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Components of Nutraceutical Value in *Physalis minima*

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Abstract

It is of utmost importance to feed the current world population by improving agricultural production with newer varieties of food crops, but what is still more important is to add nutrition into the food. Some of the plants, which are currently growing in the wastelands, contain certain phytochemicals which add to their neutraceutical and health value. These plants contain secondary metabolites which enhance the over all metabolic functions of the body. Withasteroids are one of such phytochemicals. These chemicals are almost exclusively found in plants of the *Solanaceae* family; one of which, *Physalis minima*, contains several withasteroids. The aerial parts and roots of *P. minima* have been found to contain several steroids, identification of which is been discussed in this paper. These withasteroids contribute to the functional value since incorporation of withanolides in the diet may prevent or decrease the growth of tumors in humans.

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

INTRODUCTION

Physalis Linn. (*Solanaceaus*) is a genus of herbaceous annuals or perennials, mostly natives of tropical North and South America, with a few species widely distributed in the warmer parts of the world. Some species are grown for their edible fruits. One or two species occur wild in India, while the others are cultivated (1). *Physalis minima*, is an annual herb growing wildly in India, Ceylon and tropical region of Africa. It is commonly found along the boundaries of fields, in wastelands around the houses, and on roadsides etc. where the soil is porous and rich in organic matter (2). The plant has a bitter taste and Indian popular medicine recommends it, *Inter alia*, as a diuretic and laxative and in the treatment of inflammations of the spleen (3,4). 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).

Physalis minima has been widely used in the folk medicine. The roots are used as a vermifuge, febrifuge and for diabetes. The leaves are used in the treatment of infectious hepatitis and gonorrhea while the fruits are used against dropsy, urinary diseases and gout in India (6,7). The fruits and leaves are reported to be edible. The fruits are used for preserves in some parts of Africa. The fruits are considered to be tonic, diuretic and purgative. The leaves and roots are used for medicinal purposes. The fruits of *Physalis minima* var. *indica* are used to form an ingredient in medicinal oil given for spleen disorders (8). The plant has been used in the indigenous system of medicine and the abortifacient activity of physalin has been studied previously (9).

Previous work on the *P. minima* has revealed the presence of several steroidal molecules (2-4). Particularly physalins are the appropriately oxidized ergostane skeletal C_{28} compounds where C_{20} and C_{18} are characteristically oxidized to form a γ-lactone or lactol ring (10,11). Our work on the flowers and fruits has yielded several other important biologically active steroids for the first time from this plant (*Physalis minima*: ground cherry) whose identification is discussed in the present paper. The chemotyping of the plant and its different parts is useful for identifying useful phytochemical components, facilitating its use and consumption for biological functionalities.

MATERIALS AND METHODS

Instrumentation

Melting points were taken on Fisher-Johns melting apparatus. The ^{1}H NMR spectra were recorded on 300 MHz Bruker AV-300 FT NMR spectrometer as well as ^{13}C NMR and DEPT spectra were recorded on 75 MHz Bruker AV-300 FT NMR spectrometer. ^{1}H-^{1}H COSY, HMBC and HSQC were also recorded on Bruker FT 300 MHz. All the spectra were recorded in $CDCl_3$ using

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TMS as internal standard. EI Mass spectrum was recorded on a Jeol D 300 spectrometer and FAB Mass spectra were recorded on JEOL SX 102/DA-6000 Mass Spectrometer/Data System. 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 used for this study i.e. flowers and fruits of *Physalis minima* (ground cherry) were collected in the month of September, 2004 from Lucknow, India. The weights of collected fresh plant materials were as follows: 280 g flowers and 430 g fruits.

Extraction and chromatographic separation of the constituents of chloroform extracts

Extraction and isolation of compounds from flowers: The fresh plant material (flowers) 280 g were ground (powdered) in a grinder in the presence of liquid nitrogen. The powdered plant material was extracted with 25% MeOH in water three times (500 mL first time and 300 mL each for second and third time) in the 1000 mL glass percolator by keeping at room temperature overnight. The aqueous extract was pooled together and concentrated on rotavapour at 50°C and reduced pressure.

The concentrated aqueous extract was defatted three times, with n-hexane (300 mL) by partition chromatography. The defatted aqueous extract was further partition chromatographed three times with chloroform (300 mL). The chloroform extract was treated with anhydrous sodium sulphate and dried completely on a rotavapour at 40°C and reduced pressure. Thus, 4.5 g chloroform extract was obtained whose TLC showed well-resolved and different patterns.

The chloroform extract (4.5 g) was adsorbed on silica gel (3.5 g) and chromatographed over a column of a silica gel. The extract was packed in a glass column using 250 g silica gel as the stationary phase and n-hexane as the mobile phase. The polarity was increased sequentially by adding 20, 30, 40, 50, 60, 70, 80, 90% ethyl acetate in n-hexane, then pure ethyl acetate and finally 5, 10 and 25% methanol was added in ethyl acetate. Several small fractions were collected and pooled into 6 major fractions based on their TLC pattern. Fraction 1 gave nothing of interest while fraction 2 yielded **1** (R_f : 0.64, 22.0 mg); fraction 3 after further purification gave **2** (R_f: 0.60, 18.0 mg); fraction 4 after further purification and crystallization in ethyl acetate afforded **2** (3.0 mg) and **3** (R_f: 0.52, 16.0 mg). Fraction 5 and 6 were complex mixtures.

Extraction and isolation of compounds from fruits: The fresh plant material (fruits) 430 g were ground (powdered) in a grinder in the presence of liquid nitrogen. The powdered plant material was extracted with 25% methanol in water three times (800 mL first time and 500 mL each for second and third time) in a 2000 mL glass percolator by keeping at room temperature overnight. The aqueous extracts were pooled together and concentrated on rotavapour at 50°C and reduced pressure.

The concentrated aqueous extract was defatted three times, with n-hexane (400 mL) by partition chromatography. The defatted aqueous extract was further partition chromatographed three times with chloroform (400 mL). The chloroform extract was treated with anhydrous sodium sulphate and dried completely on rotavapour at 40°C and reduced pressure. Thus, 5.0 g chloroform extract was obtained whose TLC studies showed well resolved and different patterns.

The chloroform extract (5.0 g) was adsorbed on silica gel (4.5 g) and chromatographed over a silica gel column. The extract was loaded in a glass column using 245 g silica gel as the stationary phase and n-hexane as the mobile phase. The polarity was increased sequentially by adding 20, 30, 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 six major fractions based on their TLC pattern. Fraction 1 gave nothing of interest while fraction 2 yielded **1** (R_f: 0.64, 25.0 mg); fraction 3 after further purification gave **1** (4.0 g) and **2** (R_f: 0.60, 18.0 mg); fraction 4 and 5 after crystallization in ethyl acetate afforded **2** (3.5 g) and **3** (R_f: 0.52, 8.0 mg). Fraction 6 was a complex mixture.

RESULTS AND DISCUSSION

Spectral data of the isolated compounds

Compound 1 was obtained as colorless shining crystals from chloroform, mp 282 ~ 284°C. Compound 2 was obtained as colorless shining crystals from ethylacetate, mp 283°C. Compound 3 was obtained as colorless crystals from ethyl acetate, mp 272°C. The spectral data on several analytical methods of the three compounds were shown in Table 1, and the chemical structures of three identified compounds and an acetylated compound isolated from the fruits and flowers of *Physalis minima* were shown in Fig. 1.

Table 1. The spectral data of three isolated compounds

	Compound		
	1	2	3
IR (KBr, cm^{-1})	3450, 2990, 2920, 2880, 1715, 1685, 1460, 1430, 1375, 1290, 1210, 1150, 1120, 1020, 900, 810, 620	3465, 2990, 2950, 2900, 1710, 1690, 1680, 1470, 1410, 1325, 1250, 1215, 1185, 1130, 1045, 1020, 800, 620	3430, 2970, 2940, 2880, 1710, 1690, 1450, 1410, 1320, 1225, 1200, 1185, 1125, 1030, 1025, 810, 610
EIMS (relative intensity)	*m/z* 470 (M^+, 2.5), 452 (M^+-H_2O, 2.5), 434 (M^+-$2H_2O$, 5), 344 (65), 326 (25), 292 (5), 280 (5), 261 (12.5), 169 (100), 125 (35)	*m/z* 434 $[M-2H_2O]^+$ (4); 419 [434- Me]$^+$ (2); 348 (5); 273 (10); 262 (100); 185 (10); 77 (20); 43 (25)	*m/z* 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), 95 (90), 79 (30), 67 (58), 55 (58)
^1H NMR	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) and 1.95 (s, 3H, C_{24}-CH_3)	δ 5.85 (d, 1H, J=10.0 Hz, C_2-H), 6.60 (m, 1H, C3-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 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)
^{13}C NMR	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)	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)	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)

Acetylation of compound 3 (4)

Compound 3 (5 mg) was refluxed with acetic anhydride (1.5 mL) in the presence of pyridine (0.5 mL) on a water bath for 5 hours. The reaction mixture was transferred to 10 mL water in a separatory funnel and extracted with chloroform. The chloroform extract was washed with water three times and dried over anhydrous sodium sulphate. Co-TLC was checked with the original compound and it was found that the acetate spot was above the original compound's spot, thus proving that acetylation had occurred. The acetate 4 (5 mg) gave a single spot on TLC (R_f: 0.68).

^1H NMR: 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) and C_4-$OCOCH_3$ and C_{27}-$OCOCH_3$).

Identification of these compounds isolated from the flowers and fruits of *P. minima*

The flowers and fruits of *P. minima* were investigated individually to identify their major constituents. The flowers and fruits afforded withanolide A (1), withanone (2), withaferin A (3), whose structures were elucidated by spectroscopic methods.

Withanolide A (1)

The IR spectrum of **1** showed bands at 3450 cm^{-1} (OH), 1715 cm^{-1} (α,β-unsaturated-δ-lactone), 1685

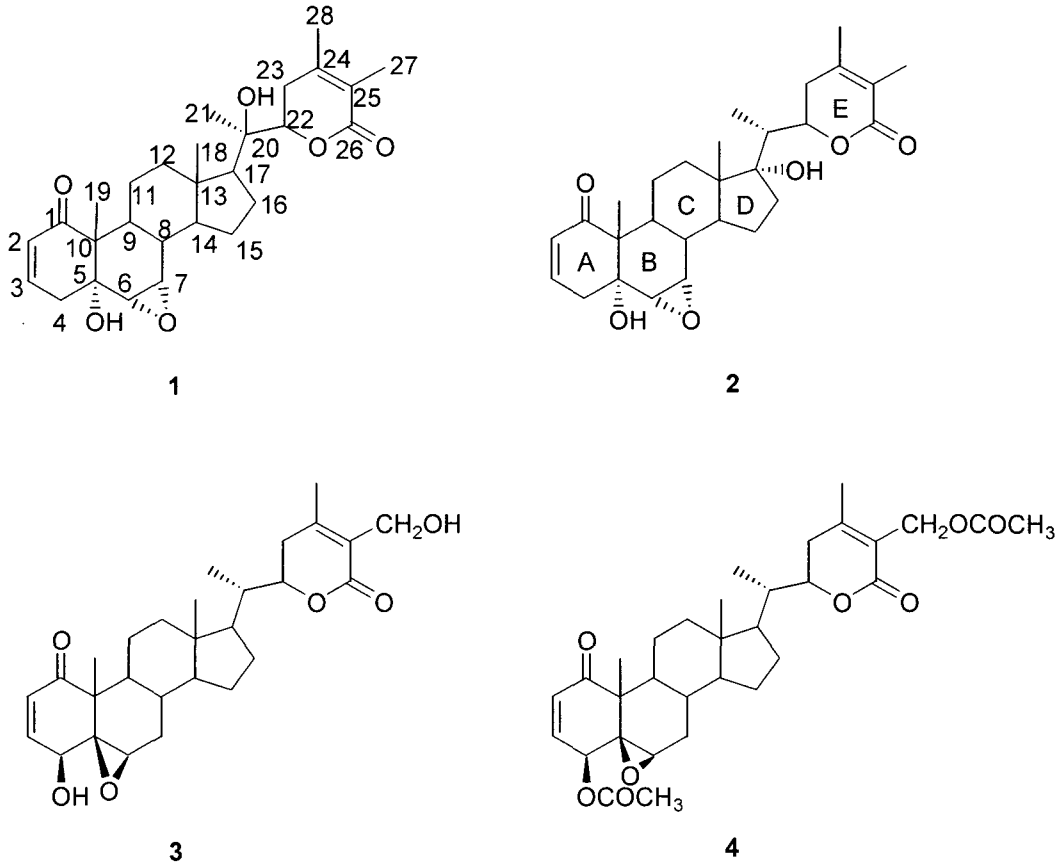

Fig. 1. The chemical structures of three identified compounds and an acetylated compound isolated from the fruits and flowers of *Physalis minima.*

cm^{-1} (α,β-unsaturated six membered ketone) and 1120 cm^{-1} for an epoxide group along with other typical signals 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 $[M]^+$ fragments at *m/z* 470 in FAB MASS corresponding to $C_{28}H_{38}O_6$, followed by further fragments at *m/z* 452 for $[M-H_2O]^+$ and at *m/z* 434 for $[M-2H_2O]^+$ along with the base peak at *m/z* 169 and by other characteristic fragments of withanolides/withasteroids.

The ^1H NMR spectrum showed the following characteristic signals for the steroidal molecule: two angular methyl singlets for H-18, H-19 were appeared at δ 0.96, 1.31 along with two olefinic methyl singlets at δ 1.89 and 1.95 for H-27 and H-28 protons, respectively. While a singlet of one methyl at δ 1.18 was assigned to H-21 methyl, which suggested that C-20 is oxygenated or substituted. Another 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 bearing the hydroxyl group. Upfield shifting of H-22 (~0.15 ppm) value also indicated that C-20 is oxygenated. The vinylic signals that appeared at δ 5.85 (d, 1H, J=10.0 Hz) and 6.58 (m, 1H) were attributed to H-2 and H-3 protons, 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 (OH) were assigned for vicinal proton of α epoxide group. The above assignment has clearly established that a 5 α-hydroxy-6 α,7 α-epoxy-2-en-1-one unit is present in the molecule, and that it is comparable to the values already available in the literature for withanolides (12,13).

The ^{13}C NMR and DEPT spectra of compound **1** also supported the withanolidal structure. The signals at δ 202.0, 129.0, 139.2, 36.9, 72.5, 57.2 and 56.60 are typical to α,β-unsaturated ketone with hydroxyl at C-5 and epoxide at C-6, C-7. Similarly, the signals at δ 81.2, 31.8, 148.2, 122.0 and 165.5 showed that C-22 and C-26 were oxidized to form a α,β-unsaturated-δ-lactone ring system in E ring of the molecule. It is obvious that C-20 is oxygenated with a β hydroxyl group, since it showed a singlet at δ 75.0. In addition, signals at δ 3.8, 14.7,

20.9, 12.3 and 20.4 correspond to five methyls, while δ 21.9, 40.6, 23.2 and 21.9 corresponded to four methylene carbons in the molecules. The singlets at δ 50.9 and 43.1 also matched for the quaternary carbons of the withasteroids.

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 the structure of compound **1** is 5α,20α-dihydroxy-6α,7α-epoxy-1-oxo-witha-2,24-dienolide, which is comparable to the values already available in the literature for withanolide A (14).

Compound **1** resisted acetylation with acetic anhydride in the presence of pyridine supporting that it does not bear any primary or secondary hydroxyl group.

Withanone 2

The IR spectrum of 2 showed bands at 3465 cm^{-1} (OH), 1710 cm^{-1} (α,β-unsaturated-δ-lactone), 1690 cm^{-1} (α,β-unsaturated ketone) and 1130 cm^{-1} for an epoxide group along with other typical signals of CH stretching and bending vibrations. Its mass spectrum showed $[M]^{+}$ fragments at *m/z* 434 for $[M-2H_2O]^{+}$ corresponding to $C_{28}H_{38}O_6$ and at *m/z* 419 along with the base peak at *m/z* 262 followed by other characteristic fragments of withanolides/withasteroids.

The ^{1}H NMR spectrum of **2** showed the following characteristic signals for the steroidal molecule: two angular methyl singlets for H-18, H-19 were present at δ 0.86, 1.28 along with two olefinic methyl singlets at δ 1.88 and 1.93 for H-27 and H-28 protons, respectively. One secondary methyl doublet at δ 1.04 (J=7.0 Hz) was assigned to H-21 methyl, which suggested that C-20 is not oxygenated or substituted. Another double triplet at δ 4.60 (J=8.5 Hz, 3.0 Hz) for H-22 clearly indicated that typical E ring lactone is present. Downfield shifting of the H-22 (~0.25 ppm) value also indicated that C-17 is oxygenated.

The vinylic signals that appeared at δ 5.85 (d) and 6.60 (m) were attributed to H-2 and H-3 protons, 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 at δ 3.05 for H-6 and double doublet at δ 3.15 for H-7 were assigned for an epoxide group along with a broad singlet at δ 3.32 (5-OH). The above assignment clearly established that 5α-hydroxy-6α,7α-epoxy-2-en-1-one unit is present in the molecule.

The ^{13}C NMR and DEPT spectra of **2** also supported the structure for withanone. The signals at δ 203.2, 128.9, 139.7, 36.7, 73.2, 57.1 and 56.30 which are typical to α,β-unsaturated ketone with hydroxyl at C-5 and epoxide at C-6, C-7. Similarly, the signals at δ 78.8, 32.7, 150.0, 121.3 and 167.2 showed that C-22 and C-26 oxidized to form α,β-unsaturated-δ-lactone ring system in the E ring of the molecule. It is obvious that C-17 is oxygenated with α hydroxyl group as it showed a singlet at δ 84.5. In addition, signals at δ 9.4, 15.0, 12.3, 14.7 and 20.5, correspond to five methyls, while δ 21.6, 32.4, 22.9 and 36.7 corresponds to four methylenes as well as the signals at δ 50.9 and δ 48.6 also matched for the quaternary carbons of the withasteroids.

Compound **2** 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 the structure of compound 2 is 5α,17α-dihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide, which is comparable to the values already available in the literature for withanone (15).

Withaferin A 3

The IR spectrum of **3** showed bands at 3430 cm^{-1} (OH), 1710 cm^{-1} (α,β-unsaturated-δ-lactone), 1690 cm^{-1} (α,β-unsaturated ketone) and 1125 cm^{-1} for an epoxide group along with other typical signals of CH stretching and bending vibrations. Its mass spectrum showed $[M]^{+}$ peak at *m/z* 470 corresponding to $C_{28}H_{36}O_5$, at *m/z* 452 for $[M-H_2O]^{+}$, at *m/z* 434 for $[M-2H_2O]^{+}$ and the base peak at *m/z* 124 along with other characteristic fragments of withanolides/withasteroids.

The ^{1}H NMR spectrum of **3** showed following characteristic signals for the steroidal molecule: two angular methyl singlets for H-18, H-19 were present at δ 0.75, 1.45 along with one olefinic methyl singlets at δ 2.02 for H-28 protons, 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 or substituted. In the ^{1}H NMR spectrum one methyl singlet was missing, which supports that one methyl is oxygenated to appear as a broad singlet of two protons at δ 4.35 for H-27 (-CH_2OH). Another double triplet at δ 4.40 (J=12.0, 3.5 Hz) for H-22 clearly indicated that typical the E ring lactone is present. The vinylic signals that appeared at δ 6.20 doublet (1H, J=10.0 Hz) and 6.98 double doublet (1 H, J=10.0, 6.0 Hz) were attributed to H-2 and H-3 protons, 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 ^{1}H-^{1}H COSY spectrum of **3**, H-3 showed correlation with H-2 at δ 6.21 and for H-4 at δ 3.76 1H,

d, J=6.0 Hz). 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 is present in the molecule which is comparable to the values of withaferin A already known compound (14).

The ^{13}C NMR and DEPT spectra of compound **3** also supported the structure for withaferin A. The signals at δ 201.8, 131.6, 143.0, 69.2, 63.2, 60.2, which are typical to α,β-unsaturated ketone with hydroxyl at C-4 and epoxide at C-5, C-6. Similarly, δ 78.0, 29.3, 153.1, 125.3 and 166.2 showed that C-2 and C-26 oxidized to form α,β-unsaturated δ lactone ring system is present in E ring of the molecule, where as δ 56.0 was assigned to the oxygenated methylene. In addition, signals at δ 11.1, 16.3, 12.8 and 19.7 corresponded to four methyls, while δ 30.8, 20.9, 38.7, 23.8 and 26.8 corresponds to four methylenes and the signals at δ 42.0 and 47.0 also matched for the quaternary carbons of the withasteroids.

On acetylation **3** with acetic anhydride in the presence of pyridine afforded a diacetate **4**, which showed two additional singlets at δ 2.06 and 2.08 for -$OCOCH_3$ in ^{1}H NMR spectrum. The 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 indicated that the molecule contains two hydroxyl groups at position H-4 and H-27. These data established that the structure of 3 is 5β,6β -epoxy-4β,27-dihydroxy-1-oxowitha-2,24-dienolide i.e. withaferin A.

In conclusion, the major parts of the plant, particularly those that are edible, in some parts of the world or in the case of certain ailment/disease are believed to have nutraceutical value. This type of qualitative and quantitative chemotyping is important since consumption of herbs and food is 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 organ and plant part specific preponderance of specific withanolides and sterols. Particularly, significant presence of withaferin A and withanolide A in parts like flowers and fruits has potential nutraceutical and commercial/industrial overtones. Withaferin A has been demonstrated to be a bio-active as immuno-modulatar and anti-tumor agent while withanolide A is one an important molecule for use in neurological disorder like Alzheimer's and Parkinson's disease. It promotes neurite growth stimulating nerve communications and incorporation of withanolides in the diet may prevent or decrease the growth of tumors in human (15,16).

Similarly, withaphysalins can be resourced in the plant particularly its stem for bioprospection leads. However, it may be mentioned that the physalin derivatives (10,11) may synergistically or antagonistically modulate the activity of the extracts or these withanolides of *P. minima*.

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