), 3.16 (dd, 1H, J=3.2, 2.1 Hz, C_7-H), 1.31 (s, 3H, C_{10}-CH_3), 0.96 (s, 3H, C_{13}-CH_3), 1.18 (s, 3H, C_{20}-CH_3), 4.21 (dd, 1H, J=12.0, 4.0 Hz, C_{22}-H), 2.46 (d, 2H, J=5.5 Hz, C_{23}-H), 1.89 (s, 3H, C_{25}-CH_3), 1.95 (s, 3H, C_{24}-CH_3)	δ 202.0 (s, C-1), 129.0 (d, C-2), 139.2 (d, C-3), 36.90 (t, C-4), 72.5 (s, C-5), 57.2 (d, C-6), 56.6 (d, C-7), 35.4 (d, C-8), 35.8 (d, C-9), 50.9 (s, C-10), 21.9 (t, C-11), 40.6 (t, C-12), 43.1 (s, C-13), 52.2 (d, C-14), 23.2 (t, C-15), 21.9 (t, C-16), 56.4 (d, C-17), 13.8 (q, C-18), 14.7 (q, C-19), 75.0 (s, C-20), 20.9 (q, C-21), 81.2 (d, C-22), 31.8 (t, C-23), 148.2 (s, C-24), 122.0 (s, C-25), 165.5 (s, C-26), 12.3 (q, C-27), 20.4 (q, C-28)
Withanone	δ 5.85 (d, 1H, J=10.0 Hz, C_2-H), 6.60 (m, 1H, C_3-H), 2.80 and 2.46 (dd, 2H, J=10.0, 4.5 Hz, C_4-H), 3.32 (br s, 1H, C_5-OH), 3.05 (d, 1H, J=3.2 Hz, C_6-H), 3.15 (dd, 1H, J=3.2, 2.1 Hz, C_7-H), 1.28 (s, 3H, C_{10}-CH_3), 0.86 (s, 3H, C_{13}-CH_3), 1.04 (d, 3H, J=7.0 Hz, C_{20}-CH_3), 4.60 (dt, 1H, J=8.5, 3.0 Hz, C_{22}-H), 2.56 (d, 2H, J=5.5 Hz, C_{23}-H), 1.88 (s, 3H, C_{25}-CH_3), 1.93 (s, 3H, C_{24}-CH_3)	δ 203.2 (s, C-1), 128.9 (d, C-2), 139.7 (d, C-3), 36.7 (t, C-4), 73.2 (s, C-5), 57.1 (d, C-6), 56.3 (d, C-7), 35.2 (d, C-8), 36.0 (d, C-9), 50.9 (s, C-10), 21.6 (t, C-11), 32.4 (t, C-12), 48.6 (s, C-13), 45.8 (d, C-14), 22.9 (t, C-15), 36.7 (t, C-16), 84.5 (s, C-17), 9.4 (q, C-18), 15.0 (q, C-19), 42.9 (d, C-20), 12.3 (q, C-21), 78.8 (d, C-22), 32.7 (t, C-23), 150.5 (s, C-24), 121.3 (s, C-25), 167.2 (s, C-26), 14.7 (q, C-27), 20.5 (q, C-28)
Withaferin A	δ 6.20 (d, 1H, J=10.0 Hz, C_2-H), 6.98 (dd, 1H, J=10.0, 6.0 Hz, C_3-H), 3.76 (d, 1H, J=6.0 Hz, C_4-H), 3.24 (br s, 1H, C_6-H), 0.75 (s, 3H, C_{13}-CH_3), 1.45 (s, 3H, C_{10}-CH_3), 1.02 (d, 3H, J=7.0 Hz, C_{20}-CH_3), 4.40 (dt, 1H, J=12.0, 3.5 Hz, C_{22}-H), 2.50 (br s, 2H, C_{23}-H), 4.35 (br s, 2H, C_{25}-CH_2OH), 2.02 (s, 3H, C_{24}-CH_3)	δ 201.8 (s, C-1), 131.6 (d, C-2), 143.0 (d, C-3), 69.2 (d, C-4), 63.2 (s, C-5), 60.2 (d, C-6), 30.8 (t, C-7), 29.3 (d, C-8), 43.6 (d, C-9), 42.0 (s, C-10), 20.9 (t, C-11), 38.7 (t, C-12), 47.0 (s, C-13), 55.5 (d, C-14), 23.8 (t, C-15), 26.8 (t, C-16), 51.3 (d, C-17), 11.1 (q, (C-18), 16.3 (q, C-19), 38.3 (d, C-20), 12.8 (q, C-21), 78.0 (d, C-22), 29.3 (t, C-23), 153.1 (s, C-24), 125.3 (s, C-25), 166.2 (s, C-26), 56.0 (t, C-27), 19.7 (q, C-28)
Withaphysalin B	δ 6.03 (d, 1H, J=10.0 Hz, C_2-H), 6.9 (m, 1H, C_3-H), 3.15 (d,1H, J=3.5 Hz, C_6-H), 5.17 (s, 1H, C_{18}-H), 1.26 (s, 3H, C_{19}-CH_3), 1.47 (s, C_{21}-CH_3), 4.58 (dd, 1H, J=11.5, 5.0 Hz, C_{22}-H), 2.56 (d, 2H, J=5.5 Hz, C_{23}-H), 1.86 (s, 3H, C_{27}-CH_3), 1.97 (s, 3H, C_{28}-CH_3)	–
Acetylation of withaphysalin B	δ 6.1 (d, 1H, J=10.0 Hz, C_2-H), 6.95 (m, 1H, C_3-H), 3.18 (d, 1H, J=3.5 Hz, C_6-H), 6.15 (s, 1H, C_{18}-H), 1.16 (s, 3H, C_{19}-CH_3), 1.45 (s, C_{21}-CH_3), 4.55 (dd, 1H, J=11.5, 5.0 Hz, C_{22}-H), 2.56 (d, 2H, J=5.5 Hz, C_{23}-H), 1.87 (s, 3H, C_{27}-CH_3), 1.98 (s, 3H, C_{28}-CH_3), 2.02 (s, 3H, C_{18}-$COCH_3$)	–
Acetylation of withaferin A	δ 6.26 (d, 1H, J=10.0 Hz, C_2-H), 7.06 (dd, 1H, J=10.0, 6.0 Hz, C_3-H), 4.67 (d, 1H, J=6.0 Hz, C_4-H), 3.24 (br s, 1H, C_6-H), 1.40 (s, 3H, C_{10}-CH_3), 0.71 (s, 3H, C_{13}-CH_3), 1.0 (d, 3H, J=7.0 Hz, C_{20}-CH_3), 4.42 (dt, 1H, J=12.0, 3.5 Hz, C_{22}-H), 2.53 (br s, 2H, C_{23}-H), 4.86 (br s, 2H, C_{25}-CH_2OCOCH_3), 2.06 (s, 9H, C_{24}-CH_3, C_4-$OCOCH_3$, C_{27}-$OCOCH_3$)	–

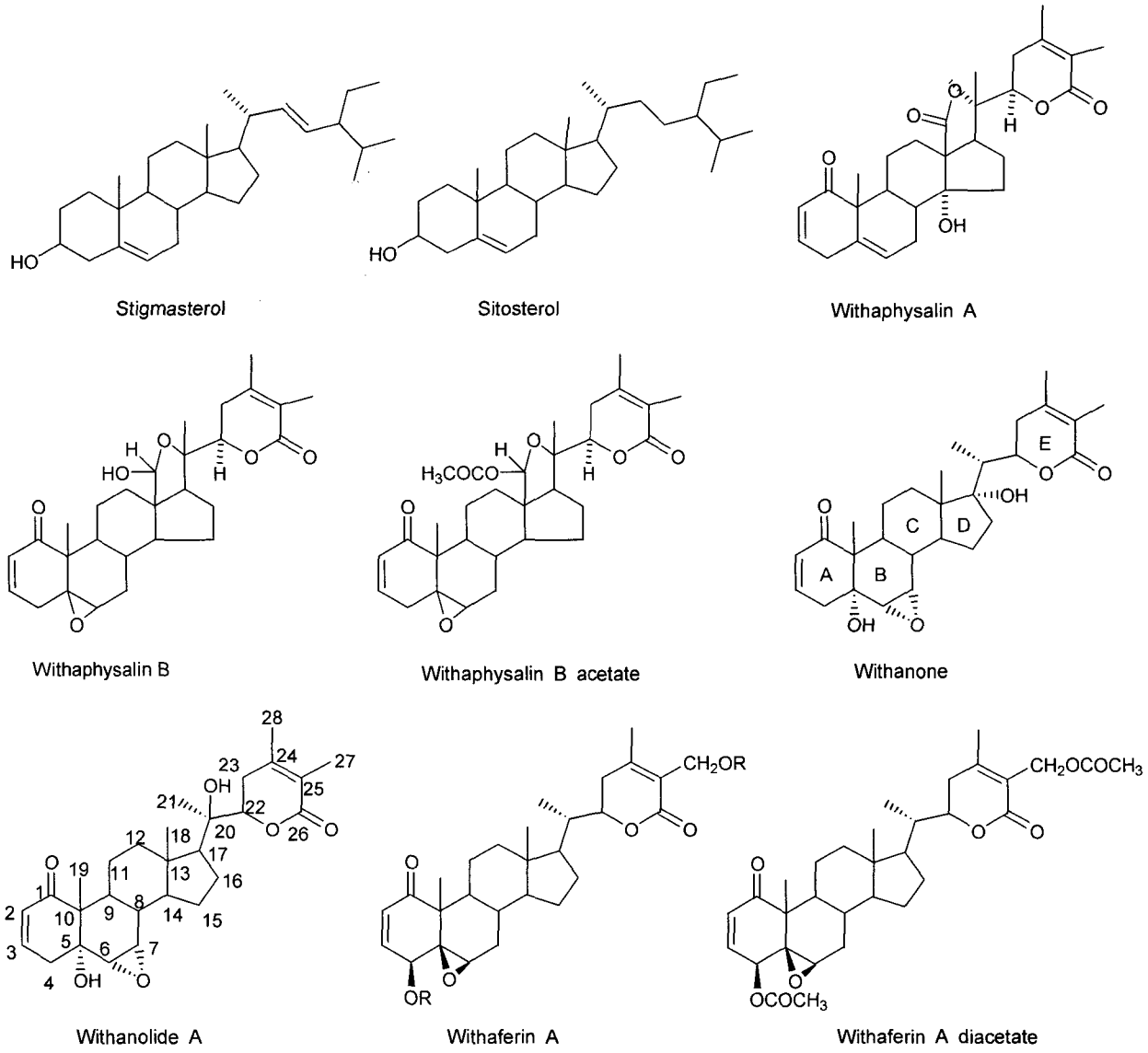

Fig. 1. The chemical structures of seven identified compounds and two acetylated compound isolated from theleaves, stem and roots of *Physalis minima.*

lation with acetic anhydride in the presence of pyridine supporting that it does not bear any primary or secondary hydroxyl group. The values of ^{1}H NMR and ^{13}C NMR were further confirmed by 2D NMR i.e. ^{1}H-^{1}H COSY, HMBC and HSQC. These data established that structure is 5 α,20 α-dihydroxy-6 α,7 α-epoxy-1-oxo-witha-2,24-dienolide and that it is comparable to the values already available in the literature for withanolide A (13).

Withanone: The IR spectrum showed bands for hydroxyl group (3465 cm^{-1}), lactone (1710 cm^{-1}), ketone (1690 cm^{-1}) and epoxide (1130 cm^{-1}) along with other typical bands of CH stretching and bending vibrations. Its mass spectrum showed $[M]^{+}$ fragments at *m/z* 434 for $[M\text{-}2H_2O]^{+}$ corresponding to $C_{28}H_{38}O_6$. The ^{1}H NMR spectrum was found very close to withanolide A, only difference was observed for methyl signal at C-20 that appeared doublet (J=7.0 Hz) δ 1.04 in place of singlet at δ 1.18 while typical H-22 signal appeared as double triplet at δ 4.60 (J=8.5 Hz, 3.0 Hz) indicated that typical E-ring lactone is present with C-17 bearing hydroxyl group while C-20 in not oxygenated. Rests of signals were almost similar to withanolide A (1). The ^{13}C NMR and DEPT spectra also supported the structure for withanone with five methyls, six methylenes, nine methine and eight quaternary carbon signal. It is obvious that C-17 is oxygenated with α hydroxyl group as it showed a singlet at δ 84.5 while C-20 is not oxygenated, as singlet at δ 75.0 was absent.

This compound did not acetylate with acetic anhydride in the presence of pyridine supporting that it does not

bear any primary or secondary hydroxyl group. These data established that structure is 5 α,17 α-dihydroxy-6 α, 7 α-epoxy-1-oxowitha-2,24-dienolide and that it is comparable to the values already available in the literature for withanone (14).

Withaferin A: The IR spectrum showed bands for hydroxyl group at (3430 cm^{-1}), lactone (1710 cm^{-1}), ketone (1690 cm^{-1}) and epoxide (1125 cm^{-1}) along with other typical bands for CH stretching and bending vibrations. Its mass spectrum showed $[M]^+$ peak at *m/z* 470 corresponding to $C_{28}H_{36}O_5$. The ^1H NMR spectrum showed two angular methyl singlets at δ 0.75, 1.45 for H-18, H-19 along with one olefinic methyl singlets at δ 2.02 for H-28, respectively. One secondary methyl doublet at δ 1.02 (J=7.0 Hz) was assigned to H-21 methyl, which confirmed that C-20 is not oxygenated. One methyl singlet was missing which support that is oxygenated to appear as a broad singlet of two protons at δ 4.35 for H-27 (-CH_2OH). The typical double triplet at δ 4.40 (J=12.0, 5.0, 3.5 Hz) for H-22 clearly indicated that typical E- ring lactone is present. The vinylic signals that appeared at δ 6.20 (1H, d, J=10.0 Hz) and 6.98 (1H, dd, J=10.0, 6.0 Hz) were attributed to H-2 and H-3, respectively, in a steroidal 2-en-1-one system. The downfield shifting of H-2 and H-3 suggested that C-4 in A ring is also oxygenated which was evident by the double doublet signal for H-3 and a doublet (J=6.0 Hz) at δ 3.76 for H-4. In the ^1H-^1H COSY spectrum, H-3 showed correlation with H-2 at δ 6.21 and for H-4 at δ 3.8. A broad singlet appeared at δ 3.2 for H-6 clearly indicating that a 4β-hydroxy-5β,6β-epoxy-2-en-1-one unit exist (9). The ^{13}C NMR and DEPT spectra also supported the withaferin A structure. The assignment of each carbon is presented in Table 2.

On acetylation with acetic anhydride in the presence of pyridine it afforded a diacetate, whose ^1H NMR spectrum showed two additional singlet at δ 2.06 and δ 2.08 for -$OCOCH_3$ and downfield shifting of the doublet for H-4 from δ 3.76 to 4.67 and the broad singlet of H-27 from δ 4.35 to 4.85 which confirmed that the molecule contains two hydroxyl groups at position H-4 and H-27. These data established that structure is 5β,6β -epoxy-4β,27-dihydroxy-1-oxowitha-2,24-dienolide i.e. withaferin A (9,15).

In conclusion, the major parts of the plant particularly fruits that are edible in some parts of the world while leaves and roots are used in case of ailment/disease. This type of comparative (qualitative and quantitative) study is important as consumption of herbs and food as plant part, organ and even tissue- specific. Therefore, phytochemical description need to be made available in the same frame rather then for the plant as a whole often practiced in chemical works. The study shows that plant part specific preponderance of specific withanolides and sterols. Particularly, significant presence of withaferin A and withanolide A in parts like leaves and roots have potential antioxidant, anti-stress, nutraceutical and commercial overtones. Withaferin A has been elucidated to be bio-active as COX-2 enzyme inhibitor, immuno- modulatar and anti-tumor while withanolide A is one of the lead molecule for use in neurological disorder like Alzheimer's and Parkinson's disease. It promotes neurite growth stimulating nerve communications (15-17). Similarly, withaphysalins can be resourced in the plant particularly its stem for bioprospection leads and incorporation of sterols in the diet may prevent or decrease the growth of tumors in human. Therefore, natural products or chemicals with the potential to scavenge singlet species could reduce biological disorders that limit the progression of various aging-related diseases (18).

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