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Chemical constituents from the fruits of *Withania coagulans* (Stocks) Dunal

SHOWKAT RASSOL MIR[1], MOHAMMED ALI[1]*✉, MOHAMMAD WARIS[1] AND SHAHNAZ SULTANA[1,2]

[1]Phytopharmaceuticals Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, P.O. Hamdard Nagar, New Delhi-110062, India
[2]Present address: College of Pharmacy, Jazan University, Jazan, Saudi Arabia

ABSTRACT

Withania coagulans (Stocks) Dunal (family: Solanaceae) is a rigid undershrub found in Iran, Afghanistan, Pakistan, northern India and Nepal. Its fruits are used to treat asthma, biliousness, flatulent colic, cough, diabetes, dyspepsia, liver complaints, intestinal infections, skin rashes, stomachache, strangury and wounds. The air-dried fruits of *W. coagulans* were exhaustively extracted with methanol in a Soxhlet apparatus. The concentrated methanol extract was adsorbed on silica gel to be chromatographed on a silica gel column. The column was eluted with dichloromethane, ethyl acetate and methanol successively to isolate ten new phytoconstituents characterized as (3*R*,4*R*)-dihydroxyadipic-γ,γ′-dilactone (*n*-hexa-1(3),4(5)-diolide, **2**), (20*S*,22*R*)-1-oxo-witha-2,24-dienolide (withacoagulanide A, **3**), (20*S*,22*R*)-1-oxo-witha-24-enolide (withacoagulanide B, **4**), (20*S*,22*R*)-1-oxo-witha-6β-ol-2,24-dienolide **(**withacoagulanide C, **5**), (20*S*,22*R*)-1-oxo-witha-3β,5β-diol-24-enolide (withacoagulanide D, **6**), (20*S*,22*R*)-1-oxo-witha-6β-ol-2,24-dienolide-6β-D-arabinopyranosyl-2′-(2′′-methoxy)-benzoate (withacoagunalide C 6-arabinosyl 2′-*O*-anisate, **7**), (20*S*,22*R*)-1-oxo-witha-3β-ol-24-enolide-3β-*O*-D-galactoyranosyl-(2′→1′′)-β-*O*-D-galactopyranoside (3-*O*-digalactosyl withacoagulanide B, **8**), 1-oxo-3-seco-witha-21, 27, 28-trioic acid-24-ene-6β-ol-19(8), 18(11)-diolide-6β-*O*-D-galacuronopyranoside (3-secowithacoagulanolide 6β-olyl galactourinoside, **10**), (20*S*,22*R*)-1-oxo-witha-6β-ol-2,24-enolide-6β-*O*-D-(4′-acetoxy arabinopyranosyl-(2′→1′′)-(3′′,4′′-diacetoxy arabinopyranosyl)-2′′-(2′′′-methoxy)-benzoate (withacoagulanide C 6β-olyl diarabinosyl 2′′-(*O*)-anisate, **11**) and *n*-hexanoyl-β-*O*-D-xylopyranosyl-(2′→1′′)-β-*O*-D-xylopyranosyl-(2′′→1′′′)-β-*O*-D-xylopyrano- side (caproyl trixyloside, **12**) along with the known rare chemical compounds identified as cetyl palmitate (hexadecyl hexadecanoate, **1**) and glyceryl-1,2-dihexadecanoate-3-phosphate (glyceryl-1,2-dipalmityl 3-phosphate, **9**). The structures of isolated phytoconstituents were established on the basis of analysis of spectral data and chemical means.

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1. Introduction

The medicinal plants are useful to treat and manage various health problems. Global estimate indicates that 80% of the world populations are using traditional medicines, which are mainly derived from medicinal plants listing over 20,000 species (WHO 1993; Chaudhari, 2006). The human beings are dependent on botanicals and herbs for their food and health care issues (Mohammadhosseini et al., 2019). Natural products utilized in the modern therapy are the result of an approach that has been adopted during the past 50 years. Many phytochemical constituents exhibit bioactivities and impart several health benefits. Promising phytochemicals have been developed from the medicinal plants for many health problems (Gupta et al., 1994). Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). Natural products play an important role in drug development programs in the pharmaceutical industry (Baker et al., 1995). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. Most of the vegetable plants contained dietary antioxidants which are useful to reduce the risk of several diseases. Many phytochemical constituents exhibit bioactivities and impart several health benefits. Many food constituents play a vital role as essential nutrients in preventing and delaying the premature onset of chronic disease late in life. Medicinal plants are used for the development of new herbal drugs. Currently, about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. The utility of herbal remedies is cost-effective, safe and without any serious adverse effect. The rural elders, farmers and tribals of the world know about the plants being used for various purposes of health since thou-

✉Corresponding author: Mohammed Ali
Tel: +919968281082; Fax: +91-11-26059663
E-mail address: maliphyto@gmail.com

sands of years (Venditti et al., 2018). In India, Ayurveda and other traditional medicinal systems utilize more than 45,000 medicinal plant species for the treatment of a variety of ailments. The interest in the area of natural product medicines is growing exponentially due to the increased awareness of people towards the adverse effects of synthetic drugs (Venditti et al., 2018).

Withania coagulans (Stocks) Dunal, syn. *Puneeria coagulans* Stocks (family: Solanaceae), is a native to Afghanistan, Iran and the Indian subcontinent. Within the *Withania* genus, *W. somnifera* (Ashwagandha) and *W. coagulans* (Ashutosh booti) are economically significant and distributed in several regions for their medicinal uses and are distributed in east of the Mediterranean region extending to South Asia. *W. coagulans* is common in many parts of Afghanistan, Pakistan, India and Nepal. In India, it occurs in Punjab, Rajasthan, Simla, Kumaun and Garhwal (Anonymous, 2009). Unfortunately, the populations of this plant species have decreased and become scattered in the wild, which can be attributed to low rates of natural regeneration from reported that the seed germination of the *Withania* is poor and mortality rate of seedlings is high under field conditions. *W. coagulans* ecotypes based on their germination responses to light and germination media (Edalatifard et al., 2014). The plant is prone to leaf spot disease caused by *Alternaria alternata*.

As there are no cultivation practices for *W. coagulans*, the plant species is harvested from wild, causing loss of natural diversity of the germplasm. The natural regeneration rate does not sustain to keep up with the exploitation rate and the plant species is threatened in its habitat and declared critically endangered. Overexploitation, hostile environmental factors, habitat disturbances and reproductive failure threaten the survival and render the plant species vulnerable to complete extinction. Hence, harvest of plant material from wild is not a viable option to meet the commercial demand and some other strategies must be devised to meet the requirements. An efficient *in vitro* regeneration system for *W. coagulans* is a pre-requisite for its sustainable utilization to meet the pharmaceutical needs (Rathore et al., 2016).

This plant carries different local names, such as Akri, Punir bandh, Paneer dodi, or Puni-ke-bij (Hindi), Khamjira (Panjabi), Tukhmekaknaje-hindi (Persian), Spiubajja (Afghanistan), Punirband or Punir-ja-fota (Sindhi), Indian Cheese maker, Indian Rennet, Vegetable Rennet (English), Asvagandha (Bengal), Kaknaj (Bombay), Javzulmizaja (Arabic), Panneru-gadda (Telgu) and Hab kaknaj (Urdu) (Kirtikar and Basu, 1999 ; Anonymous, 2009). The berries of the shrub are used for milk coagulation to prepare paneer. The milk coagulating property of the fruits is attributed to the pulp and husk of berries which contain a rennet-like protease enzyme called withanin, having milk-coagulating activity. A small portion of the fruit is mixed with a minimum amount of water or milk and is added to the milk to coagulate it. One ounce of the fruits of *W. coagulans* when mixed with 1 quart of boiling water makes a decoction, one table spoonful of which is capable of coagulating a 3.5 L of milk in just an hour. Dried capsules also retain the coagulating property in an equal degree (Anonymous, 2009).

W. coagulans is a rigid grey-tomentose undershrub, 30-120 cm tall; branches terete; leaves are lanceolate oblong, sometimes ovate, obtuse, entire, thick, rugose, narrowed at the base with very short stalked, running down into an obscure, long, indistinct petiole, densely covered with minute, gray, stellated tomentum, 2.5-7.5 cm by 1.5 cm; flowers are 7-12 mm across, yellow, dioeciously, polygamous, in axillary cymose clusters, leathery 6 mm long calyx, campanulate, clothed with fine stellate gray tomentum; teeth triangular, 2.5 mm. long, corolla stellately outside, lobes ovate-oblong, subacute, male flowers stamens about level with the top of the corolla-tube, filaments 2 mm long, glabrous; anthers 3-4 mm long. Ovary ovoid, glabrous, without style or stigma; berries globose, smooth, enclosed in calyx; seeds are dark brown, ear shaped, glabrous, dark brown, having sharp fruity smell (Fig. 1). Flowering period is from January to April and berries are ripen in May. The natural regeneration is from seed (Anonymous, 2009). *W. coagulans* is a remarkable stress-relieving, blood purifier and anti-cancer drug, used to cure debility, diabetes, failure to thrive in children, impotence, insomnia, nervous exhaustion and wasting diseases. It is externally applied to relieve inflammatory conditions, ulcers, and scabies. The plant is mixed with Ashwagandh powder and oil to make an ointment, which is lapped to cure skin diseases. It acts as an antidote to scorpion sting (Gupta and Singh, 2018). Different parts of plant are used to treat ulcers, rheumatism, dropsy, and senile debility. The fruits are alterative, anodyne, blood purifier, diuretic, emetic, hepatoprotective, hypoglycemic, hypolipidemic, milk coagulant, refrigerant, sedative, stomachic, sweet, and tonic, used against asthma, biliousness, diabetes, dyspepsia, flatulent colic, intestinal infections, liver complaints, skin rashes, stomachache, strangury and wounds. The fruits are used to prepare a composite Ayurvedic medicine 'Liv 52' which is a hepatoprotective herbal preparation and contains aqueous extracts from *W. coagulans* and *W. somnifera* (Kirtikar and Basu, 1999; Quattrocchi, 2012). The twigs are chewed for cleaning of teeth and the smoke of the plant is inhaled for relief in toothache (Dymock et al., 1972; Anonymous, 1996). The flowers are taken to control diabetes (Bown, 1995). The bitter leaves are alterative and febrifuge . The twigs are used for cleaning the teeth. The seeds are anti-inflammatory, diuretic, emetic, emmenagogue; useful in lumbago, ophthalmia and inflammation of piles (Anonymous, 2009; Khodaei et al., 2012; Nadkarni, 2002).

Antimicrobial, antiinflammatory, antiumor, hepatoprotective, antihyperglycemic, cardiovascular, immunosuppressive, free radical scavenging and central nervous system depressant activities of the plant have been reported (Maurya and Akanksha, 2010; Haq et al., 2013). Antimutagenic potential of fruits extracts of *Withania coagulans* have been reported by (Mathur and Agrawal, 2011). The fruit extracts showed the presence of alkaloids, steroids, phenolic compounds, tannins, saponins, carbohydrates, proteins, milk-coagulating enzyme, two esterases, fatty oil, an essential oil , organic acids and amino acids, viz., proline, hydroxyproline, valine, tyrosine, aspartic acid, glycine asparagin, cysteine and glutamic acid (Mathur et al., 2011). The fruits

Fig. 1. *Withania coagulans* plant and fruits.

yielded withanolides, coagulanolide (Atta-ur-Rahman et al., 2003; Maurya et al., 2008), *n*-nonacosanyl, *n*-octacosanyl and *n*-heptacosanyl linolenates, withacoagulinyl tetraglucoside, wapryloyl hexaglucoside, menthyl tetraglucoside (Ali et al., 2014), *n*-dotriacont-21-enoic, *n*-tetratriacontanoic and *n*-octatriacont-17-enoic acids, geranilan-10-olyl dihydrocinnamoate and geranilanolyl salicylic glycoside (Ali et al., 2012). The leaves produced chlorogenic acid and withanolide. The major constituents of an essential oil of the fruits were caryophyllene, longifolene, δ-cadinene, 3-carene, linoleic acid ethyl ester, 8,11-octadecadienoic acid methyl ester, 2,5-dimethyl 2-undecene, (*Z*)-oleic acid methyl ester and nonanoic acid (Bakhtawar et al., 2010). The fixed oil of the fruits consisted of linoleic (42.05%), oleic (6.9%), palmitoleic, stearic, palmitic and docosanoic acids, phthalic acid and β-sitosterol (Ali et al., 2017). A variety of withanolides (steroidal lactones) such as coagulin F, coagulin G, coagulanolide, 20beta-hydroxy-1-oxo-(22*R*)-witha-2,5,24-trienolide and withacoagulin have been isolated from the whole plant of *W. coagulans*. The plant alkaloids and steroids are responsible for hypoglycemic activity of the plant. The whole plant contained withanolides, ajugin A, β-amyrin, coagulin, coagulins A-U, methyl-4-benzoate, β-sitosterol, β-sitosterol 3-*O*-glycoside, withahejarin withapakistanin, withasomniferine-A, 14, 15β-epoxywithanolide I, 17β-hydroxywithanolide K withacoagulins A, B, C, D, E, F, withanolide F and L, coagulansins A and B, Δ^3-isowithanolide F, withanolides G-K (Choudhary et al., 1995; Atta-ur-Rahman, et al., 1998a; 1998b; 1998c; 1998d; 1999; 2003; Nur-e-Alam et al., 2003; Huang et al., 2009; Jahan et al., 2010; Khodaei et al., 2012; Haq et al. 2013). The seeds on petroleum ether extraction, give a yellow fatty oil and unsaponifiable matter. Fatty acid composition are oleic, linoleic, palmitic, stearic and arachidonic acid. The unsaponifiable matter consists of triacontane and sterols including dihydrostigmasterol and β-sitosterol. The defatted meal from the seeds contains free sugar consisting of D-galactose and D-arabinose, traces of maltose and linoleic acid (Anonymous, 1996). The leaves contain withanolides, viz., withaferin-A, 5,20α(*R*)-dihydroxy-6α,7α-epoxy-1-oxo-(5α)-witha-2,24-dienolide and epoxy-22*R*-witha-2,24-dienolide (withanone). Withaferin A was active against *Micrococcus pyogenes* var. *aureus* and *Bacillus subtilis* glucose-6-phosphate-dehydrogenase. Withaferin A has marked tumor inhibitory property when studied *in vitro* against cells derived from human carcinoma of nasopharynx (KB). It also acts as mitotic poison arresting the division of cultured human larynx carcinoma cells at metaphase. Withaferin A exhibited potent anti-arthritic and anti-inflammatory effects without any toxic effect. The animals treated with withaferin A showed weight gain in arthritic syndrome . Withaferin A inhibits angiogenesis (Mohan et al., 2002). Some withanolides have promising role in the treatment of neurodegenerative diseases (Kuboyama et al., 2005). *W. coagulans* exhibited antidiabetic activity in rats (HemLatha et al., 2004; Jaiswal et al., 2009) and antihyperglycemic effect (Mau-

rya et al., 2008). Keeping in view the high reputation and wide application of the fruits of *W. coagulans* in many indigenous medicinal systems, it has been aimed to analyze the spectral data to establish structures of the phytoconstituents isolated from the fruits of this plant procured from Delhi.

2. Experimental

2.1. General procedures

Melting points were measured using one end open capillary tubes on a thermoelectrically heated melting point apparatus (Perfit, India) without correction. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were obtained by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ and DMSO-d_6 as solvents. TMS (Fluka analytical, Sigma-Aldrich, Netherland) was taken as an internal standard and the coupling constants (*J* values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer (Waters Corp., UK) instrument equipped with direct inlet prob system. The *m/z* values of the more intense peaks are mentioned and the figures in bracket attached to each *m/z* values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size. The purity of the isolated compounds was checked on precoated TLC plates with silica gel 60 F254 (0.25 mm, Merck, Mumbai, India). The spots were visualized by exposure to iodine vapors and under UV radiations at 254 and 366 nm and spraying with ceric sulphate solution.

2.2. Plant material

W. coagulans fruits were procured from a local Khari Baoli drug market, Near Fatehpuri Mosque, Chandni Chowk, Delhi. In Delhi, the drug is supplied from the fields near Amritsar, Punjab, latitude and longitude coordinates are 31.633980°N and 74.872261°E, respectively. It was authenticated by Prof. M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen No. PRL/RMH/2010/01 was deposited at the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi for future references.

2.3. Extraction and isolation

The dried and coarsely pulverized fruits (2 kg) of *W. coagulans* were extracted exhaustively with methanol in a Soxhlet apparatus to get maximum numbers of the chemical constituents of the plant in minimum duration of time. The extracts were concentrated under reduced pressure to get a dark brown mass (261.1 g). A small portion (5 g) of the extract was analyzed chemically to determine the presence of alkaloids, amino acids, carbohydrates, fatty acids, glycerides, glycosides, proteins, flavonoids and phenolic compounds by treating with various chemical reagents. The dried extract (200 g) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. It was dried in air and chromatographed over silica gel (1.2 kg) column (1.6 m x 16 mm x 2 mm) packed in dichloromethane. The column was eluted successively in increasing order of polarity in various combinations with dichloromethane, dichloromethane-ethyl acetate (9:1, 3:1, 1:1, 1:3, *v/v*), ethyl acetate and ethyl acetate-methanol (99: 1; 49: 1; 97:3; 19:1; 93:7; 9:1; 17:3; 4:1; 3:2; and 1:1, *v/v*). The fractions (250 mL) were collected separately, concentrated and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized to get partially purified compounds. The isolated compounds were recrystallized to get the following pure compounds:

2.3.1. Cetyl palmitate (**1**)

Elution of the column with dichloromethane furnished colourless waxy mass of **1**, recrystallized from acetone-methanol (1:1), 850 mg, R_f: 0.5 (dichloromethane), m.p.: 54-55 °C; UV λmax (MeOH): 203 nm (log ε 3.2); IR νmax (KBr): 2904, 2843, 1722, 1443, 1215, 1072, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.71 (1H, d, *J* = 14.7 Hz, H2-1'a), 4.65 (1H, d, *J* = 15.9 Hz, H2-1'b), 2.31 (2H, t, *J* = 14.1 Hz, H_2-2), 1.55 (2H, m, CH_2-3), 1.33 (52H, brs, 26 x CH_2), 1.05 (3H, t, *J* = 6.9 Hz, Me-16), 0.94 (3H, t, *J* = 6.6 Hz, Me-16'); FAB MS *m/z* (rel. int.): 480 $[M]^+$ ($C_{32}H_{64}O_2$) (53.8), 239 (37.2).

2.3.2. (3*R*,4*R*)-Dihydroxyadipic-γ,γ'-dilactone **(2)**

Elution of the column with dichloromethane-ethyl acetate (9:1) yielded light brown needle shaped crystals of **2**, recrystallized from acetone, 1.48 g, R_f: 0.45 (dichloromethane-ethyl acetate, 9:1); m.p.: 155-157 °C; UV λmax (MeOH): 206 nm (log ε 4.1); IR νmax (KBr): 2904, 1751, 1716, 1440, 1349, 1238, 1185, 1146, 1079, 969 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.60 (1H, m, $w_{1/2}$ = 13.4 Hz, H-3α) 3.54 (1H, m, $w_{1/2}$ = 13.7 Hz, H-4α), 2.80 (1H, d, *J* = 11.2, Hz, H_2-2α), 2.77 (1H, d, *J* = 15.6 Hz, H_2-2β), 2.66 (1H, d, *J* = 12.8 Hz, H_2-5α), 2.60 (1H, d, *J* = 14.6 Hz, H_2-5β); ^{13}C NMR (DMSO-d_6): δ 173.88 (C-1), 171.58 (C-6), 73.37 (C-3), 72.95 (C-4), 52.44 (C-2), 43.45 (C-5); FAB MS *m/z* (rel. int.): 142 $[M]^+$ ($C_6H_6O_4$) (7.2).

2.3.3. Withacoagulanide A **(3)**

Elution of the column with ethyl acetate afforded yellow crystals of **3**, recrystallized from chloroform-methanol (1:1), 1.97 g, m.p.: 113-115 ºC; UV λmax (MeOH): 219, 278 nm (log ε 4.7, 2.8): IR νmax (KBr): 2954, 2845, 1732, 1680, 1635, 1438, 1357, 1208, 1021, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.26 (1 H, ddd, *J* = 5.4, 8.1, 15.9 Hz, H-3), 5.56 (1H, d, *J* = 8.1 Hz, H-2), 4.37 (1H, ddd, *J* = 10.2, 11.1, 4.8 Hz, H-22), 2.57 (1H, m, H_2-23a), 2.37 (1H, m, H_2-4a), 2.28 (1H, m, H_2-4b), 2.23 (1H, m, H2-11a), 2.19 (1H, m, H_2-23b), 2.04 (1H, m, H_2-12a), 1.97 (1H, m, H-20β), 1.93 (1H, m, H_2-15a), 1.85 (3H, brs, Me-28), 1.82

(1H, brm, $w_{1/2}$ = 14.2 Hz, H-5α), 1.77 (1H, m, H_2-16a), 1.74 (3H, brs, Me-27), 1.68 (1H, m, H-9α), 1.53 (1H, m, H2-11b), 1.46 (1H, m, H_2-7a), 1.43 (1H, m, H-8β), 1.41 (1H, m, H_2-7b), 1.38 (1H, m, H_2-6a), 1.35 (1H, m, H_2-16b), 1.31 (1H, m, H_2-12b), 1.23 (1H, m, H-14α), 1.25 (1H, m, H-17α), 0.97 (3H, brs, Me-19), 0.94 (3H, d, *J* = 6.5 Hz, Me-21) , 0.79 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 203.85 (C-1), 127.46 (C-2), 143.92 (C-3), 34.46 (C-4), 44.27 (C-5), 18.75 (C-6), 31.74 (C-7), 39.53 (C-8), 40.91 (C-9), 52.24 (C-10), 25.08 (C-11), 39.50 (C-12), 43.44 (C-13), 51.89 (C-14), 25.66 (C-15), 38.05 (C-16), 52.56 (C-17), 14.36 (C-18), 20.52 (C-19), 40.57 (C-20), 20.65 (C-21), 81.04 (C-22), 30.26 (C-23), 148.38 (C-24), 122.07 (C-25), 168.58 (C-26), 12.57 (C-27), 22.53 (C-28); FAB MS *m/z* (rel. int.): 424 $[M]^+$ ($C_{28}H_{40}O_3$) (6.7).

2.3.4. Withacoagulanide B **(4)**

Further elution of the column with ethyl acetate gave colourless crystals of **4**, recrystallized from acetone, 1.48 g, R_f: 0.4 (ethyl acetate), m.p.: 108-109 ºC; UV λmax (MeOH): 228, 279 nm (log ε 5.6, 0.6); IR νmax (KBr) 2921, 2850, 1708, 1695, 1635, 1439, 1380, 1205, 811 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.63 (1H, ddd, *J* = 8.1, 4.5, 9.7 Hz, H-22), 2.64 (1H, m, H_2-2α), 2.49 (1H, m, H_2-23α), 2.43 (1H, m, H_2-2β), 2.41 (1H , m, H_2-23β), 2.06 (1H, m, H-14), 1.96 (1H, m, H-20), 1.90 (3H, brs, Me-28) 1.79-1.22 (16H, m, 8 x CH_2), 1.70 (3H, brs, Me-27), 1.64 (1H, m, H-9), 1.57 (1H, m, H-17), 1.54 (1H, m, H-8), 1.46 (1H, m, H-5), 1.23 (3H, brs, Me-19), 1.07 (3H, d, *J* = 6.7 Hz, Me-21), 0.85 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 211.81 (C-1), 41.77 (C-2), 29.45 (C-3), 44.27 (C-4), 56.41 (C-5), 18.75 (C-6), 31.74 (C-7), 34.13 (C-8), 40.91 (C-9), 52.24 (C-10), 25.08 (C-11), 39.50 (C-12), 43.44 (C-13), 51.89 (C-14), 25.66 (C-15), 30.05 (C-16), 52.56 (C-17), 14.36 (C-18), 20.52 (C-19), 20.07 (C-20), 20.65 (C-21), 81.04 (C-22), 30.26 (C-23), 148.38 (C-24), 122.07 (C-25), 163.58 (C-26), 12.57 (C-27), 22.53 (C-28); FAB MS *m/z* (rel. int.): 426 $[M]^+$ ($C_{28}H_{42}O_3$) (6.2).

2.3.5. Withacoagulanide C **(5)**

Elution of the column with ethyl acetate-methanol (99:1) produced colourless crystals of **5**, recrystallized from acetone, 894 mg, m.p.: 152-153 ºC, R_f: 0.48 (ethyl acetate-methanol, 99:1), UV λmax (MeOH), 221, 274 nm (log ε 5.3, 1.1); IR νmax (KBr): 3405, 2927, 2864, 1726, 1680, 1620, 1443, 1381, 1262, 1071, 800 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.91 (1H, ddd, *J* = 3.1, 8.5, 15.2 Hz, H-3), 5.83 (1H, d, *J* = 8.5 Hz, H-2), 4.88 (1H, ddd, *J* = 9.8, 11.3, 4.5 Hz, H-22), 3.73 (1H, brm, $w_{1/2}$ = 18.1 Hz, H-6α), 2.51 (1H, m, H_2-23a), 2.43 (1H, m, H_2-4a), 2.31 (1H, m, H_2-4b), 2.19 (1H, m, H2-11a), 2.16 (1H, m, H_2-23b), 2.10 (1H, m, H_2-12a), 2.01 (1H, m, H-20β), 1.95 (1H, m, H_2-15a), 1.89 (1H, m, H_2-15b), 1.86 (3H, brs, Me-28), 1.79 (1H, brm, $w_{1/2}$ = 14.2 Hz, H-5α), 1.76 (3H brs, Me-27), 1.72 (1H, m, H_2-16a), 1.63 (1H, m, H-9α), 1.51 (1H, m, H2-11b), 1.51 (1H, m, H_2-7a), 1.46 (1H, m, H-8β), 1.42 (1H, m, H_2-7b), 1.33 (1H, m, H_2-6a), 1.31 (1H, m, H_2-16b), 1.29 (1H, m, H_2-12b), 1.26 (1H, m, H-14α), 1.23 (1H, m, H-17α), 1.18 (3H, brs, Me-19), 1.08 (3H, d, *J* = 6.9 Hz, Me-21), 0.82 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 205.16 (C-1), 131.90 (C-2), 140.43 (C-3), 35.27 (C-4), 49.10 (C-5), 71.59 (C-6), 31.80 (C-7), 31.12 (C-8), 40.58 (C-9), 45.14 (C-10), 25.72 (C-11), 37.74 (C-12), 46.33 (C-13), 49.07 (C-14), 24.91 (C-15), 27.70 (C-16), 51.18 (C-17), 18.42 (C-18), 19.36 (C-19), 20.71 (C-20), 21,12 (C-21), 79.69 (C-22), 29.53 (C-23), 148.71 (C-24), 129.12 (C-25), 167.14 (C-26), 11.26 (C-27), 22.60 (C-28); FAB MS *m/z* (rel. int.): 440 ($C_{28}H_{40}O_4$) (5.8).

2.3.6. Withacoagulanide D **(6)**

Elution of the column with ethyl acetate – methanol (97:3) gave yellow crystals of compound **6**, recrystallized from acetone-methanol (97:3), 680 mg (0.7% yield), R_f: 0.51 (acetone-methanol, (97:3). m.p.: 108 – 109 ºC; UV λmax (Me OH): 205, 268, and 325 nm (log ε 4.1, 1.1, 0.8); I.R νmax (KBr): 3408, 3310, 2935, 2845, 1708, 1695, 1640, 1382, 1238, 1134, 1077, 1030 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.82 (1H, brm , H-22), 3.20 (1H, brm, $w_{1/2}$ = 17.2 Hz, H-3α), 2.74 (1H, m, H-20), 2.53 (2H, m, H_2-2), 2.45 (1H, m, H_2-23a), 2.38 (1H, m, H_2-23b), 2.10 (2H, d, *J* = 9.3 Hz, H_2-4), 1.95 (2H, m, H_2-15), 1.91 (3H, brs, Me-28), 1.76 (3H, brs, Me-27), 1.62 (2H, m, H_2-6), 1.58 (1H, m, H-9α), 1.51 (2H, m, H2-11), 1.45 (1H, m, H-8), 1.37 (2H, m, H_2-16), 1.31 (1H, m, H-17), 1.28 (1H, m, H-14), 1.25 (2H, m, H_2-7), 1.22 (2H, m, H_2-12), 1.18 (3H, brs, Me-19), 1.05 (3H, d, *J* = 6.3 Hz, Me-21), 1.03 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 204.81 (C-1), 22.37 (C-2), 74.86 (C-3), 38.21 (C-4), 70.57 (C-5), 20.59 (C-6), 32.15 (C-7), 36.99 (C-8), 38.21 (C-9), 49.67 (C-10), 20.63 (C-11), 39.51 (C-12), 43.13 (C-13), 51.72 (C-14), 20.93 (C-15), 31.21 (C-16), 56.50 (C-17), 15.18 (C-18), 23.82 (C-19), 39.67 (C-20), 23.77 (C-21), 78.56 (C-22), 21.49 (C-23), 151.26 (C-24), 120.88 (C-25), 166.43 (C-26), 12.34 (C-27), 19.01 (C-28); FAB MS *m/z* (rel. int.): 458 [M] + ($C_{28}H_{42}O_5$) (8.9).

2.3.7. Withacoagunalide C6-arabinosyl 2′-*O*-anisate **(7)**

Elution of the column with ethyl acetate-methanol (49:1) furnished pale yellow crystals of **7,** recrystallized from acetone-methanol (1:1), 2.45 g (2.5% yield), R_f: 0.27 (ethyl acetate-methanol, 49:1), m.p.: 102-104 ºC, UV λmax (MeOH): 224, 281 nm (log ε 5.3; 0.6); IR νmax (KBr): 3525, 3427, 2930, 2871, 1709, 1680, 1640, 1451, 1382, 1221, 1133, 844 cm^{-1}; ^{1}H NMR (DMSO-d_6):δ 6.09 (1H, m, H-3), 5.86 (1H, d, *J* = 8.4 Hz, H-2), 4.67 (1H, ddd, *J* = 9.6, 4.5, 5.3 Hz, H-22), 3.81 (1H, brm, $w_{1/2}$ = 18.2 Hz, H-6α), 2.83 (2H, m, H_2-4), 2.47 (1H, m, H_2-23α), 2.41 (1H, m, H_2-23β), 2.73-1.59 (10H, m, 5 x CH_2), 2.50 (1H, m, H-20), 2.17 (1H, m, H-14), 1.98 (1H, m, H-8), 1.83 (3H, brs, Me-27), 1.76 brs (3H, brs, Me-28), 1.62 (1H, m, H-5), 1.52 (1H, m, H-9), 1.46 (1H, m, H-17), 1.01 (3H, d, *J* = 6.5 Hz, Me-21), 1.30 (3H, brs, Me-19), 0.88 (3H, brs, Me-18), 5.23 (1H, d, *J* = 7.1 Hz, H-1′), 4.75 (1H, m, H-2′), 4.45 (1H, m, H-3′), 4.14 (1H, d, *J* = 9.2 Hz, H_2-5′α), 4.03 (1H, d, *J* = 9.2 Hz, H_2-5′β), 3.56 (1H, m, H-4′), 6.94 (1H, m, H-3′′), 6.72 (1H, m, H-4′′), 6.03 (1H, m, H-6′′), 5.63 (1H, m, H-5′′), 3.35 (3H, brs, OMe); ^{13}C NMR ($CDCl_3$): δ 205.18 (C-1), 125.85 (C-2), 147.29 (C-3), 35.81 (C-4), 47.34 (C-5), 73.97 (C-6), 35.14 (C-7), 33.21 (C-8), 41.79 (C-9), 50.49 (C-10), 23.26 (C-11), 38.20 (C-12), 42.83 (C-13), 57.16 (C-14), 24.23 (C-15), 30.10 (C-16), 54.01 (C-17), 14.29 (C-18), 19.09 (C-19), 20.32 (C-20), 19.65

(C-21), 81.31 (C-22), 31.74 (C-23), 152.37 (C-24), 124.22 (C-25), 165.75 (C-26), 12.59 (C-27), 20.69 (C-28), 107.21 (C-1′), 87.89 (C-2′), 78.66 (C-3′), 81.47 (C-4′), 60.85 (C-5′), 147.29 (C-1′′), 152.81 (C-2′′), 139.32 (C-3′′), 127.29 (C-4′′), 125.86 (C-5′′), 119.06 (C-6′′), 168.53 (C-7′′), 55.01 (OMe); FAB MS *m/z* (rel. int.): 694 $[M]^+$ ($C_{42}H_{54}O_{10}$) (11.2), 439 (8.3), 295 (12.7), 275 (22.4), 151 (17.1).

2.3.8. 3-*O*-Digalactosyl withacoagulanide B **(8)**

Elution of the column with ethyl acetate-methanol (97:3) afforded brown crystal of **8,** recrystallized from acetone-methanol (1:1); 3.3 g (3.3% yield), R_f: 0.28 (ethyl acetate-methanol, 97:3), m.p.: 102-104 ºC; UV λmax (MeOH) 205, 289 nm (log ε 4.7, 0.6); IR νmax (KBr): 3425, 3367, 3280, 2931, 2855, 1712, 1708, 1645, 1435, 1362, 1239, 1077, 761 cm^{-1}; ^1H NMR (DMSO-d_6):δ 4.93 (1H, ddd, *J* = 12.6, 5.3, 5.1 Hz, H-22), 3.63 (1H, brm, $w_{1/2}$ = 18.5 Hz, H-3α), 2.93 (1H, dd, *J* =17.4,5.6 Hz, H_2-2α), 2.91 (1H, dd, *J* = 9.3, 5.5 Hz, H_2-2β), 2.63 (1H, brm, $w_{1/2}$ = 16.8 Hz, H-20 β), 2.39 (2H, dd, J = 12.6, 5.3 Hz, H_2-23), 2.61-1.13 (19H, m, 7 x CH_2 , 5 x CH), 1.90 (3H, brs, Me-27), 1.78 (3H, brs, Me-28), 1.13 (3H, brs, Me-19), 1.05 (3H, d, *J* = 6.5 Hz, Me-21), 0.96 (3H, brs, Me-18), 5.70 (1H, d, J = 7.3 Hz, H-1′), 4.27 (1H, m, H-2′), 3.74 (1H, m, H-3′), 3.55 (1H, m, H-4′), 4.41 (1H, m, H-5′), 3.23 (1H, m, H-6′), 5.43 (1H, d, J = 7.1 Hz, H-1′′), 4.14 (1H, m, H-2′), 3.71 (1H, m, H-3′), 3.40 (1H, m, H-4′), 4.43 (1H, m, H-5′), 3.20 (1H, m, H-6′); ^{13}C NMR (DMSO-d_6): δ 203.61 (C-1), 41.76 (C-2), 70.56 (C-3), 31.25 (C-4), 46.45 (C-5), 18.79 (C-6), 31.74 (C-7), 35.34 (C-8), 40.90 (C-9), 50.48 (C-10), 23.12 (C-11), 39.49 (C-12), 45.81 (C-13), 54.95 (C-14), 28.35 (C-15), 29.08 (C-16), 53.11 (C-17), 14.23 (C-18), 19.09 (C-19), 20.33 (C-20), 20.67 (C-21), 73.85 (C-22), 30.41 (C-23), 151.20 (C-24), 120.84 (C-25), 165.83 (C-26), 12.58 (C-27), 21.49 (C-28), 109.15 (C-1′, galactose), 81.37 (C-2′), 77.25 (C-3′), 63.57 (C-4′), 86.43 (C-5′), 61.59 (C-6′), 101.86 (C-1′′), 77.43 (C-2′′), 75.02 (C-3′′), 70.56 (C-4′′), 80.22 (C-5′′), 60.85 (C-6′′); FAB MS *m/*z (rel. int.): 766 $[M]^+$ ($C_{40}H_{62}O_{14}$) (10.2), 603 (8.5), 587 (13.2), 425 (13.1), 341 (17.9), 179 (23.6), 163 (19.8).

2.3.9. Glyceryl-1,2-dipalmityl 3-phosphate **(9)**

Elution of the column with ethyl acetate-methanol (19:1) produced pale yellow colored crystalline amorphous powder of compound **9,** recrystallized from chloroform-methanol (1:1) 1.3 g (1.3% yield), R_f: 0.37 (ethyl acetate-methanol, 19:1), m.p.: 105-106 ºC; UV λmax (MeOH): 204, 285 nm (log ε 4.8, 1.1); IR νmax (KBr): 3401, 2923, 2851, 1721, 1460, 1185, 720 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.90 (1H, m, H-2), 4.50 (2H, m, H2-3), 4.36 (2H, m, H2-1), 2.73 (2H, t, *J* = 11.2 Hz, H_2-2′), 2.62 (2H, t, *J* = 7.2 Hz, H_2-2′′), 2.32 (2H, m, CH_2-3′), 2.27 (2H, m, CH_2-3′′), 1.55 (4H, m, CH_2-15′, CH_2-15′′), 1.23 (44 H, brs, 22 x CH_2), 0.91 (3H, t, *J* = 6.5 Hz, Me-16′), 0.83 (3H, t , *J* = 6.3 Hz, Me-16′′); ^{13}C NMR (DMSO-d_6) δ 67.47 (C-1), 74.36 (C-2), 72.89 (C-3), 175.04 (C-1′), 56.18 (C-2′), 43.41 (C-3′), 31.74 (C-4′), 29.45 (C-5′), 29.42 (C-6′), 29.38 (C-7′), 29.31 (C-8′), 29.28 (C-9′), 29.23 (C-10′), 29.18 (C-11′), 29.14 (C-12′), 29.14 (C-13′), 29.14 (C-14′), 22.68 (C-15′), 14.41 (C-16′), 171.60 (C-1′′), 51.75 (C-2′′), 43.19 (C-3′′), 31.13 (C-4′′), 29.44 (C-5′′), 29.42 (C-6′′), 29.36 (C-7′′), 29.31 (C-8′′), 29.5(C-9′′), 29.21 (C-10′′), 29.16 (C-11′′), 29.14 (C-12′′), 29.14 (C-13′′), 29.14 (C-14′′), 22.54 (C-15′′), 14.39 (C-16′′); FAB MS *m/z* (rel. int.): 648 $[M]^+$ ($C_{35}H_{69}O_8P$) (33.5), 239 (62.1).

2.3.10. 3-Secowithacoagulanolide 6β-olyl galacturonoside (**10**)

Elution of the column with ethyl acetate-methanol (93:7) imparted yellow colored crystals of **10**, recrystallized from acetone 950 mg (0.95% yield), R_f: 0.47 (ethyl acetate-methanol, 24:1); m.p.: 114-115 ºC; UV λmax (MeOH): 204, 281, 321 nm (log ε 4.8, 2.7, 0.9); IR νmax (KBr): 3565, 3428, 3231, 2856, 2842, 1732, 1710, 1702, 1690, 1675, 1635, 1438, 1201, 1019 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.11 (1H, ddd, *J* = 7.2, 7.5, 5.7, H-22α), 4.08 (1H , brm , $w_{1/2}$ =18.8 Hz, H-11α), 3.36 (1H, brm, $w_{1/2}$ = 17.5 Hz, H-6α), 2.85 (1H, ddd, *J* = 9.6,5.7, 6.9 Hz, H_2-2α), 2.80 (1H, d, *J* = 5.7 Hz, H_2-23α), 2.77 (1H, m, H_2-23β), 2.71 (1H, m, H_2-2β), 2.74 (1H, d, *J* = 9.6 Hz, H_2-12β), 2.67 (1H, d, *J* = 5.8 Hz, H_2-12α), 2.62 (1H, dd, *J* = 4.8, 7.2 Hz, H-20β), 2.11 (1H, m, H-17α), 2.08 (1H, d, *J* = 8.5 Hz, H-9α), 1.94 (1H, m, H-14α), 1.85 (1H, m, H-5α), 1.83-1.23 (6H, m, H_2-7, H_2-15, H_2-16), 1.18 (3H, d, *J* = 6.5 Hz, Me-4), 1.05 (3H, t, *J* = 6.9 Hz, Me-3), 5.06 (1H, d, *J* = 7.3 Hz, H-1′), 4.19 (1H, m, H-5′), 3.67 (1H, m, H-2′), 3.45 (1H, m, H-3′), 3.17 (1H, m, H-4′), ^{13}C NMR (DMSO-d_6): δ 205.68 (C-1), 38.02 (C-2), 14.38 (C-3), 18.62 (C-4), 55.14 (C-5), 72.97 (C-6), 29.51 (C-7), 76.42 (C-8), 43.48 (C-9), 56.51 (C-10), 73.45 (C-11), 38.42 (C-12), 49.06 (C-13), 52.49 (C-14), 21.03 (C-15), 24.79 (C-16), 52.08 (C-17), 172.01 (C-18), 170.30 (C-19), 43.38 (C-20), 177.51 (C-21), 73.94 (C-22), 31.10 (C-23), 152.22 (C-24), 122.81 (C-25), 172.63 (C-26), 174.86 (C-27), 173.70 (C-28), 110.13 (C-1′), 77.25 (C-2′), 67.50 (C-3′),63.56 (C-4′), 88.70 (C-5′), 178.40 (C-6′); FAB MS *m/z* (rel. int.): 766 $[M]^+$ ($C_{34}H_{38}O_{20}$) (2.8).

2.3.11. Withacoagulanide C 6β-olyl diarabinosyl 2′′-(*O*)-anisate **(11)**

Elution of the column with ethyl acetate-methanol (9:1) offered yellow colored crystals of **11**, recrystallized from acetone, 1.6 g (1.6% yield), R_f: 0.55 ethyl acetate-methanol (97:3); m.p.: 106-107 ºC; UV λmax (MeOH): 205, 295, 321 nm (log ε 4.8, 1.3, 1.1); IR νmax (KBr): 3370, 2924, 2853, 1721, 1709, 1680, 1635, 1547, 1514, 1440, 1381, 1260, 1038 cm^{-1}; ^1H NMR (DMSO – d6): δ 6.68 (1H, m, H-3), 5.86 (1H, d, *J* = 10.1 Hz, H-2), 4.89 (1H, ddd, *J* = 12.7, 5.6, 5.2 Hz, H-22), 3.86 (1H, brm, $w_{1/2}$ =18.6 Hz, H-6α), 2.75 (1H, dd, *J* = 9.6, 5.3 Hz, H_2-4α), 2.68 (1H, dd, *J* = 5.5, 4.9 Hz, H_2-4β), 2.63 (1H, m, H-22 α), 2.51 (2H, dd, *J* = 12.3, 5.6 Hz, H_2-23), 2.40-1.48 (16H, m, 5 x CH_2 , 6 x CH), 1.89 (3H, brs, Me-27), 1.76 (3H, brs, Me-28), 1.18 (3H, brs, Me-19), 1.03 (3H, d, *J* = 6.7 Hz, Me-21), 0.85 (3H, brs, Me-18), 5.76 (1H, d, *J* = 7.5 Hz, H-1′), 4.11 (1H, m, H-2′), 3.76 (1H, m, H-3′), 4.32 (1H, m, H-4′), 3.60 (1H, m, H-5′), 5.13 (1H, d, *J* = 7.3 Hz, H-1′′), 4.41 (1H, m, H-2′′), 4.01 (1H, m, H-3′′), 4.20 (1H, m, H-4′′), 3.17 (1H, m, H-5′′), 7.16 (1H, m, H-3′′′), 7.23 (1H, m, H-4′′′), 6.68 (1H, m, H-6′′′), 7.08 (1H, m, H-5′′′), 3.56 (3H, brs, OMe), 2.21 (3H, brs, OAc), 2.14 (3H, brs, OAc), 2.02 (3H, brs, OAc); ^{13}C NMR (DMSO-d_6):

δ 204.12 (C-1), 145.30 (C-2), 126.27 (C-3), 34.12 (C-4), 46.44 (C-5), 72.91 (C-6), 33.93 (C-7), 31.24 (C-8), 43.06 (C-9), 54.94 (C-10), 22.54 (C-11), 38.01 (C-12), 43.26 (C-13), 56.14 (C-14), 25.05 (C-15), 29.35 (C-16), 55.26 (C-17), 14.37 (C-18), 19.10 (C-19), 20.49 (C-20), 20.64 (C-21), 79.56 (C-22), 31.08 (C-23), 151.22 (C-24), 120.84 (C-25), 166.67 (C-26), 12.55 (C-27), 20.97 (C-28), 109.13 (C-1′), 80.53 (C-2′), 73.41 (C-3′), 77.49 (C-4′), 63.54 (C-5′), 101.84 (C-1′′), 86.45 (C-2′′), 73.41 (C-3′′), 68.52 (C-4′′), 61.59 (C-5′′), 148.41 (C-1′′′), 151.85 (C-2′′′), 134.15 (C-3′′′), 125.88 (C-4′′′), 116.03 (C-5′′′), 126.27 (C-6′′′), 170.40 (C-7′′′), 174.75 (OAc), 172.26 (OAc), 175.75 (OAc), 21.44 (3 x $OCOCH_3$), 53.10 (OMe); FAB MS *m/z* (rel. int.): 964 $[M]^+$ ($C_{52}H_{68}O_{17}$) (2.1), 524 (8.1), 439 (11.7), 351 (6.3), 151 (24.2).

2.3.12. Caproyl trixyloside **(12)**

Elution of the column with ethyl acetate-methanol (17:3) provided yellow crystals of **12**, recrystallized from acetone-methanol (1:1), 680 g (0.68% yield), R_f: 0.49 (ethyl acetate-methanol, 19:1), m.p.: 103-105 ºC; UV λmax (MeOH): 206 nm (log ε 5.8); IR νmax (KBr): 3445, 3386, 3231, 3271, 2923, 2850, 1743, 1436, 1238, 1077, 1030 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.90 (1H, d, *J* = 7.5 Hz, H-1′), 4.86 (1H, d, *J* = 7.3 Hz ,H-1′′), 4.80 (1H, d, *J* = 7.5 Hz, H-1′′′), 4.50 (1H, m, H-2′), 4.38 (1H, m, H-2′′), 4.28 (1H, m, H-2′′′), 3.80 (1H, m, H-3′), 3.73 (2H, m, H-3′′, H-3′′), 3.63 (2H, brs, H_2-5′), 3.56 (4H, brs, H_2-5′′, H_2-5′′′), 3.11 (1H, m, H-4′), 3.05 (2H, m, H-4′′, H-4′′′), 2.91 (2H, t, *J* = 7.8 Hz, H_2-2), 1.91 (2H, m , CH_2-3), 1.29 (4H, m , CH_2-4, CH_2-5), 1.03 (3H, t, *J* = 6.6 Hz, Me-6); ^{13}C NMR (DMSO-d_6): δ 172.41 (C-1), 56.49 (C-2), 31.13 (C-3), 29.89 (C-4), 21.51 (C-5), 18.99 (C-6), 100.14 (C-1′), 77.25 (C-2′), 73.85 (C-3′), 70.81 (C-4′), 63.38 (C-5′), 97.37 (C-1′′), 77.19 (C-2′′), 73.57 (C-3′′), 72.40 (C-4′′), 61.70 (C-5′′), 92.69 (C-1′′′), 75.34 (C-2′′′), 73.05 (C-3′′′), 72.85 (C-4′′′), 61.70 (C-5′′′); FAB MS *m/z* (rel. int.): 512 $[M]^+$ ($C_{21}H_{36}O_{14}$) (2.8), 397 (7.3), 281 (10.4), 149 (16.7), 133 (15.1), 115 (6.0).

3. Results and Discussion

Compound **1** was a known fatty ester identified as *n*-hexadecyl hexadecanoate (palmityl palmitate) (Holman, 1981; Kung and Ciereszko, 1985) (Fig. 2).

3.1. Spectroscopic characteristics of compound **2**

Compound **2**, a dilactone, showed characteristic IR absorption bands for lactone rings at 1751 and 1716 cm^{-1}. On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **2** was determined at *m/z* 142 corresponding to a molecular formula of a hexane dilactone, $C_6H_6O_4$. The ^1H NMR spectrum of **2** exhibited two one-proton multiplets at δ 3.60 ($w_{1/2}$ = 13.4 Hz) and 3.54 ($w_{1/2}$ = 13.7 Hz) assigned to α-oriented oxymethine H-3 and H-4 protons, respectively. Four one-proton doublets at δ 2.80 (*J* = 11.2, Hz), 2.77 (*J* = 15.6 Hz), 2.66 (*J* = 12.8 Hz) and 2.60 (*J* = 14.6 Hz) were ascribed correspondingly to methylene H_2-2 and to H_2-5 protons. The ^{13}C NMR spectrum of **2** displayed signals for lactone carbons at δ 173.88 (C-1) and 171.58 (C-6), oxymethine carbons at δ 73.37 (C-3) and 72.95 (C-4) and methylene carbon at δ 52.44 (C-2) and 43.45 (C-5). On the basis of structural data analysis, the structure of **2** was elucidated as (3*R*,4*R*)-dihydroxyadipic-γ,γ′-dilactone (*n*-hexa-1(3),4(5)-diolide), a synthesized hexanyl dilactone (Fig. 2).

3.2. Spectroscopic characteristics of compound **3**

Compound **3,** named withacoagulanide A, showed a strong UV absorption at 218 nm which was characteristic of α,β-unsaturated carbonyl in a steroidal 2-ene 1-one chromophore and α,β-unsaturated lactone chromophores (Lavie et al., 1970). Its IR spectrum showed characteristics IR absorption bands for unsaturated δ lactone (1732 cm^{-1}), conjugated carbonyl (1680 cm^{-1}) and unsaturation (1635 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 424 corresponding to a molecular formula of a withanolide, $C_{28}H_{40}O_3$. The ^1H NMR spectrum of **3** displayed two deshielded signals as a triple doublet at δ 7.26 (*J* = 5.4, 8.1, 15.9 Hz) and as a doublet at δ 5.56 (*J* = 8.1 Hz) assigned to vinylic H-3 and H-2, respectively. The downfield C-22 oxymethine proton of the lactone moiety appeared as a triplet doublet at δ 4.37 (*J* = 10.2, 11.1, 4.8 Hz). Two three-proton broad signals at δ 1.85 and 1.74 were due to C-28 and C-27 methyl protons attached to the vinylic C-24 and C-25 carbons, respectively. Two three-proton broad signals at δ 0.97 and 0.79 and a three-proton doublet at δ 0.94 (*J* = 6.5 Hz) were accounted correspondingly to tertiary C-19 and C-18 and secondary C-21 methyl protons. The stereochemistry at C-22 was assigned on biogenetic grounds and by chemical shift comparisons (Lavie et al., 1970). The ^{13}C NMR spectrum of **3** supported the presence of 28 carbon resonances including five methyl, eight methylene, nine methine and six quaternary carbons. Downfield carbon signals at δ 203.85 for C-1, 127.46 for C-2, 143.92 for C-3 were indicative of α,β-unsaturated ketone. The signals for E ring (α,β-unsaturated lactone) at δ 168.58 for C-26, 81.04 for C-22, 148.38 for C-24 and 122.07 for C-25 implied that C-26 and C-22 were oxidized to form the usual E ring of a withanolide . The methyl carbons appeared at δ 14.36 (C-18), 20.52 (C-19), 20.65 (C-21), 12.57 (C-27) and 22.53 (C-28) (Atta-ur-Rahman et al., 2003; Yang et al., 2014; Chepkorir et al., 2018). On the basis of the spectroscopic studies, the structure of **3** was fully established and determined as (20*S*,22*R*)-1-oxo-witha-2,24-dienolide, a new withanolide (Fig. 2).

3.3. Spectroscopic characteristics of compound **4**

Compound **4,** designated as withacoagulanide B, had UV absorption maxima at 224 nm suggesting the presence of an α,β-unsaturated δ-lactone distinctive of a common steroidal withanolide. Its IR spectrum displayed characteristic absorption bands for unsaturated lactone (1708 cm^{-1}), carbonyl function (1695 cm^{-1}) and unsaturation (1635 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 426 corresponding to a molecular formula of a withanolide, $C_{28}H_{42}O$. The ^1H NMR spectrum of **4** exhibited a one-proton triplet doublet at δ 4.63 with coupling interactions of 8.1, 4.5, 9.7 Hz

assigned to α-oriented oxymethine H-22 proton. Two three-proton broad singlets at δ 1.90 and 1.70 were ascribed to C-28 and C-27 methyl proton located on the vinylic carbon. A three-proton doublet at δ 1.07 (*J* = 6.7 Hz) and two broad singlets at δ 1.23 (3H) and 0.85 (3H) were due to C-21 secondary and C-19 and C-18 tertiary methyl protons, respectively. The ^{13}C NMR spectrum of **4** showed signals for carbonyl carbon at δ 203.81 (C-1), unsaturated δ-lactone carbon at δ 163.58 (C-26), vinylic carbons at δ 148.38 (C-24) and 122.07 (C-25), oxymethine carbon at δ 81.04 (C-22) and methyl carbons at δ 14.36 (C-18) 20.52 (C-19), 20.65 (C-21), 12.57 (C-27) and 22.53 (C-28) comparable with the related withanolides (Atta-ur-Rahman et al., 2003; Yang et al., 2014; Chepkorir et al., 2018). The HMBC spectrum of **4** exhibited interactions of H_2-2 and H_2-3 with C-1; H_2-3, H_2-4, and H_2-6 with C-5; and H-17, H-20 and H_2-23 with C-22. On the basis of these spectral data, compound **4** was similar to **3** with the withanolide skeleton structure. The difference occurred in the existence of the vinylic linkage at C-2(3) position. Between C-24 and C-25, usual double bond for withanolides was present. This was evidenced through C-26 absorbing at δ 163.58. Consequently, compound **4** was identified as (20*S*,22*R*)-1-oxo-witha-24-enolide, a new withanolide (Fig. 2).

3.4. Spectroscopic characteristics of compound **5**

Compound **5**, designated as withacoagulanide C, had UV absorption maxima at 221 nm for α,β-unsaturated δ-lactone and displayed characteristic IR absorption bands for a hydroxyl group (3405 cm^{-1}), unsaturated lactone (1726 cm^{-1}), carbonyl function (1680 cm^{-1}) and unsaturation (1620 cm^{-1}). On the basis of its mass and ^{13}C NMR spectra, the molecular ion peak of **5** was determined at *m/z* 440 consisting of a molecular formula of the withanolide, $C_{28}H_{40}O_4$. The ^{1}H NMR spectrum of **5** exhibited two one-proton deshielded signals as a double doublet at δ 6.71 (*J* = 8.5, 3.1 Hz) and as a doublet at δ 5.83 (*J* = 8.5 Hz, H-2) assigned to vinylic H-3 and H-2 protons, respectively. A one-proton triplet doublet at δ 4.88 (*J* = 9.8, 11.3, 4.5 Hz) was ascribed to the downfield C-22 oxymethine proton of the lactone moiety. A one-proton broad multiplet at δ 3.73 with half-width of 18.1 Hz as due to α-oriented carbinol H-6α proton. Two three-proton broad singlets at δ 1.86 and 1.78 were attributed to C-27 and C-28 methyl protons attached to the vinylic C-24 and C-25 carbons, respectively. Two three-proton broad singlets at δ 1.18 and 0.82 and a three-proton doublet at δ 1.08 (*J* = 6.9 Hz) were accounted correspondingly to tertiary C-19 and C-18 and secondary C-21 methyl protons. The stereochemistry at C-22 was assigned on biogenetic grounds and by chemical shift comparisons (Lavie et al., 1970). The ^{13}C NMR spectrum of **5** showed downfield carbon signals at δ 205.16 (C-1), 131.90 (C-2) and 140.43 (C-3) supporting the presence of α,β-unsaturated ketone. The signals for vinylic carbons at δ 148.71 (C-24) and 129.12 (C-25), oxymethine carbon at δ 79.69 (C-22) and lactone carbon at δ 167.14 (C-26) suggested α,β-unsaturated lactone in the E ring. The signal at δ 71.59 was due to carbinol C-6 carbon; the methyl carbons appeared at δ 18.42 (C-18), 19.36 (C-19), 21.12 (C-21), 11.26 (C-27) and 22.60 (C-28) (Atta-ur-Rahman et al., 2003; Yang et al., 2017; Chepkorir et al., 2018). The HMBC spectrum of **5** showed correlations of H-2 and H-3 with C-1; H_2-4, H-5, and H_2-7 with C-6; and H-17, H-20 and H_2-23 with C-22. These evidences led to formulate the structure of **5** as (20S,22*R*)-1-oxo-witha-6β-ol-2,24-dienolide (6β-hydroxywithacoagulanide A), a new withanolide (Fig. 2).

3.5. Spectroscopic characteristics of compound **6**

Compound **6**, named withacoagulanide D, had UV absorption maxima at 268 and 325 nm characteristic for withanolides. Its IR spectrum showed distinctive absorption bands for δ-lactone absorption (1708 cm^{-1}), carbonyl group (1695 cm^{-1}) and unsaturation (1640 cm^{-1}). The mass spectrum of **6** displayed a molecular ion peak at *m/z* 458 consistent with a molecular formula of a withanolide, $C_{28}H_{42}O_5$. The ^{1}H NMR spectrum of **6** showed a one-proton broad multiplet at δ 4.82 assigned to carbine H-22 proton, a one proton broad multiplet at δ 3.20 with half-width of 17.2 Hz ascribed to α – oriented carbinol H-3 proton, two three-proton broad singlets at δ 1.91 and 1.76 due to C-28 and C -27 methyl protons located on the vinylic carbons, a three-proton doublet at δ 1.05 (*J* = 6.3 Hz) accounted to C-21 secondary methyl protons and two three-proton broad singlets at δ 1.18 and 1.03 attributed to tertiary C-19 and C-18 methyl protons, respectively. The ^{13}C NMR spectrum of **6** exhibited signals for carbonyl carbon at δ 204.81 (C-1), lactone carbon at δ 166.43 (C-26), carbinol carbons at δ 74.86 (C-3) and 70.57 (C-5), carbine carbon at δ 78.56 (C-22), vinylic carbons at δ 151.26 (C-24) and 120.88 (C-25) and methyl carbons between δ 23.82-12.34. The HMBC spectrum of **6** exhibited correlations of H_2-2 and H-3 with C-1; H-3, H_2-4, and H_2-6 with C-5; and H-17, H-20 and H_2-23 with C-22. On the basis of spectral data analysis, the structure of **6** has been elucidated as (20*S*,22*R*)-1-oxo-witha-3β, 5β-diol-24-enolide, a new withanolide (Fig. 2).

3.6. Spectroscopic characteristics of compound **7**

Compound **7**, named withacoagunalide C6-arabinosyl 2′-*O*-anisate, gave positive tests for glycosides and showed UV absorption maxima at 224 and 281 nm typical for withanolides. Its IR spectrum exhibited absorption bonds for hydroxyl groups (3525, 3427 cm^{-1}), unsaturated δ lactone (1709 cm^{-1}), carbonyl function (1680 cm^{-1}) and unsaturation (1640 cm^{-1}).

On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **7** was established at *m/z* 706 consistent with a molecular formula of a withanolide glycosidic ester, $C_{41}H_{54}O_{10}$. The ion peaks arising at *m/z* 151 [C_2′-C_7′′ fission, $C_8H_7O_3]^+$, 279 [C_1′-O fission, $C_{14}H_{15}O_6]^+$, 439 [M-279]$^+$ and 295 [C6-O fission, $C_{14}H_{15}O_7]^+$ suggested that an ortho-anisate substituted pentoside was linked with the withanolide. The ^{1}H NMR spectrum of **7** showed a one-proton multiplet at δ 6.09 and a one-proton doublet at δ 5.86 (*J* = 8.4 Hz) in the deshielded region assigned to vinylic H-3 and H-2 protons, respective-

ly. A one-proton triplet doublet at δ 4.67 (*J* = 9.6, 4.5, 5.3 Hz) was ascribed to the C-22 oxymethine proton of the lactone moiety. A one-proton broad multiplet at δ 3.81 with half-width of 18.2 Hz as due to α-oriented oxymethine H-6α proton. A one-proton doublet at δ 5.23 (*J* = 7.1 Hz) was accounted to anomeric H-1′ proton. The other sugar proton appeared as a one-proton multiplets at δ 4.75 (H-2′), 4.45 (H-3′), 3.56 (H-4′) and as one-proton doublets at δ 4.14 (*J* = 9.2 Hz) and 4.03 (*J* = 9.2 Hz) accounted to sugar oxymethylene H_2-5′ protons. Four one-proton multiplets at δ 6.94, 6.72, 6.03 and 5.63 were associated correspondingly with the aromatic H-3′′, H-4′′, H-6′′ and H-5′′ protons. A three-proton broad singlet at δ 3.35 was accompanied to the methoxy protons. Four three-proton broad singlets at δ 1.83, 1.76, 1.30 and 0.88 were attributed to C – 27 and C-28 methyl protons attached to the vinylic C-24 and C-25 carbons, and to C-19 and C-18 tertiary methyl protons, respectively. A three-proton doublet at δ 1.01 (J = 6.5 Hz) was accounted to the secondary C-21 methyl protons. The ^{13}C NMR spectrum of **7** displayed signals for carbonyl carbon at δ 205.18 (C-1), vinylic carbons at δ 125.85 (C-2) and 147.29 (C-3) in the deshielded region indicating the presence of α,β-unsaturated ketone. The signals for vinylic carbons at δ 152.37 (C-24) and 124.22 (C-25), oxymethine carbon at δ 81.31 (C-22) and lactone carbon at δ 165.75 (C-26) suggested α,β-unsaturated lactone in the E ring. The signal at δ 73.97 was due to oxymethine C-6 carbon. A signal at δ 107.21 was attributed to anomeric C-1′ carbon. The other sugar carbon resonated from δ 87.89 to 60.85. Six signals in the downfield region assignable to the aromatic carbons appeared at δ 147.29 (C-1′′), 152.81 (C-2′′), 139.32 (C-3′′), 127.29 (C-4′′), 125.86 (C-5′′) and 119.06 (C-6′′). The signals at δ 168.53 and 55.01 were due to ester C-7′′ and methoxy carbons, respectively. The methyl carbons resonated from δ 20.69 to 14.29 (Atta-ur-Rahman et al., 2003; Yang et al., 2014 ; Chepkorir et al., 2018). The existence of the sugar carbon in the deshielded region at δ 87.89 suggested the attachment of the aromatic ester at C-2′ carbon. The HMBC spectrum of **7** exhibited correlations of H-2 and H-3 with C-1; H-5 and H_2-7 with C-6; H-6, H-2′ and H-3′ with C-1′; H-17, H-20 and H_2-23 with C-22; and H-2′ and H-6′′ with C-7′′. Acid hydrolysis of **7** yielded 6β-hydroxywithacoagulanide A, $[M]^+$ at *m/z* 440 ($C_{28}H_{40}O_4$), β-D-arabinose, m.p.: 156-159 °C; $[\alpha]D^{20}$-103° (*c* 10, H_2O), R_f: 0.42 (*n*-butanol-pyridine-water, 3:1:1) and *o*-anisic acid (2-methoxybenzoic acid), m.p.: 101-103 °C. On the basis of the forgoing account, the structure of **7** has been formulated as (20*S*,22*R*)-1-oxo-witha-6β-ol-2,24-dienolide-6β-*O*-D-arabinopyranosyl-2′-(2′′-methoxy)-benzoate, a new withanolide glycosidic anisate (Fig. 2).

3.7. Spectroscopic characteristics of compound **8**

Compound **8,** named 3-*O*-Digalactosyl withacoagulanide B, gave positive test for glycoside and had distinctive UV absorption maximum at 289 nm for withanolides. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3425, 3367, 3280 cm^{-1}), δ-lactone (1712 cm^{-1}), carbonyl fraction (1708 cm^{-1}) and unsaturation (1645 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **8** was established at *m/z* 766 consistent with a molecular formula of a withanolide diglucoside $C_{40}H_{62}O_{14}$. The ion peaks arising at *m/z* 341 [C_3-O fission, $C_{12}H_{21}O_{11}]^+$, 425 $[M-341]^+$, 179 [C_2′-O fission, $C_6H_{11}O_6]^+$, 587 $[M-179]^+$, 163 [C_1′′-O fission, $C_6H_{11}O_5]^+$ and 603 $[M-163]^+$ indicated the attachment of a dihexoside unit with the withanolide. The ^{1}H NMR spectrum of **8** displayed a one-proton broad multiplet at δ 3.63 with half width of 18.5 Hz assigned to oxymethine H-3α-proton, a one-proton triplet doublet at δ 4.93 with coupling interactions of 12.6, 5.3 and 5.1 Hz ascribed to lactonic oxymethine H-22 proton, five three-proton signals as broad singlets at δ 0.96, 1.13, 1.78 and 1.90 and as a doublet at δ 1.05 (*J* = 6.5 Hz) attributed correspondingly to tertiary C-18, C-19, C-27 and C-28 and secondary C-21 methyl protons. Two one-proton doublets at δ 5.70 (*J* = 7.3 Hz) and 5.43 (*J* = 7.1 Hz) were accounted to anomeric C-1′ and C-1′′ protons, respectively. The other sugar protons appeared between δ 4.43-3.20. The ^{13}C NMR spectrum of **8** showed signals for carbonyl carbon at δ 203.61 (C-2), δ-lactone carbon at δ 165.83 (C-26), vinylic carbons at δ 151.20 (C-24) and 120.84 (C-25), anomeric carbons at δ 109.15 (C-1′) and 101.86 (C-1′′) and other sugar carbon in the range of δ 86.43-60.85. The presence of H-2′ signal in the ^{1}H NMR spectrum in deshielded region at δ 4.27 and carbon C-2′ at δ 81.37 indicated (2′→1′′) linkage of the sugar units. The HMBC spectrum of **8** showed that H_2-2 and H-3 interacted with C-1; H-3, H-2′ and H-3′ interacted with C-1′; H-2′, H-2′′ and H-3′′ interacted with C-1′′; and H-17, H-20 and H_2-23 interacted with C-22. Acid hydrolysis of **8** yielded 3β-hydroxy-withacoagulanide B, $[M]^+$ at *m/z* 442 ($C_{28}H_{42}O_4$) and β-D-galactose, R_f: 0.16 (*n*-butanol-acetic acid-water, 4:1:5, *v/v*), specific rotation $[\alpha]^{20}D$ +78° to + 81° (c = 10%, water). On the basis of the above discussion, the structure of the compound **8** has been elucidated as (20*S*,22*R*)-1-oxo-witha-3β-ol-24-enolide-3β-*O*-D-galactopyranosyl-(2′→1′′)-β-*O*-D-galactopyranoside, a new withanolide digalactoside (Fig. 2).

3.8. Spectroscopic characteristics of compound **9**

Compound **9**, showed distinctive IR absorption bands for hydroxyl groups (3401 cm^{-1}), ester linkage (1721cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **9** was determined at *m/z* 648 consistent with a molecular formula of a glyceride phosphate, $C_{35}H_{69}O_8P$. An ion peak arising at *m/z* 239 $[(CH_3CH_2)_{14}CO]^+$ indicated that palmityl groups were linked along with phosphate function to the glyceryl unit. The ^{1}H NMR spectrum of **9** displayed oxygenated methylene and methine protons as multiplet at δ 4.90 (H-2), 4.50 (H_2-3) and 4.36 (H_2-1). Two triplets at δ 2.73 (*J* = 11.2 Hz) and 2.62 (*J* = 7.2 Hz), integrating for two proton each, were ascribed to methylene (H_2-2′) and H_2-2′′ protons, respectively, adjacent to the ester functions. The other methylene protons resonated as multiplets at δ 2.32 (2H), 2.27 (2H) and 1.55 (4H) and as a broad singlet at δ 1.23 (44 H). Two three-proton triplets at δ 0.91 (*J* = 6.5 Hz) and 0.83 (*J* = 6.3 Hz) were accounted corre-

spondingly to terminal C-16′ and C-16′′ primary methyl protons. The ^{13}C NMR spectrum of **9** exhibited signals for ester carbons at δ 175.04 (C-1′) and 171.60 (C-1′′), oxymethylene carbons at δ 72.89 (C-3) and 67.47 (C-1), oxymethine carbon at δ 74.36 (C-2), methylene carbon in the range of 56.18-22.54 and methyl carbons at δ 14.41 (C-18′) and 14.39 (C-16′′). On the basis of spectral data analysis, the structure of **9** has been characterized as glyceryl-1, 2-dihexadecanoate-3-phosphate (glyceryl-1, 2-dipalmityl 3-phosphate) (Fig. 2).

3.9. Spectroscopic characteristics of compound **10**

Compound **10,** named 3-secowithacoagulanolide 6β-olyl galactourinoside, yielded effervescences with sodium bicarbonate solution and gave positive test for glycosides. Its UV absorption maxima at 281 and 321 nm indicated withanolide type of the compound. Its IR spectrum showed distinct absorption bands for γ-lactones (1732 cm^{-1}), δ-lactone (1702 cm^{-1}), carbonyl function (1710 cm^{-1}), carboxylic groups (1690, 1675 cm^{-1}), hydroxyl groups (3565, 3428, 3231 cm^{-1}) and unsaturation (1635 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **10** was determined at *m/z* 766 consistent to the molecular formula of a secowithanolide glycoside, $C_{34}H_{38}O_{20}$. An ion peak generated at *m/z* 193 [C_1′-O fission, $C_6H_9O_7]^+$ suggested the linkage of hexanose carboxylic acid to the seco-withanolide unit. The ^{1}H NMR spectra of **10** showed a one-proton triple doublet at δ 4.11 (*J* = 7.2, 7.5, 5.7 Hz) and two one-proton multiplets at δ 4.08 Hz ($w_{1/2}$ = 18.8 Hz) and 3.66 ($w_{1/2}$ = 17.5 Hz) assigned to oxymethine H-22, H-11α and H-6α protons, respectively. A one-proton doublet at δ 5.06 (*J* = 7.3 Hz) was ascribed to anomeric H-1′ proton. The other sugar proton appeared as one-proton multiplets at δ 4.19 (H-5′), 3.67 (H-2′), 3.45 (H-3′) and 3.17 (H-4′). A three-proton doublet at δ 1.18 (*J* = 6.5 Hz) and a three-proton triplet at δ 1.05 (*J* = 6.9 Hz) were due to secondary C-4 and primary C-3 methyl protons indicating seco nature of the molecule. The other methine and methylene protons resonated between δ 2.85-1.23. The ^{13}C NMR spectrum of **10** displayed signals for carbonyl carbon at δ 205.68 (C-1), δ lactone carbons at δ 172.01 (C-18), 170.30 (C-19) and 162.63 (C-26), vinylic carbons at δ 152.22 (C-24) and 122.81 (C-25), carboxylic carbons at δ 177.51 (C-21), 174.86 (C-27), 173.70 (C-28) and 178.40 (C-6′), anomeric carbon at δ 110.13 (C-1′), other sugar carbons from δ 88.70 to 63.56 and methyl carbons at 14.38 (C-3) and 18.62 (C-4).

The HMBC spectrum of **10** exhibited that H_2-2 and H_3-3 interacted with C-1; H-6, H-2′ and H-3′ interacted with C-1′; H-5′ interacted with C-6′; and H-17, H-20 and H_2-23 interacted with C-22. Acid hydrolysis of **10** produced β-D-galacturonic acid, m.p.: 159 °C, R_f: 0.17 (*n*-butanol-pyridine-water, 3:2:1.5).

On the basis of these evidences, the structure of **10** was estabilised as 1-oxo-3-seco-witha-21, 27, 28-trioic acid-24-ene-6β-ol-19(8), 18(11)-diolide-*O*-6β-*O*-D-galacuronopyranoside, a new secowithanolide galacturonoside (Fig. 2). The seco-withanolides have been reported from *Physalis* species (Fang et al., 2010; Lin et al., 2016; Sun et al., 2017).

3.10. Spectroscopic characteristics of compound **11**

Compound **11,** named withacoagulanide C 6β-olyl triacetyl diarabinosyl 2′′-(*O*)-anisate, responded positively to glycosidic tests and had typical UV absorption maxima at 295 and 321 nm for withanolides. Its IR spectrum exhibited characteristic absorption bands for a hydroxy group (3370 cm^{-1}), ester functions (1721 cm^{-1}), δ-lactone (1709 cm^{-1}), unsaturated carbonyl function (1680 cm^{-1}) and unsaturation (1635 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **11** was determined of *m/z* 964 consistent with a molecular formula of a withanolide diglycosidic ester $C_{52}H_{68}O_{17}$. The ion peaks generating at *m/z* 151 [C_2′′-O fission, C_6H_4-(OMe)COO]$^+$, 351 [C_1′′-O fission, C_6H_4-(OMe)COO-$C_5H_6(OAc)_2$, $C_{17}H_{19}O_8]^+$, 439 [C_1′-O fission, $C_{28}H_{39}O_4]^+$ and 524 [M-439]$^+$ suggested that anisic acid linked two acetylated pentosyl units were attached to the withanolide. The ^{1}H NMR spectrum of **11** showed a one-proton multiplet at δ 6.68 (1H, m, H-3) and a one-proton doublet at δ 5.86 (*J* = 10.1 Hz, H-2) assigned to ring A vinylic H-3 and H-2 protons, respectively, a one-proton triple doublet at δ 4.89 (*J* = 12.7, 5.6, 5.2 Hz) ascribed to oxymethine H-22 of δ-lactone ring, a one-proton broad multiplet at δ 3.86 with half-width of 18.6 Hz accounted to α-oriented oxymethine H-6 proton, four one-proton multiplets at δ 7.61, 7.23, 7.08 and 6.68 associated with the aromatic protons and five methyl protons as three-proton broad singlets due to tertiary methyl protons at δ 1.89 (Me-27), 1.76 (Me-28), 1.18 (Me-19) and 0.85 (Me-18) and as a three-proton doublet at δ 1.03 (*J* = 6.7 Hz) accounted to secondary C-21 methyl protons. Two one-proton doublets at δ 5.76 (*J* = 7.5 Hz) and 5.13 (*J* = 7.3 Hz) were attributed to anomeric H-1′ and H-1′′ protons, respectively. The other sugar protons appeared from δ 4.41 to 3.47. Four three-proton broad singlets from δ 3.56-2.02 were accounted correspondingly to one methoxy protons linked to the aromatic ring and three acetyl protons of the sugar units. The ^{13}C NMR spectrum of **11** exhibited signals for carbonyl carbons at δ 204.12 (C-1), δ-lactone at δ 166.67 (C-26), aromatic and vinylic carbons from δ 151.85 to 116.03, anomeric carbons at δ 109.13 (C-1′) and 101.84, other sugar carbons between δ 86.45-61.59, ester carbons in the range of δ 175.75-170.40, acetyl carbons at δ 21.44 and methoxy carbon at δ 53.10. The shifting of H-2′ in the deshielded region at δ 4.11 in the ^{1}H NMR spectrum and C-2′ at δ 80.53 indicated (2′→1′′) linkage of the sugar units. The attachment of the aromatic acid ester at C-2′′ was deduced by shifting of the sugar H-2′′ proton in the downfield region at δ 4.41 in the ^{1}H NMR spectrum and C-2′′ at δ 86.45 in the ^{13}C NMR spectrum. The existence of the sugar protons in the deshielded region at δ 4.32 (H-4′), 4.01 (H-3′′) and 4.20 (H-4′′) indicated the location of the acetoxy groups at these position. The HMBC spectrum of **11** showed correlations of H-2 and H-3 with C-1; H-6, H-2′ and H_2-5′ with C-1′; H-4′ and H_2-5′ with O-CO-Me; H-4′′ and H_2-5′′ with O-CO-Me; and H-2′′ and H-1′′′ with C-7′′′. Acid hydrolysis of **11** yielded 6β-hydroxywithacoagulanide A, [M]$^+$ at *m/z* 440 ($C_{28}H_{40}O_4$) (5.6), β-D-arabinose, [α]D^{20}-103° (*c* 10, H_2O), R_f: 0.42 (*n*-butanol–pyridine-water, 3:1:1) and *o*-anisic

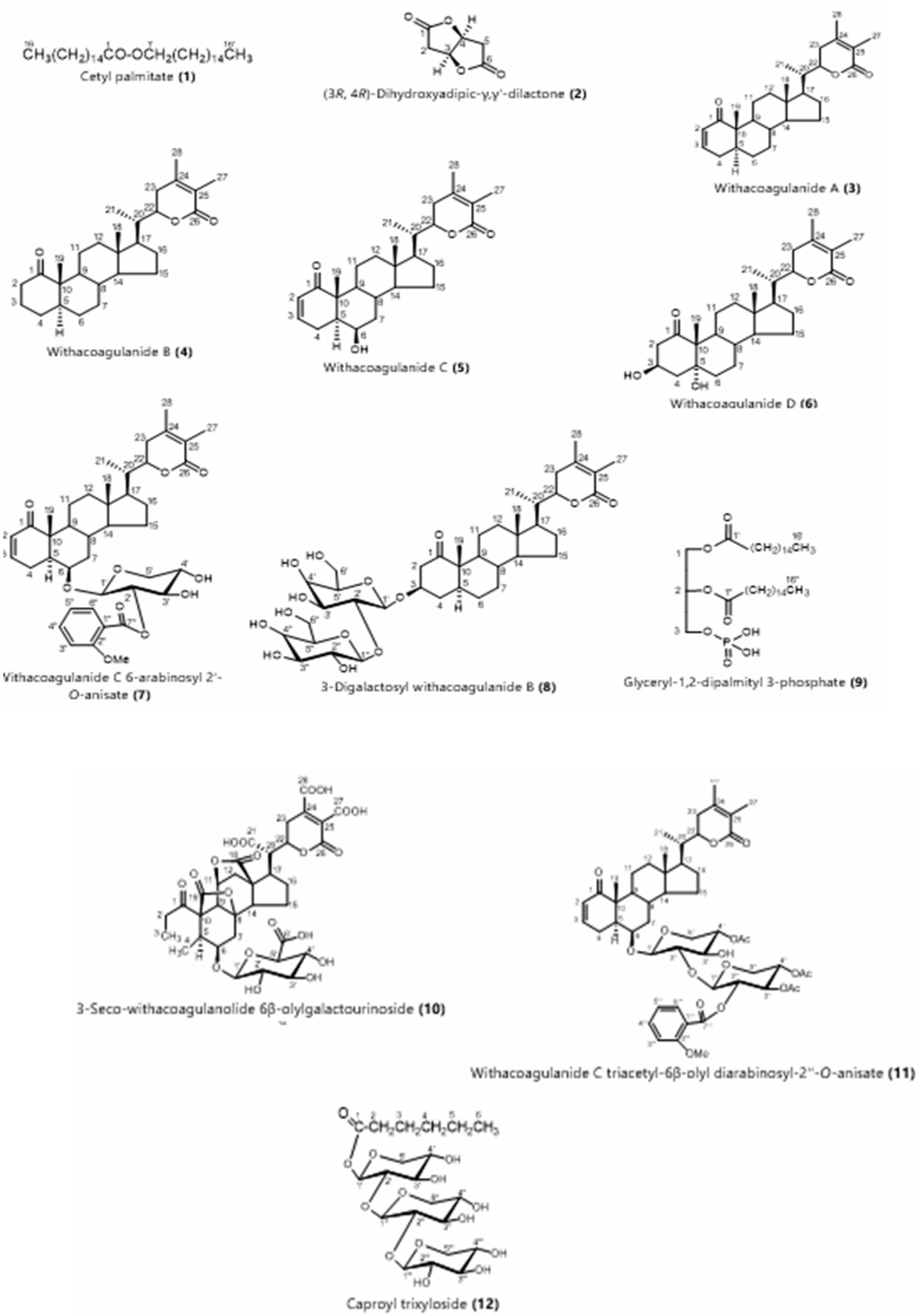

Fig. 2. Chemical constituents of **1-12** isolated from the fruits of *Withania coagulans*.

acid (2-methoxybenzoic acid), m.p.: 101-103 °C. On the basis of these evidences, the structure of **11** has been established as (20*S*,22*R*)-1-oxo-witha-6β-ol-2,24-enolide-6β-*O*-D-(4′-acetoxy arabinopyranosyl-(2′→1′′)-(3′′, 4′′-diacetoxy arabinopyranosyl)-2′′-(2′′′-methoxy)-benzoate, a new withacoagulanide C diarabinosidic ester (Fig. 2).

3.11. Spectroscopic characteristics of compound **12**

Compound **12**, named caproyl trixyloside, $[M]^+$ at *m/z* 512 ($C_{21}H_{36}O_{14}$), responded positively to glycosidic tests and had IR absorption bands for hydroxyl groups (3445, 3386, 3321, 3271 cm^{-1}) and ester function (1743 cm^{-1}). The ion peaks produced at *m/z* 133 [C_1′′′-O fission, $C_5H_9O_4]^+$, 149 [C_2′′-O fission, $C_5H_9O_5]^+$, 281 [C_2′-O fission, $C_{10}H_{17}O_9]^+$, 397 [C_1′-O fission, $C_{15}H_{25}O_{12}]^+$ and 115 [M-397, $C_6H_{11}O_2]^+$ supported that three pentosyl units were linked to a hexanoic acid unit. The ^{1}H NMR spectrum of **12** showed three one-proton doublets at δ 4.90 (*J* = 7.5 Hz), 4.86 (*J* = 7.3 Hz) and 4.80 (*J* = 7.5 Hz) assigned to anomeric H-1′, H-1′′ and H-1′′′ protons, respectively. The other sugar protons resonated from δ 4.50 to 3.05. A two-proton triplet at δ 2.91 (*J* = 7.8 Hz) and two multiplets at δ 1.91 (2H) and 1.29 (4H) were ascribed to methylene H_2-2 proton adjacent to the ester function and other methylene protons. A three-proton triplet at δ 1.03 (*J* = 6.6 Hz) was accounted to C-6 primary methyl protons.

The ^{13}C NMR spectrum of **12** displayed signals for ester carbon at δ 172.41 (C-1), anomeric carbons at δ 100.14 (C-1′), 97.37 (C-1′′) and 92.69 (C-1′′′), other sugar carbons in the range of 77.25-61.70, methylene carbons from δ 56.49 to 21.51 and methyl carbon at δ 18.99 (C-6). The appearance of H-2′ and H-2′′ as a multiplets in the deshielded region at δ 4.50 and 4.38 in the ^{1}H NMR spectrum and C-2′ and C-2′′ at δ 77.25 and 77.19 in the ^{13}C NMR spectrum, respectively, suggested (2→1) attachment of the sugar units. Acid hydrolysis of **12** yielded D-xylose, R_f: 0.81 (*n*-butonal-pyridine-water, 6:4:3, *v/v*), m.p.: 153-156 °C, $[\alpha]D^{20}$ + 91 ° (water, 10%) and caproic acid (hexanoic acid), R_f: 0.82 (methanol-acetic acid-tetralin, 10: 2: 1, *v/v*). On the basis of the foregoing account, the structure of **12** has been determined as *n*-hexanoyl-β-*O*-D-xylopyranosyl-(2′→1′′)-β-*O*-D-xylopyranosyl-(2′′→1′′′)-β-*O*-D-xylopyranoside, a new caproyl trixyloside (Fig. 2).

4. Concluding remarks

Phytochemical investigation of the the fruits of *Withania coagulans* afforded cetyl palmitate (**1**), (3*R*,4*R*)-dihydroxyadipic-γ,γ′-dilactone (**2**), eight withanolides (**3-8**, **11**), glyceryl-1,2-dipalmityl 3-phosphate (**9**) and caproyl trixyloside (**12**) for the first time. All these chemical constituents are reported for the first time from the *Withania* species.

This work has enhanced understanding about the chemical constituents of the plant. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

Conflict of interest

The authors declare that there is no conflict of interest.

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