Review Article

Plants' Steroidal Saponins - A Review on Its Pharmacology Properties and Analytical Techniques

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Abstract

The plant is a rich repository of useful secondary metabolites with profound medicinal potential. Saponins, one type of bioactive compound, are amphitheatric glycosides with one and more hydrophilic sugar and hydrophobic steroidal and terpenoid part. The former is known as steroidal saponin, and the latter is called terpenoid saponins. Steroidal saponin is mostly distributed among monocotyledon families such as *Asparagaceae*, *Amaryllidaceae*, *Dioscoreaceae*, *Smilacaceae*, and *Liliaceae*. Even though it is unusual, it could also be detected to some extent by dicotyledonous angiosperms, such as *Plantaginaceae*, *Zygophyllaceae*, *Fabaceae*, *Asteraceae*, and *Solanaceae*. It exhibits diverse pharmacological ability including antimicrobic, anti-inflammatory, cAMP phosphodiesterase inhibitory, antiadipogenic, bactericide, cardioprotective, antitumor, antidiabetic, cytotoxic activity, antifungal, antiviral, antioxidant, and hepatoprotective. Steroidal saponin timosaponin AIII from *Anemarrhena asphodeloides* has been found to possess antitumor activity. Diosgenin, another steroidal sapogenin, has the potential of preventing neurological diseases by affecting different signaling pathways, increasing bone formation, and increasing antithrombotic activity. Spicatoside A from *Liriope platyphylla* possesses anti-inflammatory, antiasthma, and antiosteoclastogenic activities. TTB2 from *Trillium tschonoskii* exhibits anticancer potential. The cell cycle arrest and ROS-dependent autophagy are induced by polyphyllin I. These diverse biological activities of steroidal saponins are attributed to the variability of their structural features. Analysis of steroidal saponins in plant materials mainly utilizes classically and advances thin layer chromatography (TLC) on normal and reverses-phase (high-performance thin-layer chromatography, densitometric TLC), gas chromatography, LC, UPLC, ultra-high-performance liquid chromatography (HPLC), supercritical fluid chromatography, and HPLC coupled to ultraviolet detector and diode array detector. HPLC coupled with MS and Nuclear magnetic resonance is used for online identification of separated saponins. The present review aims to furnish a comprehensive account of the recent advances in analytical methods of determination and medicinal applications of steroidal saponins.

Keywords: Steroidal, Saponins, Glycosides, Antitumour, Antioxidant, Analytical techniques

Introduction

Since bioactive compounds occurring in the herbal plant are popular as traditional medicine for different diseases. Currently using phytochemicals are treated to be secure and friendly for the human body. Phytochemicals are bioactive compounds naturally occurring which act as medicine and nutrient for the benefits of the human health.[1] Plants are a versatile source of different organic chemicals or phytochemicals. They comprised two groups in respect of their activity in plants as primary and secondary metabolites. The metabolites that are required to complete plant basic metabolic processes are known as primary metabolites, such as fats, carbohydrates, proteins, nucleic acid, and chlorophyll. They found throughout the plant kingdom. They are produced in large quantities and can easily extract. Secondary metabolites are not involved in primary metabolic processes but play a role to protect against abiotic and biotic stresses and ensure their existence in the environment. They usually produced in minor concentration and extraction often difficult and expensive.[2] Some examples are alkaloids, phenolics, terpenes, saponins, flavonoids, glucosides, lignans, curcumins, and plant steroids.[3,4]

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Saponins are a class of naturally occurring bioorganic compounds having steroids and terpenoids of glycosides with distinctive foaming characteristics. The name gets from the Soapwort plant (*Saponaria*), historically its roots were used as soap.[5] Hydrolysis of saponin gives a fat-soluble (hydrophobic) sapogenin and water-soluble (hydrophilic) sugar part which complement the foaming capability of saponins.[6,7] Based on the category of sapogenin, saponins split into three major kinds:

1. Steroid glycosides
2. Triterpenoid glycosides
3. Alkaloid glycosides.[8]

Many pieces of literature show that saponins are an important class of bioactive compound which possess medicinal properties. In the pharmaceutical industry, they are the substrate of many drugs. Numerous scientists' attention is seeking by steroidal saponins, a type of saponins. The various papers reported its wide spectrum pharmaceutical properties such as antimicrobic, anti-inflammatory, cAMP phosphodiesterase inhibitory, antiadipogenic, bactericide, and cardioprotective.[9-14] The various medicinal research and its results show the increasing interest in steroidal saponins which will act as bionatural compound. In this review, we briefly account for (1) the chemistry of steroidal saponins, (2) plant sources of saponins, (3) synthesis of steroidal saponins, (4) various pharmaceuticals properties, and finally, (5) different analytical techniques.

Chemistry of Steroidal Saponins

Structurally steroid glycosides or steroidal saponins are modified terpenoids that contain an aglycone and a glycone part with tetracyclic six-membered rings and bicyclic five-membered rings containing 27 carbon atoms [Figure 1].[15] Usually, aglycone part of it contains a furostanol or a spirostanol. Mostly, the glycone parts are oligosaccharides, organized moreover in a branched or linear form, linked to hydroxyl groups via a (2, 3) acetal linkage.[16] The glycone residue of steroidal glycosides made up of one to three sugar chains either linear or branched, which contain usually β-D-galactopyranosyl (Gal), β-D-mannopyranosyl (Man), α-L-rhamnopyranosyl (Rha), β-D-quinovopyranosyl (Qui), β-L-arabinofuranosyl (Ara), β-D-glucopyranosyl (Glc), β-D-xylopyranosyl (Xyl), or β-D-fucopyranosyl (Fuc) residues [Figure 2].[18]

Steroidal saponins could be grouped into three distinct classes according to their aglycone group. They could be categorized into three distinctive groups: a spirostane, a cholestane (open chain), and a furostane compound.[20] [Table 1].[22]

Occurrence and Distribution of Steroidal Saponins

Steroidal saponins are synthesized and accumulated by various plant families. They are typically distributed in members of *Asparagaceae* (*Yucca*, *Agave*, *Tupistra*, *Anemarrhena*, *Sansevieria*, *Asparagus*, *Polygonatum*, *Nolina*, *Convallaria*, *Ophipogon*, *Hosta*, *Ornithogalum*, *Ruscus*), *Amaryllidaceae* (*Allium* and *Agapanthus*), *Dioscoreaceae* (*Dioscorea*), *Smilacaceae* (*Smilax*), *Fritillaria*, *Lilium* (*Liliaceae*), *Costaceae* (*Costus*), and *Melanthiaceae* (*Paris*). Even though it is unusual, steroidal glycosides could also be detected to some extent of dicotyledonous angiosperms, such as *Plantaginaceae* (*Digitalis*), *Zygophyllaceae* (*Tribulus*, *Zygophyllum*), *Fabaceae* (*Trigonella*), *Asteraceae* (*Vernonia*), and *Capsicum*, *Lycopersicon*, *Solanum* (*Solanaceae*).[14,19,23-26]

Besides plants, some animals also act as a source of it. They have been spotted in marine sponges and starfish.[27-29]

Biosynthesis of Steroidal Saponin

Plants represent the primary producer of steroidal saponins, the majority of monocotyledonous species. Steroidal saponin produces two portions, glycone and aglycone parts during hydrolysis. The aglycone backbone is derived from 2,3 oxidosqualene, a linear precursor of 30C molecules. The synthesis of the committed precursor of steroidal saponins releases three methyl groups to form a 27C aglycone backbone.[30,31]

The steroidal saponin aglycone backbone is an isopentenyl pyrophosphate (IPP) which is synthesized from acetyl-CoA via a mevalonic acid pathway and MEP (2-C methyl-D-erythritol 4-phosphate) pathway, in cytoplasm and plastids, respectively. The acetyl CoA converted to IPP (5C), which then isomerized to form allylic isomer dimethylallyl pyrophosphate (DMAPP) in the presence of enzyme, isopentnyldisphosphate isomerase. Then, subsequent condensation of two units of IPP and one unit of DMAPP from farmesyl pyrophosphate (FPP) catalyzed by farnesyl pyrophosphate synthetase, the intermediate precursor of finally two FPP unit, forms linear squalane (30C) by condensation reaction catalyzed by SQS (Squalane synthase), which further epoxidized by enzyme squalene epoxidase (SQE) to form 2,3 oxidosqualane. It further cyclized to form cycloartenol, catalyzed by cycloartenol synthase (CAS). The cycloartenol generated a mixture of phytosterols including cholesterol (27C), campesterol (28C), and sitosterol (29C). The series of glycosylation and oxygenation of cholesterol base to furostanol or spirosanol derivative with fused O-heterocycle in formerly core aglycone framework to form steroidal synthesis.[30-33]

Pharmacological Properties of Steroidal Saponins [Table 2]

Cytotoxic property

The cytotoxic action is performed by most of the steroidal saponins via triggering apoptosis stimulation. It also stimulates oncosis, autophagy, and repression of metastatic characteristics of the examined cells, phagocytosis, or vascularization.[18] For example, a steroidal saponin isolated

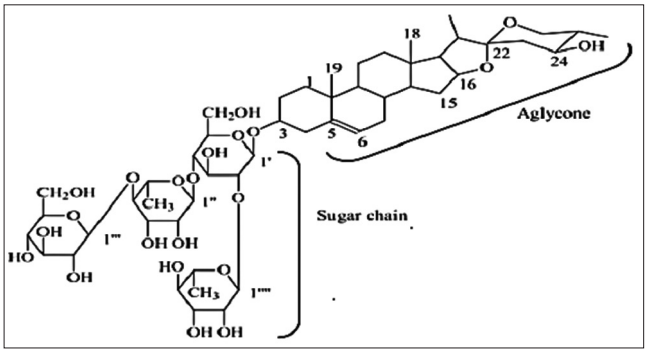


Figure 1: Chemical structure of steroidal saponins[21]

from *Paris polyphylla* stimulates apoptosis and autophagy via activating caspase 8 and 3, upregulation of Beclin1, and PARP cleavage for the former.[34] It performs cytotoxic activity through mitochondrial caspase-independent and dependent pathway, PI3K/Akt signaling, or cyclin-dependent kinase 1.[35] The commonly known glycoside–dioscin triggered both intrinsic (activation of Bak and Bid proteins and loss of mitochondrial membrane potential) and extrinsic (modulation of death ligands and receptors) apoptosis pathways which is a rare proapoptotic activity mechanism. In addition, the promyelocytes differentiate into granulocytes and monocytes induced by this compound.[18,36]

Anti-inflammatory activity

Inflammation is the response of the host to stimuli which takes place due to the pro-inflammatory cytokines such as IL1-β, TNF-α, and IL-6 produced by immune cells, recruited to wound sites. The significant anti-inflammatory activity observes due to inhibition of the inflammation mediators. The steroidal saponin diosgenin inhibits some of the inflammatory mediators derived from macrophage.[37]

Antidiabetic activity

Diabetes mellitus is one of the major concerns for a universal health issue that is distinguished by hyperglycemia that generates oxidative stress which leads to free radicals' production.[38] It leads to various complications; for instance, peripheral vascular disease, neuropathy, and retinopathy are some examples of complications resulting from diabetes.[39] Saponins use various mechanisms for lowering blood glucose level such as activation of glycogen synthesis, suppression of the activity of disaccharides, modulation of insulin signaling, regeneration of insulin action, and suppression of gluconeogenesis. For example, diosgenin displays antidiabetic effects by the mitigation of insulin resistance and hyperglycemia.[40] Gestational diabetes is also prevented by diosgenin via targeting sterol regulatory binding protein 1.[41] It prevents high glucose-induced renal tubular fibrosis.[42]

Antitumor activity

Steroidal saponin has shown antitumor activities against different kind of tumors, such as mammary carcinoma, esophageal cancer, cervix cancer, colon cancer, leukemia, gastric carcinoma, prostate cancer, lung cancer, ovarian cancer, and glioblastoma. The listed target tissues were mentioned in the review on saponins in 2016.[43] For example, dioscin steroidal saponin shows antitumor effects through activating intrinsic mitochondrial apoptosis by involving activation of caspase 9 and caspase 3 and decreasing levels of antiapoptotic proteins such as Bcl-xl, Bcl-2, McI-1, and clAP-1.[44-46] Steroidal saponins encounter antitumor activity via activating different signaling pathways and mechanisms. For example, PI3K/Akt/mTOR and p38 MAPK and JNK signaling pathways and numerous proteins, enzymes, and factors involved in antitumor activities of dioscin.[47]

Hepatoprotective property

The major organ in human for detoxification and assimilation is liver, which often faces numerous stresses that lead multiple pathogeneses. These pathological changes can exhibit cholestasis, fatty liver disease, fibrosis, and injuries. Steroidal saponin, for example, dioscin, involves different mechanisms to protect hepatocytes. It inhibits necrosis, apoptosis, inflammation, necrosis, and oxidative stress to attenuate acute liver injury caused by CCl4 and DMN.[48,49]

Antifungal

Various steroidal saponins have shown various antifungal activities. Generally, spirostanol skeleton steroidal saponin has shown high levels of antifungal activity than furostanol.[50] Distinct biochemical changes were observed during antifungal activity. For example, dioscin could be effective to show antifungal activity via inducing plasma membrane damage of *Candida albicans*,[51] cell membrane disruptive activity,[52] effective against *C. albicans* biofilms,[53,54] generated excessive ROS, and increased membrane permeability in *Saprolegnia parasitica*.[47,55] *Allium minutiflorum* produced a compound minutosides A–C which showed antifungal activity based on the concentration-dependent manner on listed fungus: *Fusarium oxysporum, Alternaria alternata, Fusarium solani, T. harzianum T39, Alternaria porri, Botrytis cinerea, Trichoderma harzianum P1, Pythium ultimum,* and *Rhizoctonia solani*.[17,19]

Antibacterial

Mohammed (2009) studied that the antibacterial activity of saponin extracted from *Tribulus terrestris* against the microorganisms examined showed inhibiting effect on both types of Gram bacteria, which show the broad-spectrum antibiotic presence or simply metabolic toxin produced by the plant. Saponins contribute to antibacterial activity maybe via membrane lysis, rather than changing the surface tension of the extracellular fluid, hence being affected by microbial population density.[57]

Cardioprotective

During the treatment of different organs, many drugs produce toxicity for the heart. Some steroidal saponins contribute to protecting the heart, such as diosgenin increased efflux of cholesterol and repressed aortic atherosclerosis.[58] It also

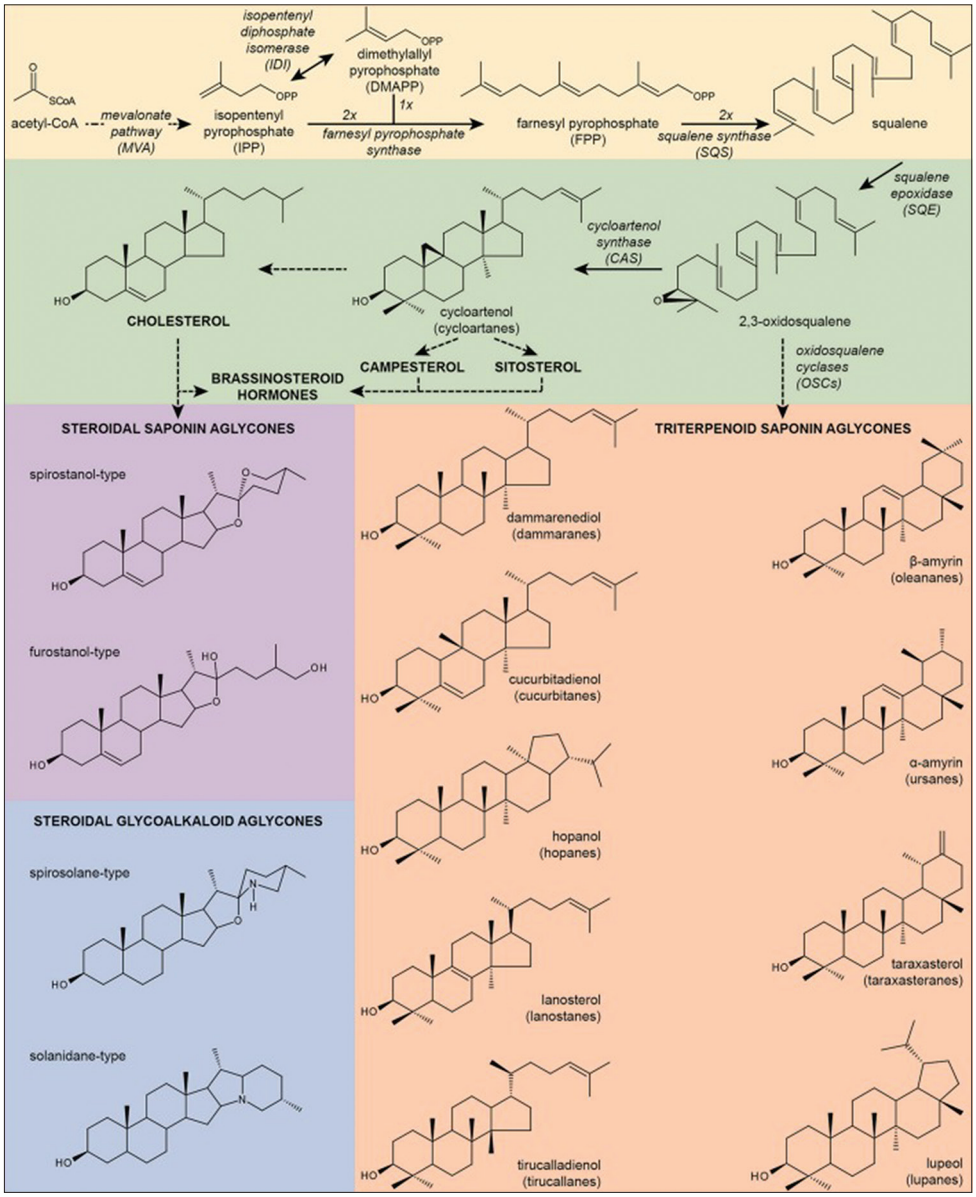


Figure 2: Overview of structural diversity in saponin aglycones[31]

contributes cardioprotective role via regulating the opening of potassium channels.[59] The combination of morroniside and diosgenin also plays a role in the prevention of myocardial injury induced by high glucose.[60,61] In addition, dioscin plays a role to suppress an angiotensin II infusion which induces cardiac hypertrophy via downregulating the MAPK and Akt/GSK3 β/mTOR pathways, which contribute to improving the impaired function of the cardiac.[62]

Antioxidant

Oxidative reactive species or oxidative stress acts as sources of many pathogenesis diseases. Steroidal saponin also acts as an antioxidant. For example, the aqueous extract of *Asparagus racemosus* root exhibits antistress activity in a mouse by inhibiting the effect of inflammatory cytokines mainly interleukin and tumor necrosis factor.[63]

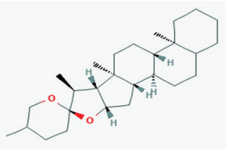
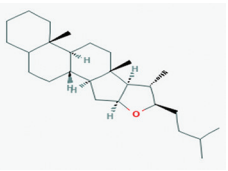
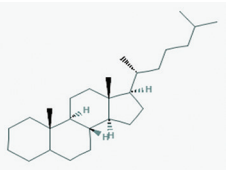
Antihypertensive property

The *T. terrestris* possesses antihypertensive activity.[64] The *Tribulus* extracts possess diuretic properties and enhance nitric oxide release from nerve endings and endothelium; it relaxes smooth muscles and enhances inhibition of the angiotensin-converting enzyme. Thus, it reduces hypertension.[65,66] However, the mechanism responsible for the antihypertensive activity is still not fully understood.

Other activities

Steroidal saponin also plays a role to control other activities such as antihyperuricemia,[47,69,70] antiviral,[47,61] antifungal,[15,16,47] antitumor,[18,31,47,61] lung protective,[47,61,74] nephroprotective,[47,63] cerebral protection,[47] antiatherosclerosis,[47,67] antiarthritic,[47] antiobesity and diabetes,[47,61,67] and antiosteoporosis.[47] In addition, they have been reported to improve sperm motility.[6]

Table 1: Summary of steroidal saponin types

Name	IUPAC name	Chemical structure
Spirostane	(1R,2S,4S,6R,7S,8R,9S,12S,13S)-5',7,9,13-tetramethylspiro [5-oxapentacyclo [10.8.0.02,9.04,8.013,18] icosane-6,2'-oxane]	
Furostane	(1R,2S,4S,6R,7S,8R,9S,12S,13S)-7,9,13-trimethyl-6-(3-methylbutyl)-5-oxapentacyclo [10.8.0.02,9.04,8.013,18] icosane	
Cholestane	(8R,9S,10S,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene	

IUPAC: International Union of Pure and Applied Chemistry Name

Different Cell Lines

- *Human cancer cell lines* - 3T3, cervical: HeLa, Caski; prostate cell: PC; liver: Hep-G2, SMMC-7721, Hep3B; gastric cell: SGC7901, SGC-7902, AGS cell, BGC-823, HGC-27; breast: BT549, MDA-MB-231, MCF7, MDA-MB-435; colon: SW480, HT-29, Caco-2, HCT 116; leukemia: HL-60, Jurkat, K562; stomach: SGC-7901, BGC-823, MGC-803; lung: A549, 95D, LU-1, NCI-H460; adenocarcinoma: MKN-7, SPCA-1 cell; glioblastoma: U87MG, U251; melanoma: A375, SK-MEL-2; ovary: SK-OV-3
- *Human normal cell lines* - Kidney embryonic: HEK293; fibroblasts
- *Animal normal cell lines* - Cardiomyoblasts: H9c2; embryonic fibroblast: 3T3.

Test for the Presence of Saponin

Foam test

About 12.5 mg standard Quil-A® saponin (≥95% purity, InvivoGen, USA) and the test samples each were taken in 250 ml measuring cylinders, separately in triplicate. Then, distilled water (87.5 ml) was added to all the measuring cylinders. After that, the measuring cylinders were shaken vigorously about 30 times by closing the mouth of a cylinder with a stopper. After shaking, the stopper was removed and the mouth of the cylinder was covered with aluminum foil. Three observations were recorded, immediately after shaking, after 30 min, and after overnight standing.[147]

Analytical Techniques

Plant extract consists of a mixture of the different bioactive compounds with distinct polarities, their partition, and characterization being a still big challenge. However, the initial steps to take advantage of the bioactive compound of plant resources are eradication, pharmaceutical screening, isolation and characterization of the active biological compound, toxicology screen, and clinical study. The primary two steps, extraction and identification are described as tedious processes of saponin from the plant material. The saponin extraction includes conventional and green technologies. The Soxhlet and reflux extraction, Maceration extraction, and subsequent extraction are the examples of conventional techniques whereas ultrasound-assisted extraction, accelerated solvent extraction, and microwave-assisted extraction are the green technologies.[7,15] Analytical methods such as high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), TLC, gas chromatography (GC), ultra-HPLC (UHPLC) associated with detectors such as tandem ultraviolet (UV) detector, evaporative light scattering detector, and diode array detector.[148] UHPLC, supercritical fluid chromatography (SFC), ultra-high-performance supercritical fluid chromatography (UHPSFC), and some different spectroscopy techniques such as nuclear magnetic resonance (NMR) and X-ray diffraction. A summary of the general techniques used for extraction and identification of steroidal saponins obtained from different plant extracts is presented in Table 3.

Thin liquid chromatography

TLC is a user-friendly, quick, and cheap technique that helps in the separation of various compounds from the mixture. It is used for the separation, identification, and characterization of steroidal saponin.[15,149] The identification of constitutes in the mixture was done by comparing Rf values of compound and known compound. In addition, some techniques involve TLC plate with one mobile and one stationary phase for confirming the identification and purity of the isolated compounds via

Table 2: List of plant species, isolated compounds, extraction methods, therapeutic uses with the mode of action, and parts used in pharmaceutical properties

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
A. obesum (Forssk.) Roem. and Schult *Apocynaceae* fruits	Cardiac glycosides, triterpenoids, and steroids (honghelin (4), obeside B (5), and obeside C (6) and doxorubicin (Control))	4, 5, 6 show high IC_{50} value against 3T3 cell line compared to drug cycloheximide, very low IC_{50} value against PC-3 and 4, 6 show the least effect against HeLa compared to doxorubicin	Methanolic extract		Cytotoxic activity	[75]
A. americana Linn. *Asparagaceae* leaf	Polyphenols, alkaloids, flavonoids, saponins, tannins, and polyphenols	Antibacterial activity comparable to gentamicin, with zones of inhibition ranging from 17 to 40 mm MIC - 2.5 mg/mL for *S. aureus*, *P. aeruginosa*, and *S. typhi* strains and 10 mg/mL for *E. coli* strains	Chloroform acetone, methanol fractions, petroleum, and ether		*A. Americana* have antibacterial activity against, *P. aeruginosa, S. typhi*, *S. aureus* gentamicin (control)	[76]
A. angustifolia Haw var. marginata *Agavaceae* leaves	3-[O-β-D-glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→3)-O]-[O-6-deoxy-α-l-mannopyranosyl-(1→4)-β-d-xylopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl) oxy]-(3β,5α,22α,25R)-26-(β-d-glucopyranosyloxy)-22-methoxy-furostane	*In vitro* and *in vivo*			Antiulcerogenic activity	[77]
A. attenuata Salm-Dyck *Agavaceae* leaves	(3β,5β,22α,25S)-26-(β-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl O-β-D-glucopyranosyl-(1-->2)-β-D-glucopyranosyl-(1-->2)-O-[β-D-glucopyranosyl-(1-->3)]-β-D-glucopyranosyl-(1–>4)-β-D-galactopyranoside	The hemolytic potential of the steroidal saponin was evaluated the anti-inflammatory activity was performed using the capillary permeability assay	80% ethanol, methanol		Anti-inflammatory	[78,180]
A. utahensis Engelm. *Agavaceae* whole plant	Spirostanol saponins and furostanol saponins	HL-60 human promyelocytic leukemia cell IC_{50} - 4.9 µg/mL			Cytotoxic activity against HL-60 human promyelocytic leukemia cells	[79]
A. ampeloprasum var. Porrum *Liliaceae* bulbs	(3β,5α,6β,25R)-6-[(β-d-glucopyranosyl) oxy]-spirostan-3-yl O-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside	Human blood cell *in vitro* for hemolytic effects Male Swiss mice (3 months old, 25-35 g) compound concentration (100 mg/kg), positive control - cimetidine (100 mg/kg)	MeOH extract		Anti-inflammatory, antiulcerogenic properties Steroidal saponin showed hemolytic effects in the *in vitro* assays (human blood cell) and demonstrated anti-inflammatory activity and gastroprotective property using *in vivo* models (male Swiss mice)	[80]
A. chinense Don *Amaryllidaceae*	(25R)-5α-spirostan-3β-yl-3-O-acetyl-O-β-D-glucopyranosyl-(1→2)-O- [β-d-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (A-24)	Human gastric cancer cell lines SGC-7902 and AGS cell lines		Downregulation of PI3K/Akt/mTOR pathway	Anticancer activity of A-24 in human gastric cancer cell lines in terms of cell proliferation, colony formation, cell cycle, induction of apoptosis/autophagy, and PI3K/Akt/mTOR pathway	[81]
A. nigrum L. *Amaryllidaceae* Roots, bulb, leaves	25(R,S)-5α-spirostan-2α,3β,6β-trio1-3-O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-d-glucopyranosyl-(1→4)-β-D-galactopyranoside (5a/5b) and 25(R,S)-5α-spirostan-2α,3β,6β-trio1 3-O-β-D-glucopyranosyl-(1→2)-O-[4-O-(3S)-3-hydroxy-3-methylglutaryl-β-D-xylopyranosyl-(1→3)]-O-	Human colon carcinoma (HT-29 and HCT 116) cell lines. Compounds 5a/5b and 6a/6b were found to be the most active with IC_{50} values 1.09 and 2.82 µM against HT-29 and 1.59 and 3.45 µM against HCT 116, respectively			Cytotoxic and antifungal activity	[19,33,82-84]

Contd...

Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
	β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (6a/6b) NigrosidesA1/A2, NigrosidesB1/B2, Aginoside, Aginoside/turoside A. 25(R,S)-5α-spirostan-2α,3β,6β-trio1-3-O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-d-glucopyranosyl-(1→4)-β-D-galactopyranoside	The *in vitro* and *in vivo* antifungal activity of aginoside was assessed and significant inhibition against phytopathogens was observed				
A. asphodeloides Bge., *Asparagaceae* rhizomes	Timosaponin AIII, Anemarsaponin F, Aneglycoside A Aneglycoside, Timosaponin, Anemarsaponin R, Timosaponin E1, Anemarsaponin B, Schidigerasaponin F2, Timosaponin D, Anemarsaponin B II Timosaponin V and W (1 and 2)	Cytotoxic activity Aneglycoside A (IC_{50}) - HepG2 - 38.4±2.4, HeLa-29.7±01.9, SGC7901 >100 Aneglycoside B (IC_{50}) - HepG2 - 41.8±3.5, HeLa - 34.2±3.6, SGC7901 >100 Timosaponin U (IC_{50}) - HepG2 - 61.8±4.1, HeLa - 39.7±3.7, SGC7901 44.5±2.0 Doxorubicin (control) - HepG2 - 8.4±2.2, HeLa - 9.0±1.4, SGC7901 - 6.7±1.8 Schidigerasaponin F2(IC_{50}) - MCF7 - 98±8.98, SW480 - 97.02±14.99, HepG2 >100, SGC7901 >100 Anemarsaponin F (IC_{50}) - MCF7 - 2.76±0.59, SW480 - 5.56±1.50, HepG2 - 11.73±1.24, SGC7901 - 8.18±0.26 Timosaponin AI (IC_{50}) - MCF7 - 6.83±1.99, SW480 - 4.17±0.72, HepG2 - 7.83±1.72, SGC7901 - 4.38±0.50 Timosaponin AIII (control) - MCF7 - 3.34±1.10, SW480 - 2.94±1.05, HepG2 - 4.96±0.93, SGC7901 - 12.15±1.36 Anemarsaponin R (IC_{50}) - HepG2 - 43.90±3.36 Timosaponin E1 (IC_{50}) - SGC7901 - 57.90±2.88 Doxorubicin (control) - HepG2 - 8.20±1.25, SGC7901 - 6.25±2.18 (IC_{50}) Timosaponin V (IC_{50}) - MCF7 - 2.16±0.19 μM, HepG2 - 2.01±0.19 μM, respectively		Apoptosis stimulation mainly intrinsic pathway; stimulation of autophagy, phagocytosis or oncogenesis, the inhibition of metastatic properties of the tested cells or angiogenesis, timosaponin AIII induced apoptosis through activating JNK or ERK signaling pathway and generating NO. However, JNK or ERK inhibited autophagy, while NO did not affect autophagy	Anemarsaponin R and timosaponin E1 displayed medium antiproliferative activities on HepG2 and SGC7901 cells human cancer lines with IC_{50} values of 43.90 and 57.90 μM, respectively Cytotoxic, anti-inflammatory, antiplatelet, antithrombotic, antidiabetic, antidepressant, improving learning, and memory-deficit activities Timosaponin AIII inhibits tumor cell proliferation by suppressing invasion and migration Timosaponin AIII induces apoptosis and triggers autophagy in cancer cells Timosaponin AIII reverses multidrug resistance in tumor cells through PI3K/Akt signaling pathway	[54,85-92,180]
A. racemosus Wild. *Liliaceae* Roots, leaves, flowers, and fruits	Flavonoids: quercetin, rutin and alkaloid, diosgenin, shatavarins I-IV, and various sterols, hyperoside, an isoflavone, flavonoids: quercetin, rutin and alkaloid asparagamine A, sarsasapogenin, adscendin (A, B, C), asparanin (A, B, C), phytoestrogens, polysaccharides, glycosides, alkaloids, triterpenes, mucilage, glycoproteins, peptides, and amino acids and a mucilage	Immunomodulatory activity - *In vitro* NK cell activity was evaluated using human PBMCs isolated from whole blood on a Ficoll–Hypaque density gradient. K562 a myeloid leukemia cell lines were used as target cells. ARC, tested over the range 0.2–50 μg/ml, showed a dose-related stimulation of NK cell activity with a peak increase of 16.9%±4.4% at 5.6 μg/ml.	Methanol, ethanol alcoholic	Inhibiting the effect of inflammatory cytokines mainly interleukin and tumor necrosis factor reduces the enhanced levels of alanine transkinase, aspartate transaminase, and alkaline phosphate in CCl4-induced hepatic damage in rats	Ulcerogenesis, antioxidant, treatment of thirst, fainting, dyspnea, and gout, emollient, cooling, nervine tonic, galactagogue, aphrodisiac, diuretic, rejuvenating, carminative, immunostimulant, antiseptic, gastroprotective, etc. and used in gynecological and nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy, ovulation-inducing activity	[63,68, 93, 176,177]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
	Shatavarin IX (1) and asparacoside (2), asparanin A (3) and shatavarin V (4)	Anti-HIV activity was measured in a human CD4+ T-cell line, CEM-GFP cells infected with HIV-1NL4.3. Hypolipidemic activity by increase the level of catalase, SOD, and ascorbic acid in hypercholesterolemic rats Cytotoxic activity - compounds 1, 2, 3, and 4 were cytotoxic toward human hepato- and prostate-carcinoma cell lines (IC_{50} 14-37 µM), while primary human fibroblasts were less vulnerable (IC_{50} 22-66 µM), i.e., every saponin glycoside showed selectivity toward carcinoma cells compared with normal fibroblasts				
A. elatior Blume, Tijdschr. Natuurl. Gesch. Physiol *Asparagaceae* Rhizome	(25R)-26-O-β-D-Glc-furost-5,20-dien3β,26-diol-3-O-β-D-Glc (1→2)- [β-DGlc-(1→3)]-β-D-Glc-(1→4)-β-D-Gal, Aspidsaponin A, Aspidsaponin A, Adriamycin (control)	(25R)-26-O-β-D-Glc-furost-5,20-dien3β,26-diol-3-O-β-D-Glc (1→2)- [β-DGlc-(1→3)]-β-D-Glc-(1→4)-β-D-Gal (IC_{50}) - A549 3.8, Caski - 7.2, HepG2 - 8.2, MCF7 - 10.7 Aspidsaponin A (IC_{50}) A549 - 5.1, Caski - 8.6, HepG2 - 11.1, MCF7 - 13.8 Adriamycin (control) A549 - 1.4, Caski - 1.5, HepG2 - 0.7, MCF7 - 1.7		Inhibitory activities against LPS-induced nitric oxide production	Cytotoxic, anti-inflammatory	[94,95]
A. letreae Aver., Tillich and T.A. Le *Asparagaceae* Whole plant	Aspiletreins A-C (1-3), together with 2H-chromen-2-one (4), and α-tocopherol (5)	Compounds 1-3 displayed moderate cytotoxicities against the LU-1, HeLa, MDA-MB-231, HepG2, and MKN-7 human cancer cell lines, with IC_{50} values ranging from 7.69±0.40 to 20.46±3.11 µM			Exhibited moderate cytotoxic activities against the LU-1, MDA-MB-231, HeLa, MKN-7, and HepG2 human cancer cell lines, with IC50 values ranging from 7.69±0.40 to 20.46±3.11 µM	[96]
B. striata (Thunb.) *Rchb.* f. *Orchidaceae* Tuber	Anthocyanins, steroids and their saponins, triterpenoids and their saponins, phenanthrene derivatives, malic acid derivatives, and bibenzyls	Spirostane steroidal saponins showed significant cytotoxicity against lung cancer cells (A-549), human gastric carcinoma cells (BGC-823), human hepatocellular carcinoma cells (HepG2), human myeloid leukemia (HL-60), MCF7, hepatocellular carcinoma cells (SMMC-7721), and colon cancer cells (W480) with IC_{50} values <30 µM	Organic or water extract		Antibacterial, wound-healing, antioxidant, anti-inflammatory, anticancer, antiaging, and cytotoxicity activities	[97]
C. asiatica (*Linnaeus*) Urban *Apiaceae* leaves	Dioscin, sapogenin, diosgenin				Antiprotozoal, the steroidal saponin dioscin was more effective at inhibiting protozoa activity than its sapogenin diosgenin	[98]
C. parqui L'Hér *Solanaceae* leaves	Parquispiroside	LC_{50} values were 100 and 111 ppm after 24 and 48 h, respectively, treatment in *C. pipiens* larvae Parquispiroside showed moderate cytotoxic activities against HeLa, HepG2, U87, and MCF7 cell lines with IC_{50} values of 3.3-14.1 µM		The cytotoxic activity comes from the interaction of saponins with membrane cholesterol which causes membrane structure perturbation and cell death	Cytotoxic, insecticidal effect	[21,99]
Chlorophytum borivilianum Santapau and R.R. Fern *Asparagaceae* Roots and leaves	Borivilianosides F, G, and H ISCB	The cytoxicity of borivilianosides F, G, and H was evaluated using two human colon cancer cell lines (HT-29 and HCT 116) The ISCB at a dose of 30 mg/kg significantly inhibited HDAC level in rat paw tissue	Methanol and water	ISCB may act by inhibiting histamine, prostaglandin, and HDAC	The cytotoxicity of borivilianosides F, G, and H was evaluated using two human colon cancer cell lines (HT-29 and HCT 116) Anti-inflammatory and antiarthritic properties	[100,101,103]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
C. deistelianum Engl. and K. Krause *Asparagaceae* Aerial part	Chlorodeistelianosides A-D as (24S,25S)-24-[(β-D-glucopyranosyl)oxy]-3β-[(β-d-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl) oxy]-5α-spirostan-12-one, (25R)-26-[(β-D-glucopyranosyl) oxy]-2α-hydroxy-22α-methoxy-5α-furostan-3β-yl β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, (25R)-26-[(β-D-glucopyranosyl) oxy]-3β-[(β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl) oxy]-5α-furost-20(22)-en-12-one and (25R)-3β-[(β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl) oxy]-5α-spirostan-12-one, and	Spirostane-type glycosides exhibited cytotoxicity on one human cancer cell line (SW480) and one rat cardiomyoblast cell line (H9c2) both cell lines with IC_{50} ranging from 8 to 10 μM			Cytotoxicity of most compounds was evaluated against one human cancer cell line (SW480) and one rat cardiomyoblast cell line (H9c2), three known spirostane-type glycosides exhibited cytotoxicity on both cell lines with IC_{50} ranging from 8 to 10 μM	[102]
Genus *Cynanchum* Linn. *Asclepiadaceae* Crude extract	C21 steroids, steroidal saponins, alkaloids, flavonoids, and terpene Caudatin3-*O*-*β*-d-glucopyranosyl-(1→4)-*α*-d-oleandropyranosyl-(1→4)-β-d-diginopyranosyl-(1→4)-*α*-d-oleandropyranoside200 12*β*-*O*-benzoyl-8*β*,14*β*,17*β*-trihydroxypregn-2,5-diene-20-one 8 12*β*-*O*-benzoyl-8*β*,14*β*,17*β*-trihydroxypregn-2,5-diene-20-one Caudatin175 caudatin-2,6-dideoxy-3-*O*-methy-*β*-d-cymaropyranoside 176	The anti-cancer activities: 12*β*-*O*-benzoyl-8*β*,14*β*,17*β*-trihydroxypregn-2,5-diene-20-one 8 (IC_{50}) HL-60 = 6.72 μM, MCF7 cell lines = 2.89 μM) Caudatin3-*O*-*β*-d-glucopyranosyl-(1→4)-*α*-d-oleandropyranosyl-(1→4)-*β*-d-diginopyranosyl-(1→4)-*α*-d-oleandropyranoside and 12*β*-*O*-benzoyl-8*β*,14*β*,17*β*-trihydroxypregn-2,5-diene-20-one show strong inhibitory activities against K-562 (IC_{50} = 6.72 μM) and MCF7 cell lines (IC_{50} = 2.49 μM), respectively Caudatin and caudatin-2,6-dideoxy-3-*O*-methy-*β*-d-cymaropyranoside are tested on SMMC-7721, MCF7, and HeLa cell lines. SMMC-7721 cells (IC_{50}) - 13.49 and 24.95 μM, respectively Then, the in vivo assay by using solid tumor model H22 in mice was performed. It was found that compounds Caudatin and caudatin-2,6-dideoxy-3-*O*-methy-*β*-d-cymaropyranoside can significantly inhibit the growth of transplantable H22 tumors in mice at doses of 10, 20, and 40 mg/kg Positive control 5-FU	Ethanol extract		Antitumor, neuroprotective, and antifungal effects	[104]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
Cynanchum komarovii Al. Iljinski *Asclepiadaceae* Whole herb	Komarosides R (1) and S (2)	Potent inhibitory activities Komarosides R (IC_{50}) - human leukemia cell line (HL-60) – 6.2 μM Komarosides S (IC_{50}) - human leukemia cell line (HL-60) - 17.6 μM Positive control 5-fluorouracil (IC_{50}) - 6.4 μM	95% ethanol		Anticancer compounds 1 and 2 showed potent inhibitory activities against human leukemia cell line (HL-60) with IC_{50} values being 6.2 and 17.6 μM, respectively, compared to the positive control 5-fluorouracil (6.4 μM)	[105]
Datura metel L. (*Solanaceae*) Whole plant, flower	3-O-β-D-Xyl-(1→2)-α-L-Rha-(1→4) [α-L-Rha-(1→2)]-β-D-Glc (25R, 26R)-spirost-5-en-3β-ol-26-acetamide, Dioscoroside D, Meteloside D (4), Meteloside E Metelosides A, Metelosides B, Metelosides C (3), Metelosides E, Baimantuoluoside J (14), Daturanolide A-C, Withanolide glycoside, Baimantuoluolines L–X (1–13)	Withanolides 6 marked cytotoxicity against five human cancer cell lines (HCT116, U87-MG, NCI-H460, BGC823, and HepG2) Compounds metelosides B, D, E and 2, 4, 5, and 6 were shown to be cytotoxic against three cancer cell lines, including HepG2, MCF7, and SK-Mel-2 cells Compounds 3, 4, and 7 exhibited modest anti-inflammatory effects through inhibition of no production in LPS-stimulated BV cells Baimantuoluolines L-X (1–13)and baimantuoluoside J (14) were evaluated for their immunosuppressive activities against mice splenocyte proliferation and antiproliferative activities against human gastric adenocarcinoma cells (SGC-7901), human hepatoma (HepG2), and human breast cancer (MCF7) *in vitro*. It was found that compounds 1-14 showed obvious immunosuppressive effects and some of them have moderated antiproliferative activities	Acid methanol	Furthermore, compounds 3, and 4 exhibited modest anti-inflammatory effects through inhibition of NO production in LPS-stimulated BV cells	Cytotoxic, coughs, bronchial asthma, and rheumatism. Daturanolide A, metaloside B, D, and E were shown to be cytotoxic against three cancer cell lines, including HepG2, MCF7, and SK-Mel-2 cells Cytotoxicity against five human cancer cell lines (HCT116, U87-MG, LPS -H460, BGC823, and HepG2) Immunosuppressive activities against mice splenocyte proliferation and antiproliferative activities against human gastric adenocarcinoma cells (SGC-7901), human hepatoma (HepG2), and human breast cancer (MCF7) *in vitro*	[73,106,107]
Dioscera species Linn. *Dioscoreaceae*	Diosgenin, dioscin	Cytotoxicity of dioscin against HaCaT cells was low, with an IC_{50} of about 100 μM The toxicity of dioscin to many cancer cells was relatively high, with IC_{50} ranging from 2 to 20 μM		Inhibit cancer cell viability via different pathways: G2/M cell arrest, induction of apoptosis and autophagy, downregulation of antiapoptotic proteins, induction of DNA damage mediated by ROS, diosgenin and its analogs in modulating important molecular targets and signaling pathways such as PI3K/AKT/mTOR, JAK/STAT, NF-κB, and MAPK, which play a crucial role in the development of most of the diseases, TNF-α, IL-1β, and IL-6 secretion in HUVECs, macrophages, NRK-52E and HK-2 cells, can be inhibited by dioscin,	Cardiovascular diseases, cancer, nervous system disorders, metabolic syndrome, inflammatory, antihyperuricemia, antiviral, antifungal, antitumor, lung-protective, hepatoprotective, nephroprotective, cardioprotective, cerebral protection, antiatherosclerosis, anti-inflammatory, antarthritic, antiobesity, antidiabetes, antioxidative stress, and antiosteophorosis	[47,108-111]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
Roots bulbs, rhizomes, tubers, flower		Autophagy also participates in dioscin-induced apoptosis, which could be detected 12 h after low-dose dioscin exposure and earlier than apoptosis in human lung cancer A549 and H1299 cells and hepatoma Huh7 cells		The mRNA levels of TNF-α, IL-1β and IL6 can also be suppressed by dioscin in mice and a rat model of multiple diseases, such as acute liver injury liver fibrosis, obesity, cerebral and intestinal I/R injury and inflammatory injuries of kidney and liver		
D. viridiflora Engl. and K. Krause *Asparagaceae* leaves	Trillin, methyl protodioscin doxorubicin (control), prosapogenin B of dioscin, prosapogenin A of dioscin, dioscin	Cytotoxicity activity Dioscin (IC_{50}) - A549 - 0.42 µg/mL., Jurkat - 1.70 µg/mL, Skov-3=1.90 µg/mL Trillin (IC_{50}) - Jurkat - 22.36±1.40, Caco-2 - 36.49±2.14, SK-OV-3 - 64.78±1.91, A549 - 14.14±0.10 Prosapogenin A of dioscin (IC_{50}) - Jurkat-2.06±0.12 Caco-2 - 2.51±0.32 SK-OV-3 - 5.69±0.88 A549 - 2.11±0.54 Prosapogenin B of dioscin (IC_{50}) - Jurkat - 21.74±1.80, Caco-2-13.72±0.84, SK-OV-3 -62.33±1.42, A549 - 42.44±1.60 Dioscin (IC_{50}) - Jurkat 1.70±0.38 Caco - 2.58±0.21, SK-OV-3 - 1.90±0.86, A549 - 0.42±0.15 Methyl protodioscin (IC_{50}) - Jurkat-4.82±0.33, Caco-2 - 16.13±0.34, SK-OV-3 - 7.07±0.39, A549 - 5.26±0.29 Doxorubicin (control) - Jurkat-0.61±0.04, Caco-2 - 2.32±1.04, SK-OV-3 - 0.84±0.08, A549 - 1.15±0.84	MeOH extract		Dioscin showed the most potent cytotoxicity against A549, Jurkat, and Skov-3 cell with IC_{50} values of 0.42,1.70, and 1.0 µg/ml, respectively	[18,112]
F. tuberosa *Agavaceae* Mature fruit	(25*R*)–6α-(β-D-glucopyranosyloxy)-5α-spirostane-3β-*O*-[(6-*O*-hexadecanoyl)-β-D-glucopyranoside]	Cytotoxic effects against various human cancer cell lines	95% MeOH extract		Steroidal saponins isolated have shown potent cytotoxic effects against various human cancer cell lines	[113]
L. candidum L, *Liliaceae* Flower	Steroids (beta-sitosterol), polysaccharides, flavonoid, pyrrole alkaloids (lilalin, jatrophan), steroidal alkaloids, and spinostane and furostane steroidal, tannins, amino acids, and organic acids	Cytotoxic effects on human breast carcinoma cell line MCF7 cells Alkaloids of showed cytotoxic activity against MCF7 with IC_{50} of 244.8 µg/ml	Methanol, ethanol, butanol	The cytotoxic effect comes from P53-mediated stimulation of apoptosis and in inducing significant oxidative stress and DNA damage, which lead to cell apoptosis or necrosis	Antitumor, anti-inflammatory, cytotoxic, hepatoprotective	[114]
Lilium sp. Tournier ex *Linnaeus* *Liliaceae* Bulb, roots	39 isospirostanol saponins, spirostanol saponins, 23 furostanol saponins, and 7 pseudospirostanol saponins	White lily (LSM) showed a radical inhibition rate of 74.7% in DPPH assay The antitumor activities of the genus *Lilium* have been confirmed in HepG2, K562, SGC-7901, A549, HGC-27, and SPCA-1 cells and in mouse models of S180, H22, and B16 SGL-rich fraction could increase glucose consumption in HepG2 cells and 3T3-L1 adipocytes and enhance 3T3-L1 preadipocyte differentiation		Inhibitory effect of inflammatory factors production to show the effect of anti-inflammation; inhibition of melanin synthesis in the skin epidermal tissues and also for skin lightening	Antitumor, hypoglycemic, antibacterial, anti-inflammatory, hypolipidemic, reducing blood lipid, antidepressant, antifatigue, and hypoxia tolerance gynecological disorders, associated with menstruation as well as against insomnia, anxiety, sinusitis, dry cough, asthma, and cardiac arrhythmias. Skin ulcers, rashes, burns, wounds, eye irritation, and inflammation. , Antioxidant, hepatoprotective, hypoglycemic, sedative-hypnotic effect, and inhibition of cAMP phosphodiesterase and Na^+-K^+ ATP	[72,73,115,116]

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Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
L. muscari (Decne.) L. H. Bailey *Liliaceae* *Roots*	i (25*R*)-ruscogenin-1-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-fucopyranoside, ii (25*S*)-ruscogenin-1-*O*-β-D-glucopyranosyl-(1→2)- [β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside	Cytotoxic activity against MDA-MB-435, 95D, HepG2, HeLa, MCF7, and A549 cell lines in an *in vitro* bioassay (25S)-Ruscogenin 1-O-β-D-Glc-(1→2)- [β-D-Xyl-(1→3)]-β-D-Glc - (IC_{50} μM) - MDA-MB - 435 - 15.99±1.03, 95D - 20.13±1.18, HepG2 - 49.68±1.57, HeLa-39.98±1.20, MCF7 - 47.30±1.56, A549 - 36.35±1.39 (25R)-Ruscogenin 1-O--β -D-Glc-(1→2)- [β -D-Xyl-(1→3)] - β -D-Glc - (IC_{50} μM) MDA-MB - 435-26.01±0.85, 95D - 30.00±0.51 HepG2 - 40.52±0.96, HeLa - 33.42±1.39, MCF7 - 39.12±1.02 A549 - 36.01±1.31 (25S)-Ruscogenin 1-O- β -D-Glc-(1→2)- [β -D-Xyl-(1→3)]- β -D-Xyl (IC_{50} μM) - MDA-MB-435 - 18.07±1.34, 95D - 25.67±0.41 HepG2 - 37.17±1.71, HeLa - 21.58±1.42, MCF7 - 45.82±1.44 A549 - 43.53±1.16 (25R)-Ruscogenin 1-O- β -D-Glc-(1→2)- [β -D-Xyl-(1→3)]- β -D-Xyl (IC50 μM) - MDA-MB - 435 - 17.68±2.50, 95D - 17.83±0.37 HepG2 - 29.48±1.64, HeLa - 22.23±1.43, MCF7 - 42.16±1.26 43. A549 - 43.20±1.53 (25R)-Ruscogenin 1-O- α-L-Rha-(1→2)- [β -D-Xyl-(1→3)]- β -D-Glc (IC_{50} μM) MDA-MB-435 - 19.63±0.76, 95D-10.82±0.18, HepG2 - 15.26±1.29, A549 - 35.56±1.46 (25S)-Ruscogenin 1-O- β -D-Glc-(1→2)- [α-L-Ara-(1→3)]- β -D-Fuc (IC_{50} μM) MDA-MB-435 - 16.34±0.60, 95D - 14.34±0.33 HepG2-27.10±0.84, HeLa - 14.76±0.52, MCF7--35.21±2.02, A549 - 24.69±0.76 (25R)-Ruscogenin 1-O- β -D-Glc-(1→2)- [α-L-Ara-(1→3)]- β -D-Fuc (IC50 μM) 95D - 22.15±1.41, HeLa - 42.56±3.75 Neoruscogenin-1-O- β -D-Glc-(1→2)-[β -D-Xyl-(1→3)]- β -D-Xyl (IC50 μM) MDA-MB-435 - 24.52±0.91, 95D - 36.12±1.08, HeLa - 24.30±1.55 Neoruscogenin 1-O-α-L-Rha-l-(1→2)- [β -D-Xyl-(1→3)]- β -D-Glc (IC50 μM) MDA-MB-435 - 17.54±1.39, 95D - 11.09±0.15 Neoruscogenin 1-O- β -D-Glc-(1→2)-[β D-Xyl-(1→3)]- β -D-Fuc (IC50 μM) MDA-MB-435 - 9.74±0.62, 95D - 10.64±0.21 HepG2-15.48±0.52, HeLa - 11.02±0.42 MCF7 - 10.02±0.73, A549 - 21.25±1.42 (25R)-Ruscogenin 1-O- β -D-Glc-(1→2)- [β -D-Xyl-(1→3)]- β -D-Fuc (IC50 μM) MDA-MB – 435 - 4.71±0.75, 95D - 11.62±2.00 HepG2 - Not active, HeLa - 26.36±2.01 MCF7 - NA, A549-α23.56±2.64 (25S)-Ruscogenin 1-O- β -D-Glc-(1→2)-[β -D-Xyl-(1→3)]- β -D-Fuc (IC50 μM) MDA-MB-435 - 5.91±0.27, 95D - 11.20±0.17, HepG2- 12.76±0.74 HeLa-8.00±0.45 MCF7-8.226±0.78 (25S)-Ruscogenin 1-O-α-L-Rha-(1→2)- [β-D-Xyl-(1→3)]- β -D-Glc (IC50 μM) MDA-MB - 435 95D HepG2 HeLa MCF7 A549 9.75±0.34, 19.58±0.67, 15.24±1.53, 14.03±0.61, 16.30±0.73, 13.99±0.64	70% ethanol extract		*In vitro* cytotoxic activity against MDA-MB-435, 95D, HepG2, HeLa, MCF7, and A549 cell lines. Compounds I and ii exhibited the best cytotoxicity against the MDA-MB-435 cell line with IC_{50} values of 4.71 and 5.91 μM, respectively	[117]

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Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
		5-Fluorouracil (control) MDA-MB-435 - 116.8±13.93, 95D - 83.55±10.66, HepG2 - 91.9±16.20, HeLa - 251.3±19.93 MCF7 - 568.3±54.37 A549 - 244.8±21.23				
O. japonicus (L. f.) Ker Gawl *Liliaceae* Roots, tubers, fibrous roots	Ophiopogonin D', Diosgenin 3-O-[2-O-acetyl-]-L-Rha-(1→2)]-*β*-D-Xyl-(1→3)-*β*-D-Glc, Pennogenin-3-yl 2-O-acetyl-α-L-Rha-(1→2)- [*β*-D-Xyl-(1→4)]-*β*-D-Glc, (25R)-26-[(O-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl)]- 3β,22α,26-trihydroxyfurost- 5-ene-3-O-[α-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside 26-[(*O*-*β*-D-glucopyranosyl-(1→6)-D-glucopyranosyl)]-barogenin - 3-*O*-[α-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside and (25*R*)-Ruscogenin-3-yl α-L-Rha-(1→2)- Diosgenin-3-yl 2-*O*-acetyl-α-l-Rha-(1→2)-[*β*-D-Xyl-(1→4)]-*β*-D-Glc 1. Pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranoside. 2. Ophiopogenin-3-*O*-α-L-rhamnopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→2)]-β-D-glucopyranoside. 3. Pennogenin-3-*O*-α-L-rhamnopyranosy l-(1→4)-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranoseyl-(1→2)]-β-D-glucopyranoside 4. Praze rigenin A-3-*O*-α-L-rhamnopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→2)]-β-D-glucopyranoside 5. Pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside 6. Prazerigenin A-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside	The cytotoxic activities of 1~3 against A375 and MCF7 cells Pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranoside MDA-MB - 435 - 1.90±0.17, HepG2 - 1.69±0.18, A549 - 4.39±0.37 Pennogenin-3-*O*-α-L-rhamnopyranosy l-(1→4)-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranoseyl-(1→2)]-β-D-glucopyranoside MDA-MB - 435 - 9.13±1.43, HepG2 - 21.18±1.87, A549 - 21.27±2.53 (25R)-Ruscogenin-1-O-α-L-Rha-(1→2)-[β-D-Xyl-(1→3)]-α-L-Ara - MDA-MB - 435 - 10.32±2.37, HepG2 NA (IC_{50} [50 IM]), A549 - 29.12±4.665-FU (control) - MDA-MB - 435 - 120±15.53, HepG2 - 87.3±12.10, A549 - 256.8±19.03 Ophiopogonin T (furostanol saponin) showed a significant protective effect against cisplatin-induced cytotoxicity in LLC-PK1 cells without affecting HepG2 cancer cell proliferation or tube formation in HUVECs *In vitro* cytotoxic activities against MDA-MB - 435, HepG2 and A549 cell line compounds 5 and 7 showed moderate cytotoxicity with IC_{50} values ranging from 9.13–29.12 μM, whereas compounds 2 and 6 were nearly inactive (IC_{50} >50 μM)		Significantly decreased not only the proliferation of MDA-MB-435 melanoma cells but also decreased the cell invasion properties, probably through the inhibition of the MMP-9 matrix metalloproteinase expression and suppression of the p38/ MAPK pathway; increased secretion of proinflammatory interleukins; induced G2/M phase arrest in the cells by decreasing the expression of cdc2 and cyclin B1	The cytotoxic activities against A375 and MCF7 showed by fibrophiopogonin A, fibrophiopogonin B, and (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)]-3β,22α,26- trihydroxyfurost-5-ene-3-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside with IC50 values A375 201.1 A375 42.06 MCF7 45.32, A375 63.43, respectively, metastasis inhibition, and angiogenesis inhibition	[18,118-122]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
P. turgidum Forsskål *Poaceae* Aerial part	16-O-β-D-glucopyranosyl-cholest-5-en-3β,16β-diol-22-one-3-O-α-l-rhamnopyranosyl-(1→2)-O-[(β-D-glucopyranosyl (1→4)]-O-β-D-glucopyranoside(1), 16-O-β-D-glucopyranosylcholest-5-en-3β,16β-diol-22-one-3-O-α-l-rhamnopyranosyl-(1→2)-O-β-D-glucopyranoside (2), and 16-O-β-d-glucopyranosylcholestan-3β,16β-diol-6,22-dione-3-O-α-l-rhamnopyranosyl-(1→2)-O-β-D-glucopyranoside (3) were isolated from a methanolic extract of *P. turgidum*. In addition four known compounds, pennogenin 3β-O-α-l-rhamnopyranosyl-(1→2)-O-[α-l-rhamnopyranosyl-(1→4)-O-α-l-rhamnopyranosyl-(1→4)]-O-β-d-glucopyranoside (4), yamogenin 3β-O-α-l-rhamnopyranosyl-(1→2)-O-[α-l-rhamnopyranosyl-(1→4)]-O-β-D-glucopyranoside (5), yamogenin 3β-O-α-l-rhamnopyranosyl-(1→2)-O-[α-l-rhamnopyranosyl-(1→4)-O-α-l-rhamnopyranosyl-(1→4)]-O-β-D-glucopyranoside (6), and pennogenin 3β-O-α-l-rhamnopyranosyl-(1→2)-O-[α-l-rhamnopyranosyl-(1→4)]-O-β-D-glucopyranoside (7) Yamogenin-3β-O-α-L-rhamnopyranosyl-(1→2)-O-[α-Lrhamnopyranosyl-(1→4)]-β-D-glucopyranoside (1), yamogenin-3β-O-α-L-rhamnopyranosyl-(1→2)-O-[α-Lrhamnopyranosyl-(1→4)-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (2), and diosgenin-17α-hydroxy3β-O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (3)	Cytotoxicity activity measured towards a panel of mammalian cell lines and 4–7 were found to be cytotoxic Anti-inflammatory activity-mouse macrophages (RAW264.7) iNOS by compounds 1–3. Compound IC50* 1 16 2 2.1 3 8.6 parthenolidea 0.32 * IC_{50} values are expressed in μM, a positive control	Methanol		Cytotoxicity toward a panel of mammalian cell lines anti-inflammatory	[123,124]
P. delavayi Franchet. *Liliaceae* *Rhizome*	Furostanol saponins Padelaosides C–F (1–4)	The cytotoxicity of all the saponins was evaluated for their cytotoxicity against human glioblastoma U87MG and human hepatocellular carcinoma Hep-G2 cell lines. The known spirostanol saponins 7 and 8 exhibited notable cytotoxicity against the two tumor cell lines with IC_{50} values of 1.13 and 3.42 μM, respectively, while the new furostanol saponins named padelaosides C–F (1–4), 3 and 4 showed moderate cytotoxicity with IC_{50} values of 15.28–16.98 μM			Furostanol saponins, named padelaosides C–F (1–4), new furostanol saponins 3 and 4 showed moderate cytotoxicity with IC_{50} values of 15.28 to 16.98 μM	[125]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
P. polyphylla Smith *var. yunnanensis* (Franch.) Hand. Mazz *Trilliaceae* Bulbs, rhizomes (Rs), leaves, and stems (LSs)	Parisyunnanoside H, Parisyunnanoside G, Parisyunnanoside I, Dichotomin C, Nuatigenin 3-O-α-L-Rha-(1→2)-β-DGlc Pseudoproto-Pb Parisyunnanoside A, Paris saponin I, Abutiloside L, Parisyunnanoside I, Cisplatin (control) Troxacitabine (control), (23S,24S)-spirost-5,25(27)-diene1β,3β,21,23α,24α-pentol-1-O-{α-LRha-(1→2)-[β D-Xyl-(1→3)]-β-DGlc}-21-O-β-D-Gal-24-O-β-D-Gal 12 steroidal saponins, chonglouosides SL-9–SL-20, dioscin and polyphyllin D	Cytotoxic activity Nuatigenin 3-O-α-L-Rha-(1→2)-β-DGlc (IC_{50}) HepG2 - of 2.9±0.5 μM, HEK293 - 5.0±0.6 μM Abutiloside L (IC50) HepG2 - 7.0±0.8, HEK293 - 12.9±2.7 Troxacitabine (control) (IC_{50}) HepG2 - 0.17±0.02, HEK293 - 0.30±0.03 While none showed anti-HCV activity at a concentration of 20 μM Total saponins Rs (IC_{50}) HL-60 - 1.77, A-549 - 1.75, SM MC772 - 5.23, MCF7 - 6.62, SW480 - 3.49 Total saponins LSs (IC_{50}) HL-60 - 9.54, A-549 - 9.3, SM MC772 - 12.61, MCF7 - 8.12, SW480 - 11.25 Cisplatin (control) (IC_{50}) HL-60 - 0.87, A-549 - 6.48, SM MC772 - 3.77, MCF7 - 6.4, SW480 - 4.18 (23S, 24S)-spirost-5,25 (27)-diene-1β,3β,21,23α,24α-pentol-1-O-{α-L-Rha-(1→2)-[β-D-Xyl-(1→3)]-β-D-Glc}-21-O-β-D-Gal-24-O-β-D-Gal (IC50) CNE - 32.56 Parisyunnanoside I (IC_{50}) CNE - 33.1 Cisplatin (control) (IC_{50}) CNE - 9.35 Anthelmintic activity Dioscin exhibited activity against dactylogyrus intermedius EC (50) values - 0.44 mg/l Polyphyllin D exhibited activity against dactylogyrus intermedius EC (50) values - 0.70 mg/l Positive control (EC (50)) value=1.25 mg/l Acute toxicities LC (50) of polyphyllin D and dioscin for goldfish - 1.08 and 1.37 mg/l, respectively	Methanol	Stimulate apoptosis and autophagy	Cytotoxic activity, anti-HCV effect Nuatigenin 3-O-α-L-Rha-(1→2)-β-DGlc steroidal saponin was the most cytotoxic compound overall with IC_{50} values of 2.9±0.5 μM and 5.0±0.6 μM against HepG2 and HEK293 cell lines, respectively Anthelmintic activity	[71,126-129,180]
Paris vietnamensis (Takht.) H Lin.	Pavitnosides A–D (1–4), 25(*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-l-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (5), 25(*S*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-l-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (6), 25(*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-l-rha mnopyranosyl-(1→3)-*β*-D-glucopyrano side (7) , 25(*R*)-diosgenin-3-*O*-*α*-l-rhamnopyranosyl-(1→2)-*α*-l-rhamnopyranosyl-(1→3)-*β*-d-glucopyranoside (8) , 25(*R*)-spirost-5-en-3*β*,17*α*-	The new spirostanol saponin 1 displayed weak anti-proliferative activity against U87MG cell line			Pavitnoside A displayed weak antiproliferative activity against the U87MG cell line and the known saponins 8 and 9 exhibited significant cytotoxicity against the two tumor cell lines, with IC_{50} values of 2.16–3.14 μM, but did not affect the growth of primary cultures of human astrocytes, polyphyllin VII, dioscin, polyphyllin I, progenin III were assigned as candidate ingredients accounting for the antitumor activity of RP, polyphyllin VII, polyphyllin II, dioscin and polyphyllin I play a role in the hemostatic effects	[130,183]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
Liliaceae Rhizome	diol-3-*O*-*α*-l-rhamnopyranosyl-(1→4)-[*α*-l-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (9), and 25(R)-diosgenin-3-*O*-*α*-l-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (10) 25(*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-l-rhamnopyranosyl-(1→4)-[*α*-l-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (9), 25(*R*)-diosgenin-3-*O*-*α*-l-rhamnopyranosyl-(1→2)-*α*-l-rhamnopyranosyl-(1→3)-*β*-D-glucopyranoside (8), Polyphyllin VII (P27), dioscin (P31), polyphyllin I (P33), progenin III (P34) polyphyllin VII (P27), polyphyllin II (P30), dioscin (P31), and polyphyllin I (P33)	Cytotoxic activity: Against human glioblastoma U87MG and U251 cell lines but did not affect the growth of primary cultures of human astrocytes 25(*R*)-diosgenin-3-O-*α*-l-rhamnopyranosyl-(1→2)-*α*-l-rhamnopyranosyl-(1→3)-*β*-d-glucopyranoside (IC_{50}) U251 - 2.16±0.65, U87MG - 2.33±1.03 25(*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-l-rhamnopyranosyl-(1→4)-[*α*-l-rhamnopyranosyl-(1→2)]-*β*-d-glucopyranoside (IC_{50}) U251 3.14±1.26, U87MG 2.97±0.94 ACNU (control) (IC_{50}) U251 - 0.96±0.05, U87MG - 0.88±0.04				
Petiveria alliacea L. *Phytolaccaceae* Whole plant	Aponinic glycosides, isoarborinol-cinnamate, isoarborinol-acetate, isoarborinol-triterpene, steroids, alkaloids, benzyl-hydroxy-ethyl-trisulfide, flavonoids, potassium nitrate, tannins, benzaldehyde coumarins, benzoic acid, dibenzyl trisulfide, b-sitosterol, trithiolaniacine, isoarborinol, polyphenols, glycine and glucose				CNS disorders, such as anxiety, pain, memory deficits, and seizures, as well as for its anesthetic and sedative properties	[131]
Genus *Polygonatum* Mill. *Asparagaceae* Whole plant	Steroidal saponins, homoisoflavanones triterpenoid saponins, lectins and, polysaccharides	T47D - Human breast cancer cell line RAW264.7 - Murine macrophage cells Bel-7402 - Human hepatocellular carcinoma cell line BMSCs DU145 - Human prostate cancer cell line MG-63 - Human osteosarcoma cell line PC PC12 - Rat pheochromocytoma cells			Its anti-osteoporosis, neuroprotective, immunomodulatory, anti-diabetic, and anti-fatigue effects	[132]
Smilax davidiana A. DC. *Smilacaceae* Rhizomes	Furostanol saponins Davidianoside F (6)	Davidianoside F (6) showed activity against MCF7 and HELA cell lines at the concentration of 10.2 µM and 4.3 µM, respectively Compounds 3, 5 and 7 were found to have modest anti-inflammatory effects through suppression of IL-1β production and promote the expression of IL-10 in LPS-stimulated RAW 264.7 cells			Anti-inflammatory and cytotoxic activities	[133]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
Solanum spp. *Linnaeus* *Solanaceae*, Roots, leaf, aerial, fruit, stem	Eotigogenin, torvoside N, diosgenon, nuatigenosido, chlorogenone, (5α,25S)-spirostan-3,6-dione, diosgenone, neochlorogenin solanolactosides A–C orvosides J–L Indioside H (83), borassoside E (85), indioside I (86), and yamoscin (89 Torvosides N and M Torvoside Q, 25(S)-26-O-β-d-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-O-[α-l-rhamnopyranosyl-(1→3)-O-β-d-quinovopyranoside] (1), 25(S)-26-O-β-d-glucopyranosyl-5α-furost-22(20)-en-3-one-6α,26-diol-6-O-[α-l-rhamnopyranosyl-(1→3)-O-β-d-quinovopyranoside] (2), 25(S)-26-O-β-d-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-O-β-d-quinovopyranoside (3), 5α-pregn-16-en-20-one-3β,6α-diol-6-O-[α-l-rhamnopyranosyl-(1→3)-β-d-quinovopyranoside] (4)	Indioside H (83), borassoside E (85), indioside I (86), and yamoscin (89) demonstrated cytotoxic activity against six human cancer cell lines (HepG2, Hep3B, A549, Ca9-22, MDA-MB-231, and MCF7) (IC_{50}=1.83–8.04 μg/mL) seperately Borassoside E showed inflammation inhibitory effects on SAG (IC_{50}=0.62±0.03 μg/mL) Borassoside E also inhibited elastase release with IC_{50} values - 111.05±7.37 μg/mL Indioside I presented inflammation inhibitory effects on SAG (IC_{50} - 2.84±0.18 μg/mL) Yamoscin showed anti-neutrophilic inflammatory activity against SAG with an IC50 value - 3.59 μM Torvosides N and torvosides M revealed significant cytotoxicity against MGC-803, HepG2, A549, and MCF7 as compared to the positive control, CDDP Torvosides J–L (95-97), exhibited substantial anticonvulsant activity in zebrafish seizure assays Torvosides K96 also showed considerable antifungal activity against *A. flavus* and *F. verticillioides* with MIC ranging from 31.25-250 μg/mL Compounds torvpregnanosides A and B inhibited both inflammatory mediators SAG (IC_{50}=3.49 and 2.87 μM) and elastase release (IC_{50}=2.69 and 0.66 μM) 25 (S)-26-O-β-d-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-O-[α-l-rhamnopyranosyl-(1→3)-O-β-d-quinovopyranoside] (1), 25(S)-26-O-β-d-glucopyranosyl-5α-furost-22(20)-en-3-one-6α,26-diol-6-O-[α-l-rhamnopyranosyl-(1→3)-O-β-d-quinovopyranoside] (2), 25(S)-26-O-β-d-glucopyranosyl-5α-furost-22(20)-en-3β,6α,26-triol-6-O-β-d-quinovopyranoside (3), 5α-pregn-16-en-20-one-3β,6α-diol-6-O-[α-l-rhamnopyranosyl-(1→3)-β-d-quinovopyranoside] (4), convinced cyotoxic activity against the human melanoma cell line A375, with IC_{50} values of 30 μM–260 μM			Anticancer, anti-inflammatory, antifungal, anticonvulsant, antiviral, antimelanogenesis, cytotoxic, hepatoprotective, antihypertensive	[134]
S. nigrum L. *Solanaceae* berries	Solanigrosides Y1-Y9	Solanigrosides Y1–Y9 their potential inhibitory effects on nitric oxide and IL6 and IL-1 β production inducex by LPS in macrophages cell line RAW 264.7 were evaluated Compound 1 showed significant inhibition on no production with IC_{50} values - 9.7 μM, and some compounds showed significant inhibition effects on the LPS-induced IL-6 and IL-1 β production		Their potential inhibitory effects on NO and IL-6 and IL-1β production induced by LPS in macrophages cell line RAW 264.7 were evaluated	Solanigrosides Y1 exhibited significant inhibition on NO production with an IC_{50} value of 9.7 μM, anti-inflammatory activity	[135]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
T. terrestris Linn *Zygophyllaceae* Fruits, root, and the entire plant	Desgalactotigonin, Terrestrosin B, A, C, E, and D, gitonin, F-gitonin, desglucolanatigonin, protodioscin and their respective sulfates, terrestrinins A and B, and spiro stanol type tribulosin, saponin, and beta-sitosterol-d-glucoside	Diuretic potential of *T. terrestris* has been evaluated in albino rats	An ethereal or an alcoholic	Increases secretion of luteotropic hormone from pituitary gland due to containing saponins. The luteotropic hormone is also a special stimulant for the production of testosterone and hence can improve sexual function; increased release of NO from endothelium and nerve endings; it relaxes smooth muscles and increases ACE inhibition. The mode of action of antibacterial effects of saponins seems to involve membrane lytic properties, rather than simply altering the surface tension of the extracellular medium, thus being influenced by microbial	On sexual function, diuretic effect, analgesic activity, antihypertensive property, anticholesterolemic and anticholinergic effects, antioxidant property, antibacterial effect, hypoglycemic and hypolipidemic effects. On musculoskeletal system, antitumor activity	[56,57,64,66]
T. foenum-graecum L. *Fabaceae* seed	Diosgenin 26-O-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22β,26-triol-3-O-α-L-rhamno-pyranosyl-(1″→2′)-O-[β-D-glucopyranosyl-(1‴→6′)-O]-β-D-glucopyranoside 1, minutoside B 2, and pseudoprotodioscin 3	The MeOH extract inhibited the production of phorbol-12-myristate-13-acetate-induced inflammatory cytokines such as TNF-α in cultured THP-1 cells and also restrained the intracellular synthesis of melanin in murine melanoma B16F1 cells Compounds 1 and 2 strongly suppressed the production of inflammatory cytokines, whereas 3 showed a weaker suppressing effect Melanogenesis in B16F1 cells was significantly suppressed by 1 and 3 and weakly suppressed by 2 All three compounds showed moderate cytotoxicities	95% analytical grade methanol	Antidiabetic activity as it could slow gastric emptying, inhibiting carbohydrate digestive enzymes and stimulating insulin secretion, in blocking the α-glucosidase enzyme ability to break down starch, which, in turn, would decrease the blood glucose level	α-glucosidase inhibitory activity, antidiabetic These results indicate that fenugreek extract and its active constituents could protect against skin damage	[67,136-139]
Trillium species Linn. *Melanthiaceae* Rhizome, roots, aerial part	Spirostanol and furostanol saponin pennogenin 3-*O*-α-l-rhamnopyranosyl-(1→2)[α-l-rhamnopyranosyl-(1→4)]-β-d-glucopyranoside (compound 51), 7-β-hydroxy trillenogenin 1-*O*-β-d-apiofuranosyl-(1→3)-α-l-rhamnopyranosyl-(1→2)-[β-d-xylopyranosyl-(1→3)]-α-l-arabinopyranoside, Trillenoside A (compound 52)	Cytotoxic, anti-proliferative and morphological effects on lung cancer cell line Cytotoxicity against malignant sarcoma cells 3β,25*R*-spirost-5-en-3-yl *O*-6-deoxy-α-l-mannopyranosyl-(1→2)-*O*-[6-deoxy-α-l-mannopyranosyl-(1→4)]-β-d-glucopyranoside - antifungal activity against *C. albicans* MIC (μg/mL)=1.56 Cytotoxicity against HL-60 human promyelocytic leukemia cells (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-l-rhamnopyranosyl-(1→2)-β-d-glucopyranoside (IC_{50} [μg/mL])=6.10±0.04	Ethanol, ethyl acetate and butanol, ethanol		Antifungal potential, anabolic, antidiabetic, analgesic, anti-inflammatory, and anthelmintic activities, cancers, fungal infections, inflammatory and painful disorders, cytotoxic, antiproliferative, and morphological effects on lung cancer cell line	[25]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
T. kamtschaticum Pursh *Liliaceae* Whole plant	24-O-acetyl-epitrillengenin-1-O-β-d-apiofuranosyl-(1→3)-α-Lrhamnopyranosyl-(1→2)-[β-d-xylopyranosyl-(1→3)]-α-l-arabinopyranoside 21-O-acetyl-trillenogenin-1-O-β-d-apiofuranosyl-(1→3)-4″-acetyl-α-l-rhamnopyranosyl-(1→2)-α-l-arabinopyranoside 26-O-β-d-glucopyranosyl-17(20)-dehydrokryptogenin-3-O-α-l-rhamnopyranosyl-(1→4)-β-d-glucopyranoside Trillikamtosides A–R	Cytotoxicity against human colorectal cancer cells (HCT116) IC_{50} (μM)=5.84±1.05 Cytotoxicity against human colorectal cancer cells (HCT116) IC_{50} (μM)=4.92±1.00 Cytotoxicity against human colorectal cancer cells (HCT116) IC_{50} (μM)=17.28±2.69			Cytotoxicity against human colorectal cancer cells (HCT116) IC_{50} (μM)=4.92±1.00, cytotoxicity against human colorectal cancer cells (HCT116) IC_{50} (μM)=5.84±1.05, cytotoxicity against human colorectal cancer cells (HCT116) IC_{50} (μM)=17.28±2.69, cytotoxic activity, displayed significant induced-platelet aggregation activity	[140,141]
T. govanianum Wall *Melanthiaceae* Rhizome	Govanoside B (2), protodioscin (6), pennogenin tetraglycosides (11), borassoside E (21) and borassoside D (24) govanoside B (9) and eight known, pregnachacotrioside (1), pennogenin-triglycoside (2), borassoside E (3), pennogenin-tetraglycoside (4), protodioscin (5), clintonioside B (6), pennogenin-diglycoside (7), and borassoside D (8) pennogenin (compound 35), borassoside E (compound 32), diosgenin (compound 1) 1β,3β,23*S*,24*S*)-1-[*O*-β–d-glucopyranosyl (1→3)-*O*-β-d-glucopyranosyl (1→6)-*O*-β-d-apiofuranosyl]-3,23 dihydroxyspirosta-5,25-dienyl-24-[*O*-α-l-rhamnopyranosyl (1→4)-β-d-6-deoxygulopyranoside] (govanoside A) (compound 31) boeassoside E (compound 32) 7, 8, 9-trihydroxy-(10*Z*)-10-octadecenoic acid (compound 48)	ROS inhibitory activity Antifungal activity against A. niger, *A. flavus*, *C. albicans*, *C. glabrata*, *T. rubrum*	Ethanol and water		Joint pains, wounds, and sexual disorders	[116,142]
T. chinensis Baker	Tupistrosides J–N and furostanol saponins	Tupistrosides L and tupistrosides N showed cytotoxicity against human cancer cell lines SW620 with IC_{50} values of 72.5±2.4 and 77.3±2.5 μmol·L−1, respectively Tupistrosides M showed cytotoxicity against human cancer cell line HepG2 with an IC_{50} value of 88.6±2.1 μmol·L			Cytotoxic activity	[143,144]

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Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
Liliaceae Roots and rhizome	as spirost-25(27)-en-1*β*,2*β*,3*β*,4*β*,5*β*-pentol-2-*O*-*β*-D-xylopyranoside (1), spirost-25(27)- en-1*β*,2*β*,3*β*,4*β*,5*β*-pentol-2-*O*-*α*-L-arabinopyranoside (2), spirost-25(27)-en- 1*β*,3*α*,5*β*-triol (12), spirost-25(27)-en-1*β*,3*α*,4*β*,5*β*,6*β*-pentol (13), spirost-25(27)-en- 1*β*,2*β*,3*β*,5*β*-tetraol-5-*O*-*β*-D-glucopyranoside (16), 5*β*-spirost-25(27)-en-1*β*,3*β*-diol- 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (17), (25*R*)-5*β*-spirostan-1*β*,3*β*-diol-3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (18), (25*R*)-5*β*- spirostan-1*β*,3*β*-diol-3-*O*-*β*-D-fructofuranosyl-(2→6)-*β*-D-glucopyranoside (19), 5*β*-spirost-25(27)-en-3*β*-ol-3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (20).	The antiproliferative effects against seven human cancer cell lines and inhibitory activities on NO production induced by LPS in a macrophage cell line RAW 264.7 were assayed for all the isolated compounds. Compounds 17, 19, and 21 exhibited potential antiproliferative activities against all of human cancer cell lines tested. Compound 21 showed significant inhibition on NO production with IC_{50} values of 11.5 μM				
V. amygdalina Del. *Asteraceae* Leaves	Vernonioside B2, Vernoniamyoside A–D and Vernoamyoside D Vernoniamyoside A (1) Vernoniamyoside B (2) Vernoniamyoside C (3) Vernoniamyoside D (4) Vernoamyoside D (5) Vernonioside B2 (6)	The cytotoxicity of the compounds was also tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method on the cell lines Hela, MCF7, BT-549, and MDA-MB-231 Vernoniamyoside A, inhibition (%) - BT-549 - 63.61, MDA-MB - 231 - 28.97% MCF7 - 46.54, HeLa - 42.05 Vernoniamyoside B inhibition (%) - BT-549 - 62.17, MDA-MB - 231 - 27.78%, MCF7 - 37.07 HeLa - 31.64 Vernonioside B_2 inhibition (%) - BT-549 cell lines - 34.18. MDA-MB - 231 - 32.74% MCF7 - 39.38 HeLa - 26.73 Vernoniamyoside C inhibition (%) - BT-549 - 44.00, MDA-MB - 231 - 31.53% MCF7 - 31.36 Hela - 32.93 Vernoniamyoside D inhibition (%) - BT-549 - 36.41 and MDA-MB - 231 - 33.61% MCF7 - 49.72 Hela - 21.48 Vernoamyoside D inhibition (%) - BT-549 - 51.14 MDA-MB - 231 - 30.75% MCF7 - 39.08, Hela - 35.63 Positive control inhibition (%) - BT-549 - 83.79, MDA-MB - 83.39%, MCF7 - 95.32, Hela - 92.70	Ethanol		Vernoniamyoside A, Vernoniamyoside B, and Vernonioside B_2 showed cytotoxicity towards BT-549 cell lines. Vernoniamyoside C, Vernoniamyoside D, and Vernoamyoside D showed different levels of cytotoxic activities	[26]

Contd...

Table 2: Contd...

Plant name	Compound	Cell lines and concentration	Extraction solvent	Mode of action	Properties	References
Y. parviflora F. T. Wang and Tang *Liliaceae* Whole plant	Ypsiparosides A–G	The induced rabbit platelet aggregation activities of the isolates were tested Compounds 4, 15, and 17 showed maximal platelet aggregation rates ranging from 43% to 55% at a concentration of 300 μg/mL Further experiments exhibited that compounds 4, 15, and 17 possessed EC_{50} values of 642.9, 95.3, and 300.8 μg/mL, respectively			Induce platelet aggregation	[145]

A. obesum: Adenium obesum, A. americana: Agave americana, A. angustifolia: Agave anguistifolia, A. attenuata: Agave attenuata, A. utahensis: Agave utahensis, A. ampeloprasum: Allium ampeloprasum, A. chinense: Allium chinense, A. nigrum: Allium nigrum, A. asphodeloides: Anemarrhena asphodeloides, A. racemosus: Asparagus racemosus, A. elatior: Aspidistra elatior, A. letreae: Aspidistra letreae, B. striata: Bletilla striata, C. asiatica: Centella asiatica, C. parqui: Cestrum parqui, C. borivilianum: Chlorophytum borivilianum, C. deisteliianum: Chlorophytum deisteliianum, C. komarovii: Cynanchum komarovii, D. metel: Datura metel, F. tuberosa: Furcraea tuberosa, L. candidum: Lilium candidum, L. muscari: Liriope muscari, O. japonicus: Ophiopogon japonicus, P. turgidum: Panicum turgidum, P. delavayi: Paris delavayi, P. polyphylla: Paris polyphylla, P. vietnamensis: Paris vietnamensis, P. alliacea: Petiveria alliacea, S. nigrum, Solanum nigrum, T. terrestris: Tribulus terrestris, T. foenum: Trigonella foenum, T. govanianum: Trillium govanianum, T. chinensis: Tupistra chinensis, V. amygdalina: Vernonia amygdalina, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, S. typhi: Salmonella typhi, E. coli: Escherichia coli, C. albicans: Candida albicans, C. pipiens: Culex pipiens, C. borivilianum: Chlorophytum borivilianum, A. flavus: Aspergillus flavus, F. verticillioides: Fusarium verticillioides, T. terrestris: Tribulus terrestris, C. albicans: Candida albicans, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, C. albicans: Candida albicans, C. glabrata: Candida glabrata, T. rubrum: Trichophyton rubrum, Y. parviflora: Ypsilandra parviflora, T. kamtschaticum: Trillium kamtschaticum, D. viridiflora: Dracaena viridiflora, ISCB: Isolated saponins from *Chlorophytum borivilianum*, MIC: Minimum inhibitory concentration, LPS: Lipopolysaccharide, SGL: Steroidal glycoside, iNOS: Inhibition of inducible nitric oxide synthase, BMSCs: Bone marrow stromal cells, PC: Positive control, JNK: c-Jun N-terminal protein kinase, ERK: Extracellular signal-related kinase, TNF: Tumor necrosis factor, NO: Nitric oxide, IL-1β: Interleukin 1 beta, ACE: Angiotensin-converting enzyme, CNS: Central nervous systems, mRNA: Micro RNA, HT: colorectal adenocarcinoma cell line, HCT: human colon cancer cell line, SMMC: hepatocellular carcinoma, BGC: human gastric cancer cell line, NCI: large cell lung cancer, BV: cell line for inflammation, HaCaT: human keratinocyte cell line, SK-OV: Human Ovarian Cancer Cell Line , LSM: White lilly, DPPH: 2,2-diphenylpicrylhydrazyl, MDA: Malondialdehyde, MB: Human breast cancer cell line, ACNU: Nimustine hydrochloride, HELA: Henrietta Lacks, SAG: Superoxide anion generation, CDDP: cis-Diamminedichloroplatinum, THP-1: monocyte isolated from peripheral blood from an acute monocytic leukemia patient, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ISCB: Isolated saponins from Chlorophytum borivilianum, HDAC: Histone deacetylase, ROS: Reactive oxygen species, TNF-α: Tumour necrosis factor α, HUVECs: Human umbilical vein endothelial cells, NRK: Normal Rat Kidney, HK-2: human kidney 2, MMP-9: Matrix metallopeptidase 9 , MAPK: mitogen-activated protein kinases, HL-60: human leukemia cell line*

involving phytochemical screening spray, which leads to color changes according to existing phytochemicals or observes under the UV light.

High-performance liquid chromatography

The HPLC technique is predominately used for analysis of saponin. In HPLC, the separation of chemicals is achieved by utilizing the fact that each component has different interaction properties, which is responsible for different rates of migration within the particularly given column. Generally, using a single unchanging mobile phase system is accomplished with separation and identification of phytochemicals. As per requirements, the different proportions of the organic solvent to water are used for gradient elution purposes. Diode array detector, UV detector, and evaporative light scattering detector (ELSD) are used for the identification of compound.[149]

High-performance liquid chromatography with evaporative light scattering detector

ELSD is an aerosol-based detector used to facilitate HPLC. It is used for the identification of nonvolatile sample components in volatile elute. The key advantage: it is used for the analysis of sugar. This technique used for the identification of steroidal saponin of *Yucca schidigera* is reported in Tenon.[150]

High-performance liquid chromatography/mass spectroscopy

It is used for the separation and mass evaluation of a given compound. It could be used for accurate molecular formula determination. Using HPLC/mass spectroscopy (MS), 19 compounds are discovered from the crude elicit of *Y. schidigera*.[151]

High-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectroscopy/ mass spectroscopy

It is a suitable and reliable method used to separate and identify steroidal saponins from plant extracts.

Gas chromatography/mass spectrometry

It is an analytical technique used for separation and molecular weight detection. In GC, the mobile phase is gas, and components are separated in the vapor form. Thus, MS detects the molecular weight of small fragment compounds in the gas phase. To better characterize the purified YSS, GC/MS was used.[150]

Column chromatography

Column chromatography is a widely used technique of separation purpose based on their polarity or hydrophobicity.

Spectroscopy method

Nuclear magnetic resonance spectroscopy

The NMR spectroscopic methods are widely used to determine chemical and physical properties. It is used to deduce the complete structure of steroidal saponins containing the oligosaccharide moiety and its linkage to the sapogenin residue. The two-dimensional (2D) NMR is reported to elucidating the structure of isolated compounds

Table 3: Techniques used for steroidal saponins

Techniques	Specification	Methods	Compound	Plant name	References
TLC	It is applicable to only nonvolatile compounds, thus limiting its use Its resolution capacity ranges from 10 to 50 separations The requirement of preknown R_f values presents another challenging disadvantage Time-consuming Limited quantity of separation Evaporation of mobile phase Limited reproducibility	RP-18 and silica GF254 with 20% H_2SO_4; heating time - 3 min Silica gel GF_{254} for (TLC) Silica gel plate; mobile phase:10% acetic acid in chloroform; reagents for secondary metabolites -acetonitrile, 20% w/v Na_2CO_3 and diluted Folin–Ciocaltaeu reagent Silica gel plates 60 F_{254}; mobile solvent - $CHCl_3$: MeOH: H_2O (30:15:2.5, v/v/v); reagents - *P*-anisaldehyde reagent for total saponins and Ehrlich's reagent for furostanol saponins Silica gel plates; solvent systems: (A) for steroidal saponin - $CHCl_3$/MeOH/H_2O (65:35:10, v/v/v, lower phase) (B) for sapogenin-$CHCl_3$/MeOH (95:5, v/v); Spray reagents -orcinol/H_2SO_4 for steroidal saponin 1 and$CeSO_4$ and monosaccharides for sapogenin 1a silica gel 60 F_{254}, 0.25 mm thickness; mobile solvent-$CHCl_3$/MeOH/H_2O (8:4.5:1, v/v/v); spray reagent - anisaldehyde solution in EtOH	Pavitnosides A–D Komarosides S and R, Vernoniamyoside A–D 3β,5α,6β,25R)-6-[(β-d-glucopyranosyl) oxy]-spirostan-3-yl O-β-d-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-d-galactopyranoside Alliospiroside A Dichotomin, Protodioscin, Prosapogenin A, parrisaponin, protogracillin, and gracillin, (25R)-spirost-5-ene-3β,27-diol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (12), (25R)-spirost-5-en-3β-ol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (10) (25R)-spirost-5-ene-3β,27-diol-3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13), (25R)-spirost-5-en-3β-ol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside and (25R)-spirost-5-ene-3β,27-diol-3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13)	*Paris vietnamensis* (Takht.) *Vernonia amygdalina* Del *A. ampeloprasum* L. var. porrum (L.) J. Gay *Allium fistulosum* L.—*A. cepa* *D. esculenta* (Togedokoro) (Lour.) Burk	[26,33,80,130,155]
LCMS UPLC–MS	It is used for structural determination It is a very expensive analytical tool and high maintainance cost	LC-MS analysis conditions UPLC HSS T3 column (1.8 µm, 2.1 mm×100 mm, Waters Corp.); Guard column Temperature - at 30°C; Samples amount- (5 µL); Mobile phases - 0.0.1% aqueous formic acid (A) and 100% MeCN (B); flow rate - 0.2 mL/min The MS instrument - Synapt MS system (Waters Corp.) Ionization mode - positive ESI mode; acquired data - from 100 to 1500 Da MS source temperature -120°C; desolvation temperature - was 450°C; desolvation gas flow rate-900 L/h Capillary voltage-3 kV; Cone voltage - 30 V; Collision energy - 20 eV; Instrument controlled software used - Masslynx software (Waters Corp.)	Protodioscin, Dichotomin, and protogracillin, prosapogenin A, Parrisaponin, Gracillin, (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (12), (25*R*)-spirost-5-en-3β-ol-3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (10), and (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13) and (25*R*)-spirost-5-en-3β-ol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside	*D. esculenta* (Togedokoro) (Lour.) Burk	[155]
HPLC	Lack of a universal detector Less separation efficiency than capillary gas chromatography Retention factor,selectivity, and separation power effect it result Conventional HPLC has a practical peak capacit using columns with ~20,000 plates under	Column - ODS-A (250 mm×20 mm. D, S-5 µm, 12 nm); Dionex P680 liquid chromatograph equipped with a UV 170 UV/VIS detector Detection (nm) - 206 nm and 225 nm; Sample state- semi-preparation Column - YMC-Park ODS-A (5 µm, 250 mm×10 mm I.D), LC-6AD intelligent prep. Pump; Detector-SPD-20A intelligent UV/VIS detector	Pavitnosides A-D Vernoniamyoside A–D, komarosides R (1) and S (2), Diosgenin Spirostanol, Diosgenin, furostanol Diosgenin-3-O-α-l-rhamnopyranosyl (1→4) [α-l-rhamnopyranosyl (1→2)]-β-D-glucopyranoside Ophiopogonin D, (25*R*)-26-[(*O*- *β*-D-glucopyranosyl-(1→2) *β*-D-glucopyranosyl)]- 3*β*,22α,26-trihydroxyfurost- 5-ene-3-O-[α-L-rhamnopyranosyl-(1rost-*β*-D-glucopyranoside, Pennogenin-3-yl 2-O-acetyl-*α*-L-Rha-(1→2)-[*β*-D-Xyl-(1→4)]-*β*-D-Glc,	*Paris vietnamensis Takht* *Vernonia amygdalina Del* *Dioscorea alata L.* *Dioscorea zingiberensis C. H. Wright*	[26,69,130,155,157-161]

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Table 3: Contd...

Techniques	Specification	Methods	Compound	Plant name	References
Conventional HPLC has a practical peak capacity (pusing columns with ~20,000 plates under rradient conditions - not particularly effective for very complex samples)	gradient conditions — not particularly effective for very complex samples Conventional HPLC has practical Pc of ~200 using columns with ~ 20,000 plates under gradient conditions, not particulary effective for very complex samples The results showed that (1) the detected components can be well separated and all with good correlation coefficients. The standard calibration curves were linearly good (R^2>0.999 9). The linearity was obtained over 0.04170-3.81200 µg. The average recoveries ranged from 95.91% to 103.8%. (2) There are significant differences in the content of steroidal saponins from different species	Column - Zorbax SB-C18 (4.6 mm×150 mm, 3.5 µm); Detector - DAD; Mobile phase (gradient concentration) A – methanol (M) and B – water (W) with 0.02% H_3PO_4 was 25% A+75% B for 5 min, 30% A+70% for 10 min, 45% A+55% for 30 min, and 80% A+20% B for 45 min Column-welchrom C18 (250 mm×4.6 mm I.D., 5 µm); mobile phase: Water and acetonitrile under gradient elution Column-reversed-phase C-18; mobile phase: 0.1% aqueous formic acid and acetonitrile under gradient elution conditions Column - tigerkin C (18); Mobile phase - 0.02% formic acid in water (v/v) and 0.02% formic acid in acetonitrile (v/v); flow rate - 0.5 mL/min Column-ODS (5 µm, 4.6 mm×250 mm); Lichrospher C18 (5 µm, 10 mm×250 mm); detector - RID and ELSD 2000 (evaporative light-scattering detector) ODS column ($5C_{18}$-PAQ, 20 mm×250 mm,) and guard column ($5C_{18}$-PAQ, 10 mm×10 mm); Mobile phase - H_2O/MeCN (74:26, v/v, HPLC-1), Temperature - 30°C; Flow rate - 5.0 mL/min; detection (nm) - 203 nm Column - CAPCELL PAK ADME (4.6 mm×250 mm, 5 µm); mobile phase: Acetonitrile and water; Column temperature- 21°C; flow rate - 0.8 mL min^{-1}; UV detection wavelength-203 nm. The analysis was performed on a waters Acquity H-Class™ UPLC ultrafine liquid chromatography system coupled with a PDA detector	26-[(*O*-*β*-D-glucopyranosyl-(1→6)-D-glucopyranosyl)]-barogenin - 3-O-[*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, Diosgenin 3-*O*-[2-*O*-acetyl-]-L-Rha-(1→2)]-*β*-D-Xyl-(1→3)-*β*-D-Glc, (25*R*)-Ruscogenin-3-yl *α*-L-Rha-(1→2)- Diosgenin-3-yl 2-*O*-acetyl-*α*-l-Rha-(1→2)-[β-D-Xyl-(1→4)]-β-D-Glc, (25R)-26- [(*O*-*β*-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)]- 3β,22α,26- trihydroxyfurost- 5-ene-3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside Protodioscin, Dichotomin, Prosapogenin A, parrisaponin, protogracillin, and gracillin, (25*R*)-spirost-5-en-3β-ol-3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (10), (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (12), and (*25R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13). Further, compound 6 was estimated as (*25R*)-spirost-5-en-3β-ol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside polyphyllins VII, H, VI, II, I, and V, dioscin, and gracillin	*P. polyphylla* Smith *var. yunnanensis* (Franch.) Hand. –Mazz *and* P. polyphylla *var.* chinensis *Ophiopogon japonicus* (L. f.) Ker Gawl *D. esculenta* (*Togedokoro*) (Lour.) Burk. Paridis Rhizoma	
HPLC-ESI-QTOF-MS/MS	Powerful and reliable analytical techniques for identificationof compounds This is a creadible and feasible technique to identify and seperate steroidal saponins from botanical extracts Accurate mass capability of TOF - mass error below 5 ppm has higher mass resolution, accuratity, and sensitivity TOF analyzer with higher Mass resolution, sensitivity, and accuracy can provide accurate masses of ions and molecular formula, making it one of the most desirable detection methods TOF analyzer with higher Mass resolution, sensitivity, and accuracy can provide accurate masses of ions and molecular formula, making it one of the most desirable detection methods	Column - Poroshell 120 EC-C18 (2.7 mm×100 mm, i.d., 2.7 µm); C18guard column (4.00 mm×2.00 mm); mobile phase-0.1% formic acid aqueous and acetonitrile. Flow rate-0.35 mL/min; capillary voltage-3500 V, ESI-negative-ion mode; OCT 1 RF Vpp-750 V; fragmentor-150 V; skimmer-65 V; pressure of nebulizer-35 psi; drying gas temperature- 300°C; sheath gas temperature-350°C; Nitrogen sheath and drying gas flow rate -8.0 and 11.0 L/min;collision energy-45 V;mass range recorded m /z-100–2000; Mass Tof - 10 mL of MeOH: Water (70:30, v/v) by ultrasonication for 30 min, centrifused – 12,000 rpm for 10 min; injection volume for – 3 µl	Steroidal saponin *Yucca* steroidal saponins Progracillin, pseudoproto-Pb, disoseptemloside E, paris saponin II, polyphyllin V, chonglouoside SL-5, pariposide E, chonglouoside SL-4,paris saponin VIII, parisyunnanoside J, parisyunnanoside H, Th, protodioscin, polyphyllin H	*P. polyphylla* Smith	[148]

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Table 3: Contd...

Techniques	Specification	Methods	Compound	Plant name	References
	become a powerful and reliable analytical technique for compound identification				
HPLC/ELSD	This technique is an reliable, rapid, and accurate method which give results of reproducibility and appropriate repeatability It is cost- and time-effective techniques suit to routine analysis It donot over- or under-estimate levels of steroidal saponins For quantify the steroidal saponins groups, it do not require each and every pure standard of saponins This methods yield a fingerprints of saponin specific to the plant species. Hence, it can distinguish profile of saponins from taxonomically distant species, that is why it can unravel issues of adultatation in plant via evaluated for linear response of the calibration curve, precision (intraday and reproducibility), and accuracy	Column-Atlantis T3 column (3.0 mm×150 mm, 3 m); mobile phase - A -0.1% formic acid (v/v) in water and 0.1% formic acid in acetonitrile (v/v); gradient profile- linearly from 98% A to 40% A during 0-25 min, linearly from 40% A to 20% A during 25-35 min; flow rate -0.6 mL/min; Temperature - 25°C; gas flow - 1.2 (arbitrary unit) at 50°C and 40°C Column-reversed-phase C18 column; Mobile phase-aqueous acetonitrile system	Yucca steroidal saponins	*Yucca schidigera* Roezl	[150]
HPLC–ESI-MSn Tandem method HPLC-MS HPLC–MS/MS	A rapid, simple, and sensitive validated for simultaneous determination for pharmacokinectics evalulation The linearity, precision, accuracy, and recoveries of the analysis The intra- and inter-day precisions (RSD%) were less than 13% and the average extraction recoveries ranged from 85% to 97.0% for each analyte	Identification and quantification Column-reversed-phase C-18; binary mobile phase system (gradient elution) -0.1% aqueous formic acid and acetonitrile Ionization mode-ESI-MSn in negative ion mode used for diosgenin and pennogenin Reverse phase agilent poroshell 120 EC-C18 column; mobile phase system-acetonitrile–water containing 0.1% formic acid Triple quadruple mass spectrometer with MRM and ESI-negative mode Inersil ODS-3 C_{18} column (250 mm×4.6 mm, 5 µm); mobile phase-acetonitrile and water containing 0.1% formic acid; rate gradient elution - 0.2 mL min^{-1}. Mass spectrometer - triple quadrupole tandem mass spectrometer (MRM); ESI mode-positive mode	Diosgenin-3-O-α-l-rhamnopyranosyl (1→4) [α-l-rhamnopyranosyl (1→2)]-β-D-glucopyranoside; diosgenin-and pennogenin Polyphyllin I, polyphyllin II, polyphyllin VI, polyphyllin VII, dioscin, gracillin, and internal standard ginsenoside Rb3 were m/z 899.5 >853.4, 1059.5 >1013.5, 783.4 >737.4, 1075.5 >1029.5, 913.5 >867.4, 929.5 >883.4, and 819.5 >783.4, respectively Protodioscin, huangjiangsu A, *zingiberensis* new saponin, dioscin, and gracillin, IS-ginsenoside Rb_1	*P. polyphylla* Smith var. yunnanensis (Franch.) Hand.-Mazz and *P. polyphylla* var. chinensis *P. polyphylla* Rhizome of *D. zingiberensis* C.H. Wright	[61,129,158,159]

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Table 3: Contd...

Techniques	Specification	Methods	Compound	Plant name	References
HPLC–ESI-MS/MS	It is selective, reliable, and sensitive with high accuracy, adequate extracted recoveries, and almost negligible matrix effects	Column-reversed phase C-18; mobile phase - 0.1% aqueous formic acid and acetonitrile; elution flow rate -1 mL min^{-1} with linear gradient mode. The optimal gradient elution program was as follows: 0–5 min, 20%–30% A; 5–8 min, 30%–40% A; 8–15 min, 40–60 A%. The injection volume - 10 µL. The column temperature -25°C and autosampler temperature - 4°C Heated capillary temperature- 350°C and voltage-0.8 kV; ion spray voltage-4500V and temperature-300°C; for desolvation, nitrogen flow rate 8 L/min; dried gas, nitrogen flow rate - 11 L min^{-1}; collision gas - argon with pressure-1.5 mm Torr; Software - Varian MS workstation software Inersil ODS-3 C18 column (250 mm×4.6 mm, 5 µm); mobile phase - acetonitrile and water containing 0.1% formic acid; gradient elution mode with flow rate - 1 mL/min; mass spectrometer- triple quadrupole tandem mass spectrometer with MRM; ESI - positive ion mode; mass transitions selected 888.1→1050.2 for PG and 948.2→1110.3 for Ginsenoside Rb1 The precision in RSD form range of 0.26–2.74; the accuracy in RE form range of −1.35–3.69	Diosgenin-3-O-α-l-rhamnopyranosyl (1→4) [α-l-rhamnopyranosyl (1→2)]-β-D-glucopyranoside protodioscin (PG) - 25(R)-26-O-β-D-glucopyranosyl-furost-Δ5(6)-en-3β, 22α, 26-triol-3-O-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosidein rat plasma internal standard-Ginsenoside Rb1	*P. polyphylla* Smith var. yunnanensis (Franch.) Hand.-Mazz and *P. polyphylla* var. chinensis *D. zingiberensis* C. H. Wright	[158,160]
HPTLC	The method was preliminarily validated and the determined amounts of diosgenin	Plate - Si60F254 plates; phase: A mixture of n-heptane/ethyl acetate (7:3, v/v); spraying reagent-modified anisaldehyde	Diosgenin	Fenugreek (*Trigonella foenum-graecum* L.)	[163]
GC–MS	GC–MS analysis was performed using agilent 7890A/5975C GC–MSD instrument and split (50:1) injection system. The GC was fitted with an Agilent 19091S-433HP-5MS capillary column (30.00 m×0.25 mm inner diameter, 0.25 µm phase thickness). The GC oven was Solvent delay of 5 min was employed. These compounds were identified based on their mass spectrum, molecular weight, and fragment ions obtained from the mass spectrum. These parameters were matched with those of reference compounds	Column - HP-5 capillary (30 m ×0.32 mm, 0.25 µm); initial temperature -120°C for 2 min; Programming temperature -280°C; flow rate - 10°C/min Column - HP-5 ms capillary (30 m ×0.25 mm i.d. ×0.25 µm film thickness); mass detector- inert MSD triple-axis with ion trap conditioned at 200°C, transfer line- 280°C; sample amount-2 µL (spitless mode); Initial temperature of a column- 60°C for 1 min then 5°C/min up to 250°C Carrier gas-helium at the flow -1 ml/min Electron energy-70eV (vaccum pressure-2.21e-0.5 Torr)	Pavitnosides A–D Diosgenin Protodioscin (1),[8] dichotomin (2),[9,10] and protogracillin (3) prosapogenin A (5),[15] parrisaponin (7),[16] gracillin (8),[17,18] (25*R*)-spirost-5-en-3β-ol-3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (10),[16] (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-	1. *Paris vietnamensis* Takht 2. *Dioscorea alata* L.	[130,155,157,176]

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Techniques	Specification	Methods	Compound	Plant name	References
	Obtained from National Institute of Standards and Technology 2011 database which were incorporated into the computer system of the Equipment Agilent 7890A/5975C GC–MSD instrument and split additional preparation is required for study of nonvolatile matrices Analyte must either be capable of derivatization or volatile Atmospheric gases are challenging (CO_2, N_2, O_2, Ar, CO, H_2O)[8]	Column-DB-1MS (30 m ×0.25 mm, 0.25 µm film thickness) capillary; injection temperature - 250°C; Column temperature - 180°C for 1 min, rate - 20°C/min at 280°C and rate - 2°C/min at 300°C and final rate- 300°C for 16 min. Carrier gas - He, and flow rate - 1.0 mL/min; a mass range of m/z 50–700; the interface and ion source temperature-300°C and 250°C, with a splitless injection He GC was fitted with an agilent 19091S-433HP-5MS capillary column (30.00 m ×0.25 mm inner diameter) Agilent 7890A/5975C GC–MSD GC was fitted with an Agilent 19091S-433 HP-5MS capillary column (30.00 m ×0.25 mm inner diameter, 0.25 µm phase thickness). The GC oven temperature-100°C for 4 min to final temperature – 300°C at the rate of 4 °C/min; finalisothermally temperature - 240°C for 10 min; carrier gas helium with constant flow rate - 1.5 mL/min running time - 49 min; injection volume- 1 µL; analysis of samples in the full scan mode; electron ionization energy - 70 eV; source temperature - 250°C; solvent delay time - 5 min	rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (12),[19] and (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13),[20,21] which are spirostane-type saponin. Also, compound 6 was estimated as (25*R*)-spirost-5-en-3β-ol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside Steroidal saponin Standard - ginsenoside Rb1	3. *D. esculenta* (Lour.) Burk. 4. *Cassytha filiformis* L.	
Spectroscopic NMR UV IR		^{1}NMR spectrometer- Bruker AVANCE III 600 MHz spectrometer (Zurich, Switzerland); solution-CD_3COCD_3; internal standard-TMS 1D and 2D-NMR spectral were measured with a spectrometer- Bruker AVANCE-500 spectrometer (Bruker Corporation, Karlsruhe, Germany); solution-CD_3OD; internal standard-TMS ^{13}C and ^{1}H NMR spectra were recorded by spectrometer- JEOL ECA 500 spectrometer (500 MHz for ^{13}C and 125MHz for ^{1}H); solution-pyridine-*d5* solution; chemical shift - ^{13}C NMR (123.5 ppm) and ^{1}H NMR (7.20 ppm) using pyridine-*d5*; Mass spectrometry - JEOL JMS-T100LP spectrometer 1D and 2D NMR spectra measured by spectrometer-Varian UNITYINOVA 600 spectrometer; solution-C_5D_5N; internal standard-Tetramethyl silane NMR spectra were measured by spectrometer-Bruker AVANCE III 600 spectrometers (600 MHz for ^{1}H and 151 MHz for ^{13}C; Bruker BioSpin Corp., Billerica, MA, USA); internal standard - TMS; Spectrometer - JEOL JMS-700 spectrometer (Tokyo, Japan); Ionization mode-negative ion mode. Solution-C5D5N (Aldrich, HSQC, HMBC, COSY, TOCSY, and COSY are used to measured signal for ^{1}H and ^{13}C in 2D NMR The spectrometer used for UV spectra - Shimadzu UV-2600 PC spectrophotometer The spectrometer used to measure IR spectra - Shimadzu IRPrestige-21 spectrophotometer (Shimadzu Corporation, Tokyo, Japan)	(25*R*)–6α-(β-D-glucopyranosyloxy)-5α-spirostane-3β-*O*-[(6-*O*-hexadecanoyl)-β-D-glucopyranoside] trillikamtoside R8 Vernoniamyoside A–D, komarosides R (1) and S (2), Aspiletreins A–C (1-3) Anemarsaponin B (5) timosaponin D (6), timosaponin E1 (7) anemarsaponin B II (8) diosgenin, spirostanol, Furostanol 26-O-β-D-glucopyranosyl-(25R)-5-ene-furost-3β, 17α, 22α, 26-tetrol-3-O-α-L-arabinofuranosyl- (1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (2, parisyunnanoside A), 26-O-β-Dglucopyranosyl-(25R)-5, 20 (22)-diene-furost3β, 26-diol-3-O-α-L-arabinofuranosyl-(1→4)-[αL-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (7, parisyunnanoside B), and (25R)-spirost-5-ene3β, 12α-diol-3-O-α-L-rhamnopyranosyl-(1→4)-αL-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (13), parisyunnanoside C Protodioscin (1), dichotomin (2), and protogracillin (3) prosapogenin A (5), parrisaponin (7),[16] gracillin (8), (25*R*)-spirost-5-en-3β-ol-3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (10),[16] (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (12),[19] and (25*R*)-spirost-5-ene-3β,27-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (13)[20,21] which are spirostane-type saponin. Also, compound 6 was estimated as (25*R*)-spirost-5-en-3β-ol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside	*A. letreae* *A. obesum* (Forssk.) Genus *Trillium Linn.* *P. turgidum* Forssk *Smilax davidiana* A. DC. *T. kamtschaticum* Pursh *V. amygdalina* Del *C. komarovii* Al. Iljinski *A. letreae* *Anemarrhena asphodeloides Bge.* *D. zingiberensis* C. H. Wright *P. polyphylla* Smith *D. esculenta* (Lour.) Burk.	[7,9,25,26,33,96,105,123,133,140,141,155,162,169]

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Techniques	Specification	Methods	Compound	Plant name	References
Ultra-high-performance liquid chromatography ultraviolet mass spectroscopy	This method is sensitive, simple, and specific with short analysis time	Column- Shim-pack XR-ODS III column (150 mm×2.0 mm, 2.2 µm); mobile phase-acetonitrile and 0.1% formic acid solution; gradient profile- 17% A, 0-1.5 min; 17-23% A, 1.5-4.0 min; 23% A, 4.0-8.7 min; 23-38% A, 8.7–18 min; 38–60% A, 18-25.6 min; 60-17% A, 25.6-28 min; re-equilibration time-4 min; total running time-32 min; Mobile phase flow rate -0.45 mL/min; detector- UV detector; detection (nm) - 203 nm; column temperature - 45°C Mass spectrometer nebulizing gas - nitrogen at flow rate -3.0 L/min; drying gas - Nitrogen, flow rate -15.0 L/min; capillary voltage- 4.5 kV; ionization mode-ESI^-; desolvation temperature- 250°C; heat block temperature-100°C; acquired data range- from 100 to 1000 amu	Polyphyllin I and polyphyllin II	*P. polyphylla* Smith *var. yunnanensis* (Franch.) Hand.-Mazz and *P. axialis (PA)*	[166]
UPLC/Q-TOF-MS/MS UHPLC-ELSD	It was applied for characterization and separation purposes. This method was useful for the identification of steroidal glycosides even when reference standards were unavailable, by using fragmentation patterns. Consequently, saponins were recognized or tentatively elucidated in crude extracts from *D. zingiberensis* based on their retention times, the mass spectrometric fragmentation patterns, and MS and MS/MS data For the qualitative and quantitative determination of steroidal saponins	2.Column -Agilent Poroshell 120 EC-8, 2.1 mm×100 mm, 2.7 µm (p/n 695,775-906); Column temperature - 35°C; sample volume - 2 µL; Mobile phase A: 0.1% formic acid in water B): 0.1% formic acid in acetonitrile; flow rate 0.25 mL/min; gradient time (min) % A % B 0.00, 75, 25, 5.00, 70, 30, 7.00, 55, 45, 10.00, 40, 60, 15.00, 0 100, 20.00, 0 100 wash 5 min with 100% B equilibration 75% A/25% B, for 3 min total running time -15 min Ionization mode- positive and negative mode Carrier gas-drying N2 gas at 250°C for 15 min The agilent 1290 infinity LC System and an Agilent 6500 Series accurate-mass Q-TOF LC/MS Weight of dried rhizomes of *T. govanianum* - 100 mg; extaction solution- ethanol-water (80:20, 10 mL); by ultrasonic treatment at 40°C for 30 min. The prepared sample was analyzed by UHPLC-QTOF-MS/MS and UHPLC-ELSD UHPLC-ELSD method showed good linearity (R^2 ≥0.993), limit of detection (0.92-4.09 µg/mL), limit of quantification (3.-–13.5 µg/mL), precision (intraday RSDs <4.3% and interday RSDs <5.5%), and accuracy (84.0-110.3%)	Diosgenin, spirostanol, Furostanol Steroidal saponin [govanoside B (2), protodioscin (6), pennogenin tetraglycosides (11), borassoside E (21) and borassoside D (24)]	D. zingiberensis ℃. H. Wright *P. polyphylla* Smith *var. yunnanensis* (Franch.) Hand. - Mazz and *P. polyphylla* var. chinensis *T. govanianum*	[128,142,162,165]
UPLC/Q-TOF MSE	For rapid identification of chemical constituents in complex samples such as TCM by UPLC/Qtof MS^E, in the mean time without tedious purification from crude extact and the time-consuming	UPLC/Q-TOF MSE^s is used to characterize and identify the steroidal saponins structure from the of *Anemarrhena asphodeloides* rhizomes The techniques involved both negative and positive ion modes for the identification of fragmentation patterns. Through a single injection, give information about both fragment ion and intact precursor through this strategy extract. Based upon the correct mass, fragment ions, and retention times of peaks, and compare with standards references and known steroidal saponins	Steroidal saponins	*Anemarrhena asphodeloides* Bge.	[169]

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Techniques	Specification	Methods	Compound	Plant name	References
UPLC-ESI/QTOF/MS	It is powerful tool for rapid identification of steroidal saponins in *T. tschonoskii* Avoiding tedious and time-consuming isolation of pure constituents	UPLC-ESI/QTOF /MS is a method used for the identification of steroidal saponin Column-reversed-phase C18 - column; binary solvent phase system (gradient elution)-water and acetonitrile with formic acid; ionization module - both positive and negative ion modes. Based upon retention times, exact mass and mass fragment were identified or tentatively elucidated from *T. tschonoskii*	Steroidal saponin	*T. tschonoskii* Maxim	[170]
Polarimeter	It required large volume of sample with high concentration for dertermine the optical and specific rotation Main drawbacks is that it only analyzed optical active compounds MCP polarimeters measuring range extends from −89.9 OR to +89.9 OR This is the range that can be unambiguously measured with a polarimeter	Polarimeter used to the measured optical rotation Polarimeter- A Perkin-Elmer 241 MC digital polarimeter (German Perkin-Elmer Corporation, Boelingen, Germany). Polarimeter - An Automatic polarimeter (Hackettstown, NJ, USA) used for measurement of *Vernoria amygdalina* plant	Pavitnosides A–D Vernoniamyoside A–D, komarosides R (1) and S (2)	1. *Paris vietnamensis Takht* 2. *Vernonia amygdalina* Del	[26,130]
Column chromatography	It is time-comsuming process It has low separating power relative to advanced separation techniques Required more expensive and larger quantities of solvents are essential	1. Column-ODS Silica gel (Lichroprep RP-18, 40-63 µm, Merck Inc., Darmstadt, Germany) Silica gel H (10-40 µm, Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (GE-Healthcare, Uppsala, Sweden) 2. Column-Silica gel columns (2.8 cm×90 cm); mobile phase-$CHCl_3$/MeOH/n-BuOH/H_2O (10:5:1:4, v/v/v/v; 2 l; fraction of 25 ml) and column-Sephadex LH-20 (3.8 cm×65 cm); mobile phase-MeOH (1 l; fraction of 25 ml) 3. Column-C300 silica gel column chromatography (3 cm×60 cm; AG Tokyo, Japan); gradient solvent - CHCl3, CHCl3:MeOH (9:1–1:9); elution solvent-MeOH, and MeOH:H2O (9:1–7:4); chromatogram developed by -$CHCl_3$:MeOH:H_2O (30:15:2.5, v/v/v) 4. Macroporous resin SP825; silica gel SP825 column (10 cm×80 cm); mobile phase-Me_2CO-H_2O (1:4, 3:7, 2:3, and 1:1, 25,000 mL each). Column-ODS-A silica gel (120 Å, 50 µm; YMC ODS silica gel (3 cm×28 cm) and elute solution-Me_2CO-H_2O (1:4)	Pavitnosides A–D, (3β,5α,6β,25R)-6-[(β-d-glucopyranosyl) oxy]-spirostan-3-yl O-β-d-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-d-galactopyranoside 4. 26-O-β-D-glucopyranosyl-(25R)-5-ene-furost-3β, 17α, 22α, 26-tetrol-3-O-α-L-arabinofuranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (2, parisyunnanoside A), 26-O-β-D-glucopyranosyl-(25R)-5, 20 (22)-diene-furost3β, 26-diol-3-O-α-L-arabinofuranosyl-(1→4)-[αL-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (7, parisyunnanoside B), and (25R)-spirost-5-ene3β, 12α-diol-3-O-α-L-rhamnopyranosyl-(1→4)-αL-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (13, parisyunnanoside C)	*Paris vietnamensis* Takht *A. ampeloprasum var.* porrum *Allium fistulosum* L. *P. polyphylla* Smith	[33,80,129,130,152]
SFC	It has better efficient for separation and useful to separate weakly polar compounds High separation efficiency at low costs and without the need of toxic solvents	The mobile phase-CO_2 (mobile phase A) and methanol (containing 0.2% $NH_3 \cdot H_2O$ and 3% H_2O) (mobile phase B); The back pressure-11.03 MPa (isobarically)	Furostanol saponin	*D. zingiberensis* C. H. Wright	[69,87]
UHPSFC		Column-HSS C18 SB column or a Diol column; Co solvent-methanol	Spirostanol saponins	*Radix hedysari*	[165,166]

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Techniques	Specification	Methods	Compound	Plant name	References
UHPLC	It has low dispersion and pressure limit 15,000–19,000 psi with use of smaller internal diameter column packed with sub 2 µM particles	A BEH C18 column; mobile phase - water (with 0.1% formic acid) and acetonitrile It can differentiate the variation in aglycones and the presence of double bonds in aglycones affect the result	Spirostanol saponin	*Radix hedysari*	[165,166]
HPCPC	For quick isolation and purification of saponin glycosides in *Asparagus racemosus* Willd. root It has proven to be rapid for separate complex mixtures of phytochemicals yielding quantities suited to biological studies	A two-phase solvent system: CHC_{13}-MeOH-water (4:4:2, v/v); mode of elution-descending mode, yielding asparacoside (2) and shatavarin IX (1) For yielding asparanin A (3) and shatavarin V (4), solvent system- CH_2Cl_2- MeOH-water (4:4:2, v/v), were separated by repeated HPCPC fractionation using followed by either gel-filtration or TLC. Their structures were determined by NMR spectroscopy and ESI/MS'	Shatavarin IX (1) and asparacoside (2) in one step Asparanin A (3) and Shatavarin V (4)	*Asparagus racemosusWilld*	[176]
HPLC-Q-TOF-MS/MSA	It was developed and validated for simultaneous determination	Extracted with 70% MeOH	Five saponin glycosides, asparacoside, shatavarin IX, shatavarin IV, asparanin A, and shatavarin V in A	*Asparagus racemosusWilld*	[177]
HPLC-Q-TOF-MS/MS	It has accuracy, flexibility, selectivity, and sensitivity for reliable routine quaesility control The method was validated through intra- and inter-day precision, with the RSD - <6%, LOD - <10, and LOQ - 50 ng. Overall recoveries ranged - from 95% to 105%, with RSD ranging - from 0.7% to 4.5%				
Ultra high-performance liquid chromatography coupled with triple quadrupole mass spectrometry	A rapid and validated for simultaneous determination of four active steroidal saponins All calibration curves showed good linear regression (r>0.9985) within the test range. The limits of detection and quantification were in the range of 0.02-4.40 and 0.04–22.0 ng/mL, respectively	Agilent zorbax eclipse plus C18 column (2.1 mm×50 mm, 1.8 µm); gradient elution - acetonitrile-0.1% formic acid in water	Active steroidal saponins, i.e., dichotomin (1:), pennogenin 3-O-α-l-arabinofuranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→2)]-β-d-glucopyranoside (2:), pennogenin 3-O-α-l-rhamnopyranosyl-(1→2)-[α-l-rhamnopyranosyl-(1→4)-α-l-rhamnopyranosyl-(1→4)]-β-d-glucopyranoside (3:) and diosgenin 3-O-α-l-Rhamnopyranosyl-(1→2)-[α-l-Rhamnopyranosyl-(1→4)-α-l-Rhamnopyranosyl-(1→4)]-β-d-Glucopyranosidein (4:)	*Ypsilandra thibetica Franch*	[178]
Ultra-performance liquid chromatography-tandem mass spectrometer	Used for analysis of major steroidal saponins from *P. polyphylla* in biological samples The inter/intra-day precision, accuracy, recovery, matrix effect, and stability were evaluated per the FDA guidance. The method showed linearity in the concentartion ranges −2.4-1250 ng/mL The intraday and interday precisions (RSD) - <15.0%; Analytes extraction recoveries - 83.8%–109.4%; matrix effects ranged from 87.4% to 105.4%	A UItra BiPh column (100 mm×2.1 mm, 5 µm); mobile phase - acetonitrile/0.1% formic acid in water; mass spectrometer - Waters XEVO TQ mass spectrometer via MRM. ESI-positive scan mode		From *P. polyphylla* in plasma samples	[179]

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Techniques	Specification	Methods	Compound	Plant name	References
HPLC-ELSD-ESI-MS analysis	This method used for quantitative analysis It is preliminarily validated in terms of specificity, intraday and interday precision, LOQ, calibration/linearity, and LOD	18 column (150 mm×2.1 mm, 3 µm); mobile phase- A – water:formic acid (99.9:0.1, v/v) and B – acetonitrile:formic acid (99.9:0.1, v/v); Column temperature - 20°C ; flow rate - 0.2 mL/min ; Injection volume - 1 µL mobile phase- water/acetonitrile with addition of 0.1 % formic acid; detection - ELS; evaporator temperature - 40°C; signal strength - 4; nebulizing gas (N^2) flow rate - 1.8 L/min; ESI-MS mode - PI and NI ion modes; full scane range - m/z 800–1600 ; moniter -SIM The MS detector parameters were: ESI voltage-4.5 kV; nebulizing gas (N^2) flow 1.5 L min-1; desolvation line temperature-250°C; block temperatures were 200°C; adrying gas flow (N^2) detector voltage - 2 kV; drying gas flow (N^2) - 10 L/ min	25(27)-eno-protoneogitogenin-S1,proto-lilagenin-S1/ proto-yuccgenin-S1, proto-neogitogenin-S1/ proto-gitogenin-S1 (trigoneoside Ia/trigoneoside Ib), proto-neogitogenin-S3/proto-gitogenin-S3 (trigoneoside XVIIa/trigoneoside XVIIb), proto-neogitogenin-S4/proto-gitogenin-S4, proto-lilagenin-S2/proto-yuccagenin-S2, proto-gitogenin-S5/ proto-neogitogenin-S5, proto-neogitogenin-S2 (trigoneoside Xa), proto-gitogenin-S2 (trigoneoside Xb), 25(27)-eno-proto-diosgenin-S6 (trigoneoside VI)/(proto-sceptrumgenin)-S6, proto-yamogenina-S7 (trigoneoside Va), proto-diosgenin-S7 (trigoneoside Vb), proto-diosgenin-S8/S9/proto-yamogenin-S8/ S9,proto-tigogenina-S10/proto-neotigogenina-S10, proto-yamogenin-S11 (trigoneoside XIIIa), proto-diosgenin-S11 (trigoneoside XIIIb), proto-diosgenin-S1/ proto-yamogenin-S1/proto- makrantogenina-S1/22-deoksy-trigoneoside IIIa/IIIb, proto-yamogenin-S12 (trigoneoside Iva), proto-neotigogenina-S1/ proto-tigogenina-S1 (trigoneoside IIa/IIb), proto-diosgenin-S12 (glycoside F), proto-yamogenin-S13 (trigonelloside C protoneodioscin), proto-diosgenin-S13 (compound C, protodioscin), proto-yamogenin-S2 (trigofenoside A) ,glycoside D, proto-neotigogenina-S2/ proto-tigogenina-S2 (trigoneoside IIIa/IIIb)	*Fenugreek (Trigonella foenum-graecum, Fabaceae*)	[163]
Ultra-high-performance liquid chromatography-evaporative light scattering detector	Effiicient for qualitative and quantitative determination of steroidal saponins in *T. govanianum*	Weight of dried rhizomes of *T. govanianum* - 100 mg; extaction solution- ethanol-water (80:20, 10 mL); by ultrasonic treatment at 40°C for 30 min. The prepared sample was analyzed by UHPLC-QTOF-MS/MS and UHPLC-ELSD UHPLC-ELSD method showed good linearity (R^2 ≥0.993), limit of detection (0.92-4.09 µg/mL), limit of quantification (3.1-13.5 µg/mL), precision (intraday RSDs <4.3% and interday RSDs <5.5%), and accuracy (84.0–110.3%)	Govanoside B (2), protodioscin (6), pennogenin tetraglycosides (11), borassoside E (21) and borassoside D (24)	*T. govanianum*	[142]

T. govanianum: *Trillium govanianum*, *P. polyphylla: Paris polyphylla, T. tschonoskii: Trillium tschonoskii, D. zingiberensis: Dioscorea zingiberensis, Dioscorea esculenta*, TLC: Thin-layer chromatography, UPLC: Ultra-performance liquid chromatography-tandem, HPLC: High-performance liquid chromatography, ESI: Electrospray ionization, QTOF: Quadrupole time-of-flight, MS: Mass spectrometry, ELSD: Evaporative light scattering detector, HPTLC: High-performance thin liquid chromatography, GCMS: Gas chromatography-mass spectrometry, NMR: Nuclear magnetic resonance, UV: Ultraviolet spectroscopy, IR: Infrared spectroscopy, TOF: Time-of-flight tandem, UPLC/Q: Ultra-performance liquid chromatography and hybrid quadrupole, SFC: Supercritical fluid chromatography, UHPSFC: Ultra-high-performance supercritical fluid chromatography, UHPLC:s Ultra-high-performance liquid chromatography, HPCPC: High-performance centrifugal partition chromatography, Pc: peak capacity, MRM: Multiple reaction monitoring, PI: Positive, NI: Negative, RSD: Relative standard deviation, LOD: Limits of detection, LOQ: Limits of quantification, DAD: Diode array detector, RID: Refractive index detectors, HP: High-Performance, NMR: Nuclear magnetic resonance, IS: Internal standard, LCMS - Liquid chromatography–mass spectrometry, MSA- Measurement System Analysis, MSD – Mass Selective Detector, TCM-Traditional Chinese medicine, FDA- Food and Drug Administration, RP- RhizomaParidis ,EtOH- ethanol, LC-Liquid Chromatography, HSS-High Strength Silica, MeCN -methyl cyanide, YMC – Product name, ODS-Octadecyl-silica, PAQ- Product name, CAPCELL PAK ADME Type is a HPLC column, PDA-Photodiode array ,EC- column name, OCT- Optical coherence tomography, MHz- Mega Hertz, TMS- Tetramethylsilane, HSQC-Heteronuclear single quantum coherence, HMBC -Heteronuclear Multiple Bond Correlation, COSY-Correlated Spectroscopy, TOCSY -Total Correlation Spectroscopy, QTOF-Quadrupole Time of Flight

from *Allium fistulosum*. The pyridine-d5 solution at 500 and 125 MHz is used for getting spectra in the ^{13}C and ^{1}H NMR, respectively, which are used for the study of the *A. fistulosum*.[152] The spectroscopic methods including 1D and 2D NMR were used for the study of *Yucca filamentosa L. Panicum turgidum*.[153]

Infrared spectroscopy

Infrared (IR) spectroscopy is a simple and reliable technique used for the detection of the functional groups present in the compound. This technique also play role in the identification of steroidal saponins.

Near-infrared reflectance spectroscopy

Near-IR reflectance spectroscopy is an analytical technique used to quickly determine the compound chemical and physical properties without altering the sample. It is used to determine the ginsenoside Rg1 and Re found in Chinese medicine.[154]

Ultraviolet spectroscopy

It is widely used for the quantitative analysis of different analytes. In this techniques, analyte can be gases and solid.

Polarimeter

A polarimeter is a device for determining the polarization direction of the light or the rotation of an optically active substance. The JASCO DIP-1000 digital polarimeter was used to deduce optical rotation.[152]

Matrix-assisted laser desorption ionization-time of flight mass spectrometry

Matrix-assisted laser desorption ionization-time of flight MS is a widely used technique for analysis purposes. This instrument has a wide range of analytes including oligonucleotides, and proteins.

Gravimetry

One of the first methods that were developed decades ago for measurements of saponin is the Gravimetry method (Hahrbone, 1973). It is based on the saponin's specificity for n-butanol. However, this technique is not suitable for extraction due to its poor specificity and could not visible in the chromatogram. Most important is requires a large amount of petrol-derived organic solvent in each sample which is why it is not considered ecofriendly

Ultra-high-performance liquid chromatography

It is chiefly used to separate components of the sample in lesser time with better resolution. It requires a small volume of samples for analysis. The analysis of the MD sample is well suited to it.

Ultra-high-performance supercritical fluid chromatography

It likely performed speedily and automatically. It is worthwhile for the spirostanol saponins separation that varies in sugar chains and shares the same aglycone. It is easily affected by the position and the number of hydroxyl groups in aglycones. It is a powerful technique with better resolution.

Supercritical fluid chromatography

It could be fruitful to separates the furostanol saponins which shared a portion of the same aglycone through the difference in sugar chains. It was the sensitized type of sugars and their number. It could be useful for separating hydrophilic furostanol saponins.

Conclusion

Steroidal saponins are made up of glycosides groups that contain lipophilic components and lipophobic components which are broadly dispersed among monocotyledonous families. It belongs to secondary metabolites. The majority of the world still entrusts folk plant medicine for the treatment of various diseases. Many plants contain saponins, which can generally show account for their remedial action. Diverse pharmacological properties of steroidal saponin have been reported including cytotoxic, antiviral, nephroprotective, hepatoprotective, antitumor, antimicrobial, cardioprotective, antihyperuricemia, antimicrobial, and antifungal. It can be a probable lead molecule in the research field of drug development. Several analytical techniques are used for the quantification of steroidal saponin such as HPTLC, HPLC, LC-MS, and GC-MS. However, recently, some new techniques were also introduced to analyze steroidal saponins such as UHPSFC, SFC, and UHPLC. On the other hand, this review includes the biosynthetic pathway of steroidal saponin in plants which can contribute a significant role to develop new drugs via using synthetic biology approaches. It also includes plant species with their parts showing pharmacological properties, mode of action, various compounds, such as polyphyllin I, dioscin, timosaponin AIII, diosgenin, Paris saponin II, and dioscin, sound to be especially rising as future antitumor agents. Saponins have vast chemical diversity which seeks the interest of the researcher. This review may be helpful for further research in the qualitative and quantitative analysis and is expected to give a wide range of applications of steroidal saponins.

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Conflicts of interest

There are no conflicts of interest.

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