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Review Article

Biologically Active Saponins of Genus *Cestrum* L.: A Comprehensive Review

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ABSTRACT

Genus *Cestrum* L. belonging to family Solanaceae comprises from 250 to 300 species of flowering plants native to warm temperate to tropical regions of America. Shrubs of *Cestrum* L. species are known as Jessamines due to their highly fragrant flowers. They are planted not only for their ornamental uses but also for their valuable and diverse medicinal effects. In many African, Asian and American countries, folk medicine practitioners used different *Cestrum* L. species for their important ethno-pharmacological effects and diverse biological properties. In the last decades, fifty-two saponins, mainly of steroidal nucleus, have been isolated form certain *Cestrum* L. species and are responsible for numerous important biological activities e.g. cytotoxic, spermicidal, anti-microbial and pesticidal activities. In this updated review till 2022, we highlighted the pharmacological importance of those steroidal saponins, their biosynthetic pathway and the relation between the chemical structure and biological activity.

Keywords: *Cestrum; steroidal saponins; cytotoxic activity; pesticidal activity; biosynthesis*.

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1. Introduction

Medicinal plants are considered as a valuable resource to cure illness and ailments. They may provide a cheaper and much safer alternative for synthetic drugs. The genus *Cestrum* L. belongs to family Solanaceae and comprises about 250 to 300 species, distributed worldwide including herbs, shrubs and trees up to 12 m in height, *Cestrum* L. species are recognized by actinomorphic flowers, pentasepalous, tubular corolla and berry like fruits **[1]**.

The taxonomical classification of *Cestrum* is **[2]**:

Kingdom: Plantae

Subkingdom: Tracheobionta -Vascular plants

Superdivision: Spermatophyta - Seed plants

Division: Magnoliophyta - Flowering plants

Class: Magnoliopsida - Dicotyledons

Subclass: Asteridae

Order: Solanales

Family: Solanaceae – potato family

Genus: *Cestrum* L.

The genus is widely known not only for its ornamental uses, but also for its valuable medicinal uses. In many African, Asian and American countries, *Cestrum* L. species have been known for their important ethno-pharmacological uses and diverse biological properties **[3]**. In Ecuador, *C. auriculatum* L'Hér is used for scurvy and diarrhoea **[4]** and in Peru, it is used to treat wounds, fever and high blood pressure **[5]**. In the traditional Indian ayurvedic medicine, *C. diurnum* L. is used for the enhancement of calcium levels in the body **[6]**. *C. nocturnum* L. is being used in the folkloric Pakstinian therapy for the treatment of gastric ulcer **[7]**. Its efficacy in burn healing has been reported in Chinese folk medicine **[8]**. In Peru, it is used as abortifacient **[9]**. *C. parqui* L'Hér is used traditionally for fever and different skin diseases such as allergies, herpes and impetigo **[10]**. In Maya tribe, the traditional healers used the leaves of *C. schlechtendahlii* G. Don for the treatment of warts and infected wounds **[11]**. Mapuche a group of inhabitants in Chile and southwestern Argentina, used *C. parqui* L. leaves for the treatment of allergies, herpes, impetigo, and headache **[12]**.

Saponins, lignans, flavonoids, phenolic compounds, volatile oils and trace amounts of alkaloids were reported in genus *Cestrum* L. **[13]**. In the current updated review, we focus on the saponin content of the genus, their phytochemical structures, and their diverse pharmacological activities. Saponins are secondary glycosidic metabolites that are widely distributed in higher plants families **[14]** and are also found in some animal sources, like marine invertebrates **[15]**. They are biosynthesized by plants as a part of plant defence mechanism **[16]**, thus their content and distribution in a plant may vary in response to a herbivorous attack, pathogenic infection or even due to symbiosis influence **[17]**. Biosynthetic pathway of these compounds starts by an isoprene unit and ends up, after a series of enzymatically catalysed chemical reactions, in triterpenoidal saponins (30 C) or steroidal saponins (27 C) according to the genetic map of the plant. Thus, similar plant orders possess similar classes of saponins **[18]**. Despite the large structural diversity of saponins, they possess the same amphiphilic nature due to the presence of a hydrophilic sugar moiety and a hydrophobic aglycon or sapogenin. This characteristic feature of saponins explains many functions they exert such as foaming and haemolytic activities **[19]**.

The research strategy in this review depends on collecting and summarizing data from published articles or book chapters from different data bases, including Google Scholar, Scirus, PubMed, and Science Direct from 2007 till 2022, followed by discussing the relation between the reported biological activity and saponins structure aiming to highlight the structure activity relationship of *Cestrum* L. saponins.

2. Phytochemistry and biosynthesis

Investigation of genus *Cestrum* L. saponins **(Table 1, Fig. 1-3)** revealed that they are mainly of a steroidal aglycon (27 C), although it was reported that both *C. elegance* Brongn. and *C. nocturnum* L. possess saponins of triterpenoidal oleanane aglycone **(Fig. 4) [20, 21]**. Both aglycone types are biosynthesized from a common (30 C) precursor named 2,3-oxidosqualene that either cyclized by the aid of oxidosqualene cyclase enzymes into triterpenoidal aglycone or lose 3 methyl groups and convert into cycloartenol leading to the formation of (27 C) skeleton of steroidal saponins **[22] (Fig. 5**). Structurally, steroidal saponins are distinguished by polycyclic aglycon divided into three distinct groups: spirostane, furostane, and open-chain cholestane **[23] (Fig. 5)**. In the genus *Cestrum* spirostane and furostane types are more common than cholestane type. Spirostanol

saponins contain a bicyclic spiroacetal moiety at 22nd carbon that involves the steroid E and F rings while furostanol saponins bear a hemiacetal, methyl acetal, or 20-22 unsaturation at this position. Both types of saponins are closely related to each other where at former biosynthetic steps, glycosylation of the hydroxyl group at C26 takes place catalysed by the enzyme UDPGlc to form furostanol 26-β-D-glucoside. If enzymatic removal of the C26 glucose moiety occurs, a spontaneous cyclization will also happen leading to formation of the heterocyclic ring of spirostane **[24]**. Open chain cholestane saponins have been reported recently from *C. newelii* Veitch. **[25]**, they are considered as a distinct point in the biosynthesis pathway where cycloartenol metabolite undergoes de-methylation followed by hydroxylation of C26 which prevents ring E closure **[26]**.

P450 is another important family of enzymes responsible for the oxidation and hydroxylation of sapogenin at different positions leading to a large structural diversity of the final compounds **[27]**. Disogenin is a spirostanol sapogenin with OH group at C3 and protodisogenin is its furastanol analogue, pennogenin with dihydroxyl groups at C3 and ruscogenin with di-hydroxyl groups at C1 and C3 **[28]**.

Concerning the sugar moieties attached to the steroidal sapogenin, they play crucial role in solubility and biological activity. In *Cestrum* L. species, furostanol saponins possess a glucose moiety at C 26. At C3 of both furostanol and spirostanol sapogenins, the sugar residues attached can be galactose, glucose, xylose and rhamnose in straight or branched chain. Glycosylation is the last step in the biosynthetic pathway, where sugar residues are attached to the sapogenin at specific position catalysed by a group of glycosyltransferase enzymes (UGTs) and despite the importance of these enzymes only few of them have been identified **[29]**.

The importance of studying biosynthetic enzymes arises from the recent approach of using transgenic yeast strains for *in vitro* production of saponin, which is more beneficial than extracting saponins from plants by the traditional techniques, since massive amounts of pharmacologically active entities can be produced with specific structure required for optimum biological effect **[30]**.

Table 1. Reported saponins of Genus *Cestrum*

Species	Investigated extract/organ	Compound name	Ref.
C. diurnum L.	Methanolic extract of leaves	• Cesdiurin I **(1)** • Cesdiurin II **(2)** • Cesdiurin III **(3)**	**[51]**
C. elegance Brongn.	Aqueous methanolic extract of flowers	• 25R)-6α-[20]-5α-spirostane-3-*O*-β-D glucopyranoside **(4)** • (25R)-6α-[(β-D-glucopyranosyl)oxy]-5α-spirostan-3-*O*-β-D glucopyranosyl (1"→3')-*O*-β-D-glucopyranosid **(5)** • 3,23-di-hydroxyolean-12-en-28-oic acid-3-*O*-α-$^{1}C_{4}$ rhamnopyranosyl (1"→6')-*O*-β-$^{4}C_{1}$ glucopyranoside **(6)**	**[21]**
C. hedindium Francey.	Methanolic extract of aerial parts	• Cestruside A **(7)** • Cestruside B **(8)**	**[43]**
C. levigatum Schltdl.	Ethanolic extract of stem	• (25-R,S)-5α-Spirostan-2α,3β-diol 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl -(1→4)-β-D-galactopyranoside **(9,10)**	**[52]**

		• (25-R,S)-5α-Spirostan-2α,3β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranoside **(11,12)** • 26-*O*-β-D-Glucopyranosyl-(25-R,S)-5α-furost-20-ene,2α,3β-diol 3-*O*-β-D-galactopyranoside **(13,14)** • 26-*O*-β-D-Glucopyranosyl-(25-R,S)-5α-furost-20-ene,2α,3β-diol 3-*O*-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside **(15,16)** • (25R,S)-5α-spirostan-2α,3β-diol-3-*O*-β-D-galactopyranoside **(17,18)**	
C. newelli Veitch.	Methanolic extract of leaves	• (24S,25S)-2α,12β,24-trihydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside **(19)** • (24S)-2α,12β,24-trihydroxyspirosta-5,25(27)-dien-3β-yl-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside **(20)** • 2α-hydroxyspirosta-5,25(27)-dien-3β-yl-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside **(21)** • 26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxyfurosta-5,25(27)-dien-3β-yl-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside **(22)** • 26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxyfurosta-5,25(27)-dien-3β-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside **(23)** • 26-[(β-D-glucopyranosyl)oxy]-2α-hydroxyfurosta-5,20(22),25(27)-trien-3β-yl-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside **(24)** • 26-[(β-D-glucopyranosyl)oxy]-2α-hydroxyfurosta-5,20(22),25(27)-trien-3β-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside **(25)** • (22S)-25-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside **(26)** • (22S,25R)-26-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-y- *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside **(27)** • (22S,25R)-26-[(β-D-glucopyranosyl)oxy]-16,22-dihydroxycholest-5-en-3β-yl-*O*-α-L-rhamnopyranosyl- (1→2)-*O*-[α-L-rhamnopyranosyl- (1→4)]-β-D-glucopyranoside **(28)** • (25R)-26-[(β-D-glucopyranosyl)oxy]-3-[(*O*-α-L-rhamnopyranosyl- (1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-d-glucopyranosyl)oxy]-cholest-5-ene-16,22-dione **(29)**	**[24]**
C. nocturnum L.	Methanolic extract of leaves	• Nocturnoside A **(30)**	**[53]**
	Methanolic extract of leaves	• Nocturnoside B **(31)**	**[54]**

	Methanolic extract of leaves	• 3-O-β-D-xylopyranoside-olean-12-en-28-oic acid-28-O-β-arabinopyranosyl-(1-3)-β-D-galacto-pyranosyl-(1-2)-β-D-glucopyranosyl-(1-4)-β-D-glucopyranosyl ester **(32)** • Karativoside A **(33)**	**[20]**
C. parqui L'Hér	Methanolic extract of leaves	• Parquispiroside **(34)** • Parquifuroside **(35)** • Capsicoside D **(36)** • 22-OMe-capsicoside D **(37)**	**[55]**
C. ruizteranianum	Aqueous methanolic extract of fruits	• Pennogenin 3-*O*-β-chacotrioside **(38)** • (25R,26R)-spirost-5-ene-3 β,17α,26-triol 3-*O*- β -chacotrioside **(39)** • protodioscin **(40)** • Methyl protodioscin **(41)** • 26-*O*- β –D- glucopyranosyl- (25R)-furost-5-ene-3 β,17 α ,22 , 26-tetraol 3-*O*- β -chacotrioside **(42)** • 26-*O*- β -D-glucopyranosyl-22-methoxy-(25R)-furost-5-ene-3 β 26-diol-3-*O*- α -L-rhamnopyranosyl-(1→4)- α -L-rhamnopyranosyl-(1→4)-[α -L-rhamnopyranosyl-(1→2)]- β -D-glucopyranoside **(43)** • 26-*O*- β -D-glucopyranosyl-(25R)-furost-5-ene-3β,22,26-triol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside **(44)**	**[56]**
C. schlechtendahlii G.Don	Aqueous ethanolic extract of leaves	• (25R)–1β,2α–dihydroxy–5 α–spirostan–3–β–*O*–α–L–rhamnopyranosyl–(1→2)–β–D–galactopyranoside **(45)** • (25R)–1β,2α–dihydroxy–5α–spirostan–3–β-*O*–β–D–galactopyranoside **(46)**	**[57]**
C. sendtenerianum Francey.	Ethanolic extract of leaves	• Spirosota-5,25(27)-diene-1β,2α,3β,12β-tetrol, 3-*O*-β-D-galactopyranoside **(47)**	**[58]**
	Ethanolic extract of leaves	• 1β,2α- dihydroxy spirosta-5,25(27)-dien-3β- *O*-α-D-rhamnopyranosyl-(1→2)-β-L-galactopyranoside **(48)** • (25R)-1β,2α-dihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside **(49)** • 1β,2α-dihydroxy-5α-spirost-25(27)-en-3β-yl *O*-α-Lrhamnopyranosyl-(1→2)-β-D-galactopyranoside **(50)** • (25R)-1β,2α-dihydroxy-5α-spirostan-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside **(51)** • 1β,2α-dihydroxy spirosta-5,25(27)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside **(52)**	**[59]**

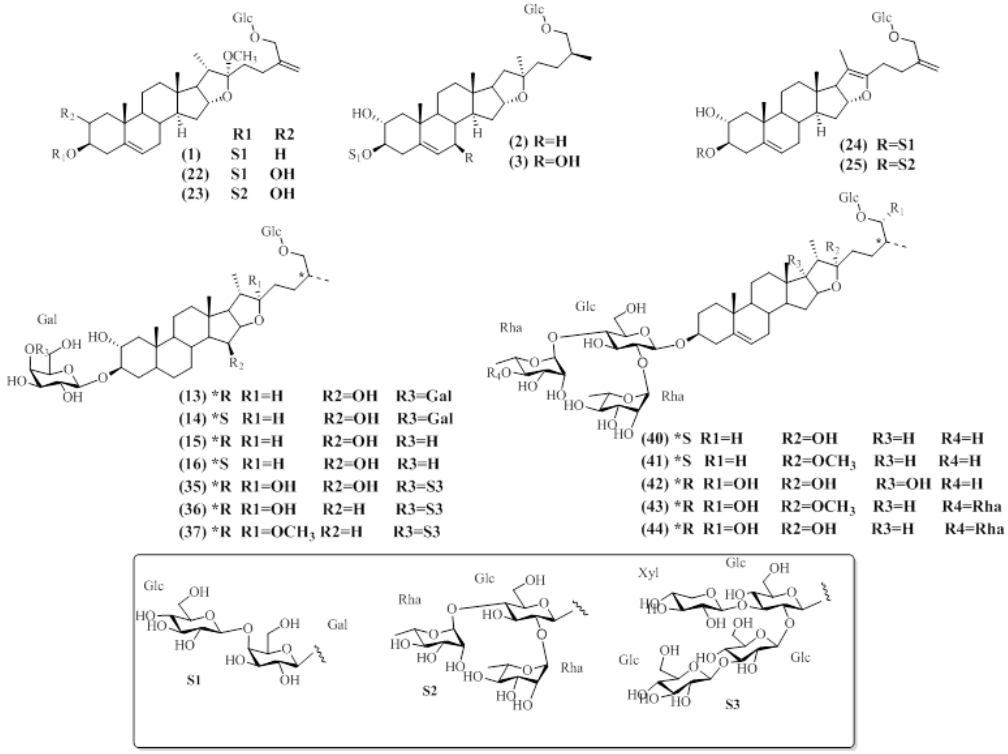

Fig. 1. Furostanol steroidal saponins of genus *Cestrum* L.

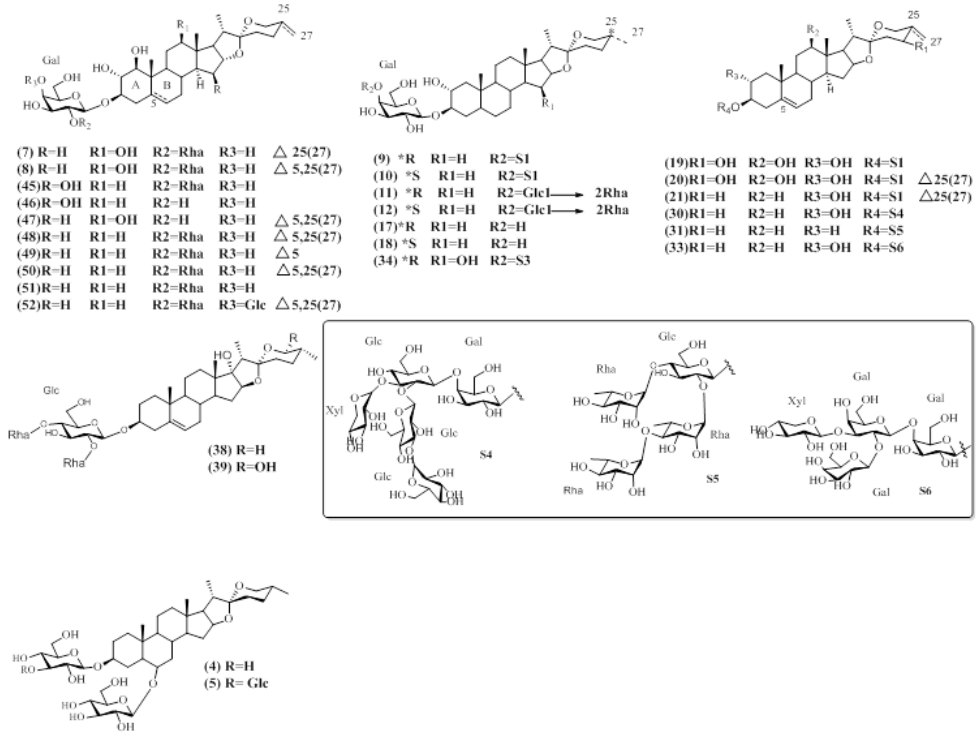

Fig. 2. Spirostanol steroidal saponins of genus *Cestrum* L.

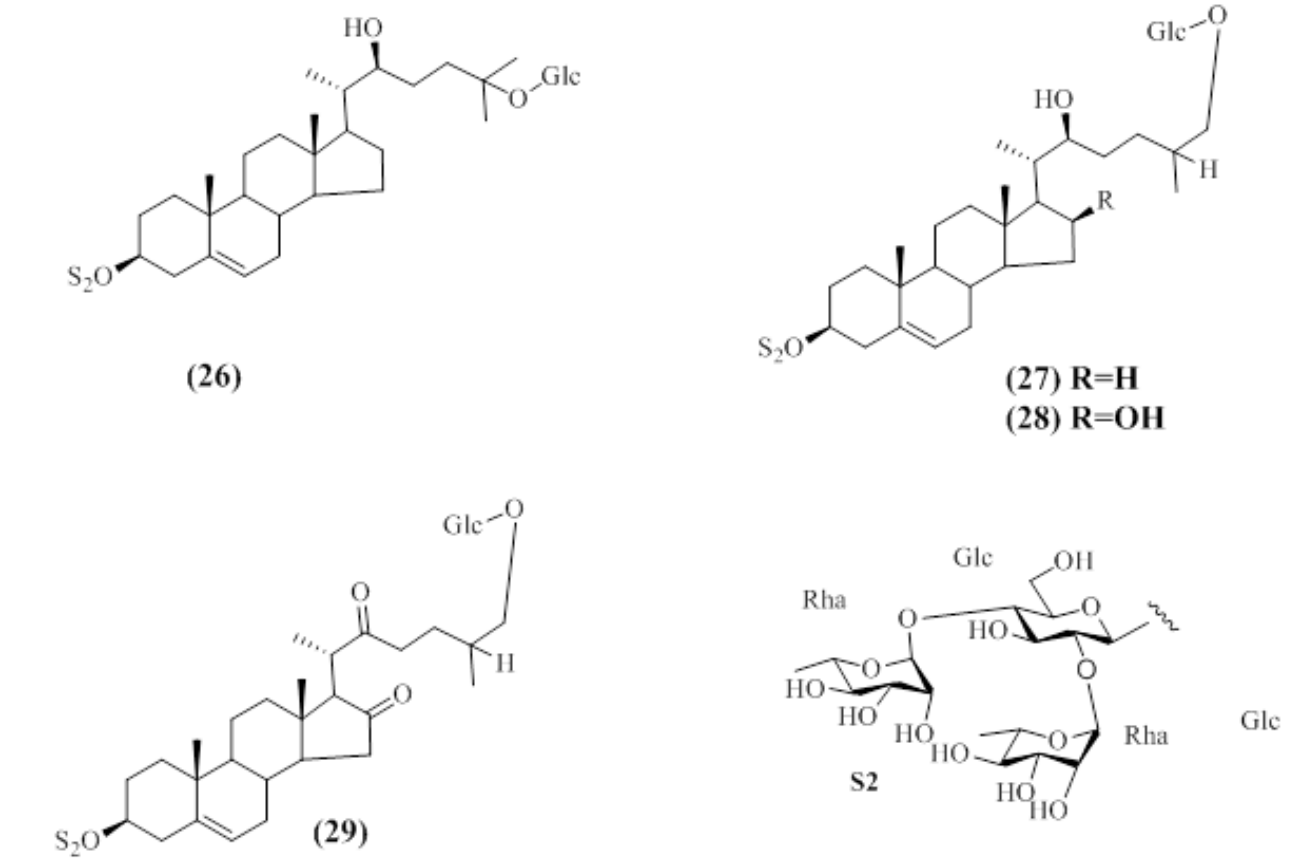

Fig. 3. Cholestan steroidal saponins of genus *Cestrum* L.

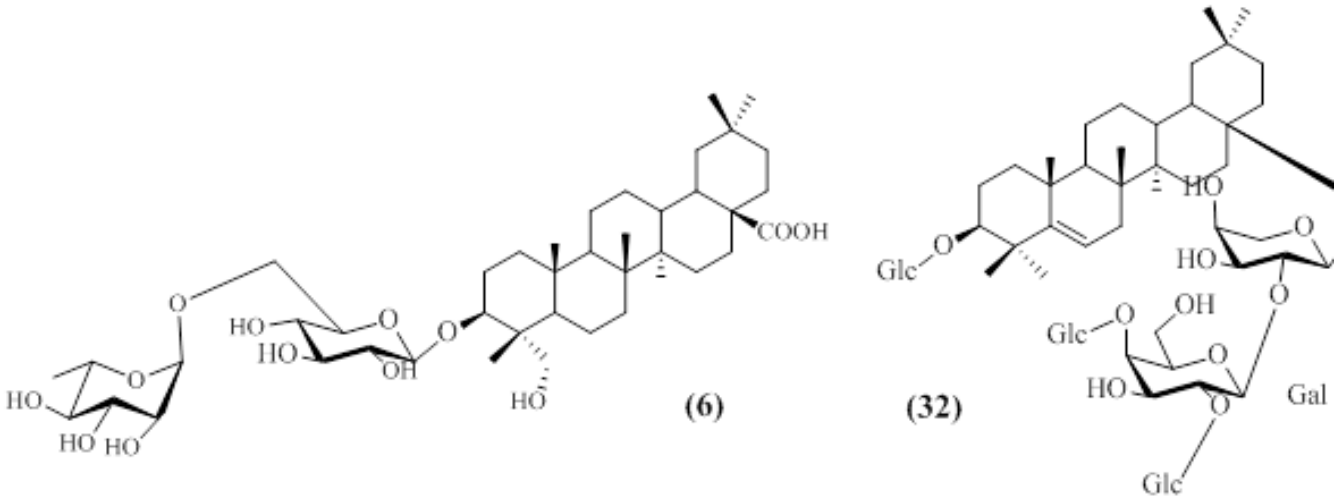

Fig. 4. Oleanane triterpenoidal saponins of genus *Cestrum* L.

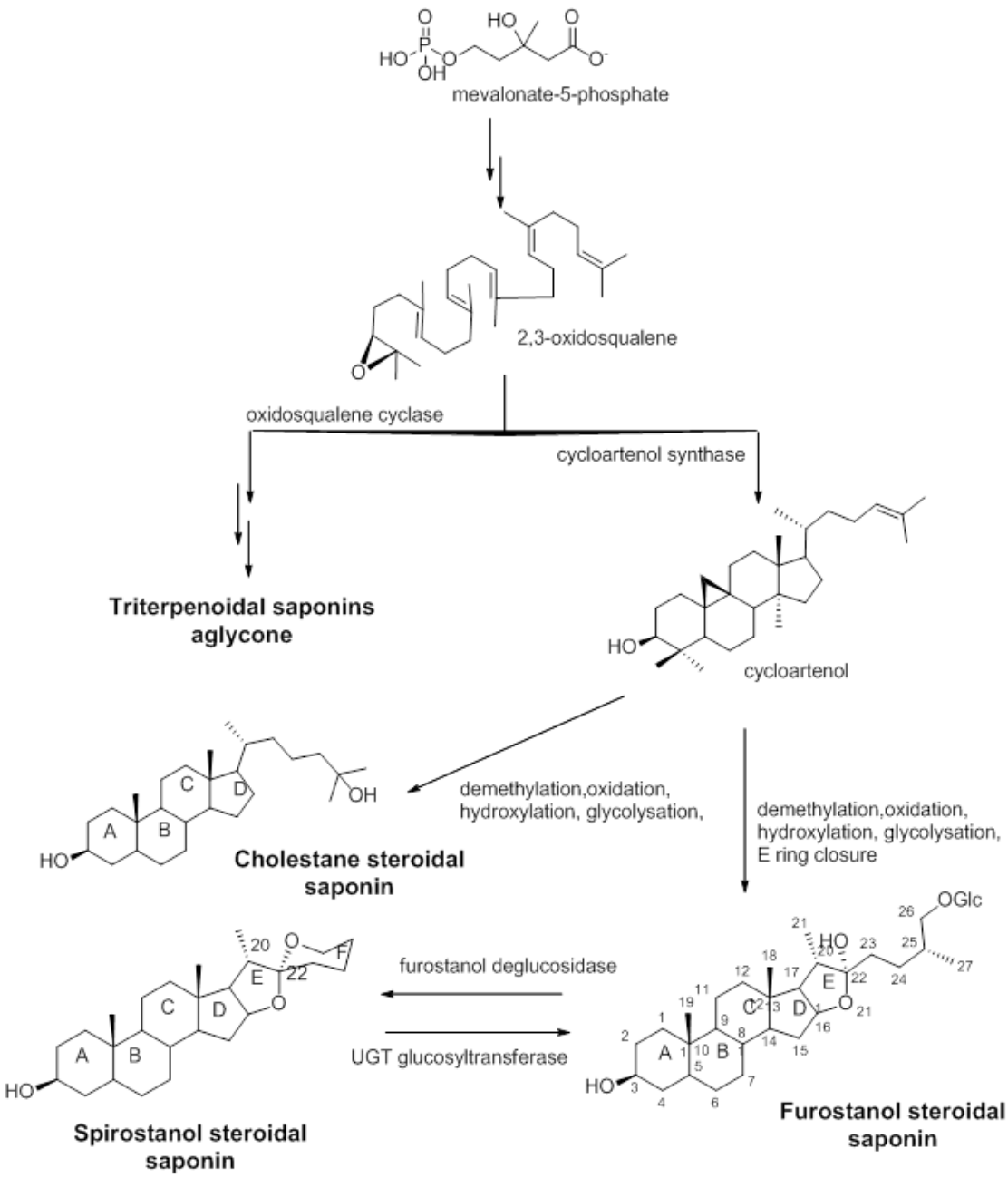

Fig. 5. Biosynthesis of steroidal saponins

3. Biological Activities

Steroidal saponins attracted the scientists' attention over the past few decades due to their multiple biological activities and their role as a precursor for the synthesis of steroidal drugs. *Cestrum* L. species exhibit many important biological activities that can be attributed to their saponin content either the saponin rich fraction or the pure isolated compounds **(Table 2)**.

Table 2. Biologically active saponins of Genus *Cestrum*

Biological activity	Species	Investigated extract/ compund	Efficacy	Ref.
1-Cytotoxic activity	*C. laevigatum* Schltdl.	Isolated steroidal saponins **(9-18)**	• Test was performed on HCT-116, OVCAR-8, HL-60 and SF-295 cell lines. • **(9,10)** exhibited high cytotoxic activity to HL-60, IC_{50} = 2.22 μg mL^{-1} and moderate activity against other cell lines, IC_{50} from 6.88 to 10.8 μg mL^{-1}. • **(11,12)** showed moderate activity to all cell lines, IC_{50} ranging from 7.28 to 15.30 μg mL^{-1}. • **(17,18)** showed moderate activity against HL-60, • OVCAR-8 and HCT-116, IC_{50} from 11.3 to16.7 μg mL^{-1}, and were inactive against SF-295 cell lines. • **(13,14,15,16)** were inactive to all. • Doxorubicin IC_{50} values are 0.02, 0.26, 0.12 and 0.24 μg mL^{-1} ,respectively.	**[52]**
	C. parqui L'Hér	Isolated steroidal saponins **(34-36)**	• Test was performed on Hela, HepG2, U87, and MCF7 cell lines. • **(34)** exhibited moderate activity,IC_{50} are 7.7, 7.2, 14.1 and 3.3 μM, respectively. • **(35,36)** were inactive. • Cisplatin IC_{50} values are 39.2, 14.6, 7.3 and 23.0 μM, respectively.	**[55]**
	C. sendtenerianum Francey.	Isolated steroidal saponins **(52)**	• **(52)** exhibited weak cytotoxic activity • against HL-60 human promyelocytic leukemia cells, IC_{50} = 7.7 mg mL^{-1}. • Etoposide IC_{50} = 0.75 mg mL^{-1}	**[59]**
2-Anti-bacterial activity	*C. elegance* Brongn.	Isolated saponins **(4-6)**	• **(6)** exhibited stronger antibacterial activity against Gm +ve bacteria *Staphylococcus aureus* than **(4)** and **(5)** with clear zone of diameter 25 mm, 23 mm and 22 mm, respectively compared to 29 mm for penicilline. • **(4)** exhibited stronger antibacterial activity against Gm -ve bacteria *pseudomonas aureginosa* than **(5)** and **(6)** with clear zone of diameter 19 mm, 17 mm and 17 mm, respectively compared to 23 mm for streptomycine.	**[21]**
	C. laevigatum	Isolated steroidal saponins **(9-18)**	• The assayed microorganisms were *P. aeruginosa*, • *S.aureus* and *B. subtilis.* • All compounds were inactive • Cefepime was used as positive control	**[52]**
	C. parqui L'Hér	Crude saponin extract CSE	• The tested bacteria were *P. aeruginosa, E. coli, Staph. aureus and Enterococcus faecalis*. • CSE showed no activity even at conc. up to 100 mg mL^{-1}	**[38]**
3-Anti-fungal activity	*C. elegance* Brongn.	Isolated saponins **(4-6)**	• **(6)** exhibited a very potent antifungal activity against *Candida albicans* with inhibition zone of diameter 34 mm compared to 22 mm for neomycine, while **(4-5)** showed weak activity with inhibition one of 19 mm and 18 mm, respectively. • **(4-6)** showed weak activity against *Aspergillus niger* with inhibition zone of diameter 15 mm, 16 mm and 14 mm, respectively compared to 26 mm for cyclohexamide.	**[21]**

	C. laevigatum Schltdl.	Isolated steroidal saponins **(9-18)**	• The antifungal susceptibility test was performed on *C. parapsilosis, C. krusei* and *C. albicans*. • **(9,10)** exhibited strong activity against *C. albicans* • and weak activity against *C. parapsilosis* with MIC 3.9 and 7.0 μg mL^{-1}. • **(17,18)** showed moderate activity against *C. kuresi* and *C. albicans* with MIC 31.2 and 7.0 μg mL^{-1}. • **(11,12)** showed strong activity against *C. krusei* and moderat against *C. albicans* with MIC 7.8 and 7.7 μg mL^{-1}. • **(13,14,15,16)** were inactive. • Fluconazole MIC values are 0.125 , 16.0 and 4.0, respectively.	**[52]**
	C.parqui L'Hér	Crude saponin extract CSE	• The antifungal activity was tested against *Fusarium solani* and *Botrytis cinerea* (plant pathogens),*Trichoderma viride* which exhibited a very high sensitivity to CSE, IC_{50} = 210 ppm. • *F. solani* and *B. cinerea* were found to be insensitive	**[38]**
	C. schlechtendahlii G.Don	Isolated steroidal saponins **(45-46)**	• Antifungal activity was assested against different fungal strains. • **(45)** inhibited growth of *Saccharomyces cerevisiae*, MIC range of 15-25μM, and *Fusarium graminearum* MIC range of 132–198 μM. • **(46)** showed no antifungal activity. • Genticin MIC range is 45-231 and 27-54 μM, respectively	**[57]**
4- Antiviral activity	*C. elegance* Brongn.	Isolated saponins **(4-6)**	• At a conc. of 1.56 μg/ml, **(4)** showed a potent antiviral activity against hepatitis A virus of 73.4 % while **(5-6)** exhibited no antiviral effect.	**[21]**
5-Spermicidal activity	*C. parqui* L'Hér	Raw saponins (RS) obtained from leaves	• The effect of RS on sperm motility and viability was observed at differenet doses and time intervals. • The maximal spermicidal effect was observed at a dose of 250 μg mL^{-1}.	**[39]**
6-Anti-inflammatory and analgesic effect	*C. hediundinum* Francey.	Isolated steroidal saponins **(7,8)**	• **(7,8)** inhibited PGE_1 and PGE_2-induced contractions of guinea pig ileum in the Magnus method test at conc. of 30 and 60 μM, respectively, which is responsible for the anti-inflammatory and analgesic effect of the extract.	**[43]**
7-Pesticidal activity	*C. parqui* L'Hér	Crude saponin extract (CSE)	• Molluscicidal activity of CSE was tested against harmful phytophagous mollusc, *Theba pisana*. • It showed strong activity on adult and jeuvenile forms with a LD_{50} of 36.13 and 6.02 μg/cm^2, respectively.	**[47]**
	C. parqui L'Hér	Crude saponin extract (CSE)	• Insecticidal activity of CSE was assested aginst two crops devastating insects *Spodoptera littoralis* and *Tribolium confusum.* • CSE showed high toxicity that was proven histologically by the dissection of insects gut.	**[46]**

C. parqui L'Hér	Crude saponin extract (CSE)	• Larvicidal activity of CSE was tested against *Culex pipens* the vector of Nile west virus. • The LC_{50} values were 100 and 111 ppm after 24 and 48 hr, respectively of treatment.	**[49]**
C. parqui L'Hér	Butanol fraction (saponin rich)	• Saponin rich fraction excerted antieffedant effect on adult and different larval satges of *Hylurgus ligniperda* leading to nutritive deficiency and significant weight loss inhibiting the evolution to next stage or cause death.	**[50]**

3.1. Cytoyoxic activity

Regarding the biological activities of *Cestrum* saponins, the most prominent pharmacological effect of the pure isolated compounds was the cytotoxic activity **[31]**. Cell death is triggered via stimulation of apoptosis, autophagy and phagocytosis, as well as inhibition of metastatisis process of the tested cells and their angiogenesis **[32]**. Fourteen compounds were assested for cytotoxic activity over different cell lines, HCT-116, OVCAR-8, HL-60, SF-295, Hela, HepG2, U87 and MCF7. Nine saponins exhibited from weak to high activity, while five saponins were completely inactive. Investigating the chemical structures of those compounds showed that spirostanol structure is essential for cytotoxic activity where the loss of ring F in compounds **(13-16, 35, 36)** led to complete loss of activity. This is in accordance with the data reported previously **[33, 34]**. Furthermore, it was observed that forcompounds **(9-12, 17, 18)**, having the same sapogenin, the presence of a trisacharide side chain enhanced the cytotoxicity when copared to the monosacharide unit. The trisacharide chain formed of galactose increased the activity more than that of rhamnose .

3.2. Anti-bacterial Activity

C. elegance Brongn. isolated saponins showed a moderate antibacterial activity against both Gm + ve and Gm – ve bacteria, while other tested saponins and saponin crude extract did not exhibit any effect. It can be explained that a large concentrtaion of saponin is required to interact with bacterial membrane, mainly the lipopolysaccharide layer in Gm –ve bacteria, forming pores and reducing the bacterial growth. It is postulated that these pores may facilitate the penetration of larger molecules of antibiotics **[35, 36]**.

3.3. Anti-fungal Activity

Steroidal saponins exhibited a strong anti-mould activity as they interact with ergosterol in fungi membrane, leading to pore formation in membrane lipid bilayer and thus its deformation, that consequently results in cell death **[37]**. As previously mentioned, furostanol saponins **(13-16)** are inactive. Saponins with one sugar residue **(17, 18)** showed a weaker activity in comparison with their di- or tri-sacharide analogues. Ahmed et al. reported that hydrolysis of a crude saponin extract led to loss of the anti-fungal activity emphasiing the importance of sugar side chain **[38]** . Oleanae saponin **(6)** exhibited very potent effect even more than the standard drug used **[21]**.

3.4. Spermicidal activity

The charecteristic interaction between saponins and cell membrane also explains the spermicidal activity *C. parqui* L'Hér raw saponin, where a clear damage in human sperm head was observed microscopically. This effect is time and dose dependant **[39]**.

3.5. Anti-inflammatory activity

Steroidal saponins are well known for their anti-inflammatory effect **[40-42]**. In Genus *Cestrum*, only compounds **(7, 8)** were tested and showed anti-inflammatory and analgesic effect **[43]**. It is worth mentioning that both compounds are spirostanols.

3.6. Pesticidal Activity

Using plants as natural pesticidal agents is strongly encouraged as they are safer than synthetic pesticides, cheaper and more environment friendly **[44]**. In traditional medicine, plants have been long used as insecticide, larvicide and molluscicide to compete crops destructive pests and vector-transmiting pathogens harmeful to humans and animals **[45]**. Crude saponin extract of *C. parqui* L'Hér effectively killed *Spodoptera littoralis*, an insect that destroies cotton crops, either by injection or oral ingestion. Dissection of treated insects revealed necrosis at the site of CSE adminstration **[46]**. In the insecticidal test applied on *Tribolium confusum*, a control group administrated CSE mixed with cholesterol where mortality rate decreased significantly showing the antagonistic effect between steroidal saponins and cholesterol **[46]** and this was also reported before **[38]**. *Theba pisana* is a phytophagous mollusc that attacks plants like asparagus, artichoke, bean, alfalfa, beet and vine causing great economic loss. It was reported that CSE of *C. parqui* is more toxic than that of *Quillaja saponaria* **[47]**. It is supposed that saponins cause excessive dehydration of snail's body. Mosquito-borne disease is a massive global health burden, with almost 350 million people infected with mosquito-borne diseases across the world in 2021. Vector "mosquito" control is cosidered the best way to prevent these diseases **[48]**. *Culex pipens* is the vector mosquito for Nile west virus infecting humans causing meneigitis and encephalitis. CSE of *C. parqui* L'Hér showed a strong larvicidal activity against *Culex pipens* offering a safer and more active solution, to compete this pest, rather than synthetic insecticidal agents which the mosquitoe acquired resistance against them **[49]**. It also exhibited a cidal effect against various larval stages of *Hylurgus ligniperda* that infect pine logs exported from Chile causing great economic loss, and thus offers a safer and more environment friendly alternative for methyl bromide fumigation **[50]**.

Conclusion

Genus *Cestrum* has long been used in traditional medicine for many purposes. Phytochemical studies on plants of the genus revealed that they are rich in steroidal saponins which are responsible for diverse pharmacological activities such as cytotoxic, pesticidal, anti-inflammatory, spermicidal..etc. Genetic studies highlighted a group of important enzymes involved in the biosynthesis of saponins and emphasized the role of environmental factors regulating the biosyntheic pathway. The advancement in synthetic biotechnology tools allowed scientists to control the production of secondary metabolites, targeting the synthesis of more valuable molecules that can be used as drug precurssors.

Abbreviations

HCT-116, Human colon carcinoma; OVCAR-8, Human ovarian carcinoma; HL60, Human leukemia; SF-295, Human glioblastoma; Hela, Human papilloma virus; HepG2, Human hepatocyte carcinoma; U87, Human primary glioblastoma MCF7, Human breast cancer; UDPGlc, Uridine diphosphate glucose.

Declarations

Ethics approval and consent of participation

Not applicable

Consent of publication

Not applicable

Data and materials availability

All data produced or analyzed throughout this study are included in the current manuscript.

Competing interests

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4. References

1. J. N. Fregonezi, T. Fernandes, J. M. D. Torezan, A. O. S. Vieira, A. L. L. Vanzela. Karyotype differentiation of four Cestrum species (Solanaceae) based on the physical mapping of repetitive DNA. Genet.Mol. biol. 2006; 29: 97-104
2. Montero-Castro JC, Delgado-Salinas A, De Luna E, Eguiarte LE. Phylogenetic analysis of Cestrum section Habrothamnus (Solanaceae) based on plastid and nuclear DNA sequences. Syst. Bot.2006; 31: 843-850
3. M. M. El-Demerdash, A. S. El-Sayed, N. M. Georg, A. Abou-Elnour, H. Nosier.Biosystematic studies of some Egyptian species of Cestrum (Solanaceae). Mol. Biol. Rep. 2021; 45: 4497-4515
4. R. W. Bussmann, D. Sharon, 'Traditional medicinal plant use in Loja province, Southern Ecuador. J. Ethnobiol. Ethnomed. 2006; 2: 44-50
5. R. W. Bussmann, D. Sharon.Traditional medicinal plant use in Northern Peru: tracking two thousand years of healing culture. J. Ethnobiol. Ethnomed. 2006; 2: 47-53
6. Srikanta, S. H. Nagarajappa, G. Viswanatha, M. Handral, R. Subbanna, R. Srinath, G. Hiremath. Anti-osteoporotic activity of methanolic extract of an Indian herbal formula NR/CAL/06 in ovariectomized rats. Zhong Xi Yi Jie He Xue Bao 2011; 9:1125-32
7. U. Saleem, N. Ali, B. Ahmad. Protective and curative effects of Cestrum nocturnum on rabbit kidney. Bangladesh J. Pharmacol. 2017; 12: 284-91
8. M. A. Khan, H. Inayat, H. Khan, M. Saeed, I. Khan, I. Rahman. Antimicrobial activities of the whole plant of Cestrum nocturnum against pathogenic microorganisms. Afr. J. Microbiol. Res. 2011; 5: 612-616
9. R. W. Bussmann, D. Sharon. Medicinal plants of the Andes and the Amazon-The magic and medicinal flora of Northern Peru. Ethnobot. Res. Appl. 2016; 15: 1-29
10. D. Shehnaz, F. Hamid, F. T. Baqai, V. Uddin Ahmad. Effect of the crude extract of Cestrum parqui on carrageenin-induced rat paw oedema and aggregation of human blood platelets. Phytother. Res. 1999; 13: 445-47
11. J. B. Alcorn. Huastec Mayan Ethnobotany. Austin. University of Texas Press. Annals of the Missouri Botanical Garden 1984; 82:34-46.
12. D. Estomba, A. Ladio, M. Lozada. Medicinal Plants used by a Mapuche Community near Junin de los Andes, Neuquén. Bol. Latinoam. Caribe Plantas 2005; 4:107-112
13. A. S. Begum, M. Goyal. Research and medicinal potential of the genus Cestrum (Solanaceae)-A review. Pharmacon. Rev. 2007; 1: 320-32
14. A. Singab, D. Bahgat, E. Al-Sayed, O. Eldahshan. Saponins from genus Albizia: phytochemical and biological review. Med Aromat Plants S 2015; 3, 2167-0412.

15. A. Rao, D. Gurfinkel. The bioactivity of saponins: triterpenoid and steroidal glycosides. Drug met. drug interact. 2000; 1 : 211-36

16. R. E. Hoagland, R. M. Zablotowicz, K. N. Reddy, in Saponins Used in Food and Agriculture, Springer, 1996: 57-73

17. A. Szakiel, C. Pączkowski, M. Henry, Influence of environmental biotic factors on the content of saponins in plants. Phytochem.Rev. 2011; 10, 493-502

18. J.P. Vincken, L. Heng, A. de Groot, H. Gruppen. Saponins, classification and occurrence in the plant kingdom. Phytochemistry 2007; 68, 275-297.

19. I. Podolak, A. Galanty, D. Sobolewska. Saponins as cytotoxic agents: a review. Phytoch. Rev. 2010; 9, 425-474.

20. H. Inayat, I. Khan, V. U. Ahmad, M. Rani, M. A. Khan. Isolation And Structure Elucidation of a New Oleanane Type Glycoside From The Aerial Portion Of Cestrum Nocturnum. Bull Chem Soc Ethiop 2020; 34 : 141-48

21. S. M. Nasr, N. M. Elwan, M. Abdel-Motagaly, A.-W. A. Abdel-Aziz, M. Ghareeb. Phytochemical investigation and differential effects of Cestrum elegans isolated compounds as antimicrobial and virucidal against hepatitis A virus. Egypt. J. Chem. 2021; 64:3729-3738

22. J. M. Augustin, V. Kuzina, S. B. Andersen, S. Bak. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry 2011; 72, 435-457.

23. V. L. Challinor, J. J. De Voss. Open-chain steroidal glycosides, a diverse class of plant saponins. Nat. prod. rep. 2013; 30: 429-54

24. N. P. Sahu, S. Banerjee, N. B. Mondal, D. Mandal. Steroidal Saponins. In The Chemistry of Organic Natural Products Springer Vienna, 2008, 45-141

25. T. Iguchi, N. Takahashi, Y. Mimaki. A Total of Eight Novel Steroidal Glycosides Based on Spirostan, Furostan, Pseudofurostan, and Cholestane from the Leaves of Cestrum newellii. Molecules 2020; 25, 44-62.

26. A. Osbourn, R. J. Goss, R. A. Field. The saponins–polar isoprenoids with important and diverse biological activities. Nat. prod. rep. 2011; 28: 1261-68

27. M. A. Schuler, 'Plant cytochrome P450 monooxygenases.Crit rev plant sci 1996; 15: 235-84

28. P. Agrawal, D. Jain, R. Gupta, R. Thakur. Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry 1985; 24, 2479-2496.

29. S. Upadhyay, G. S. Jeena, R. K. Shukla. Recent advances in steroidal saponins biosynthesis and in vitro production. Planta 2018; 248, 519-544.

30. E. O. Fukushima, H. Seki, S. Sawai, M. Suzuki, K. Ohyama, K. Saito, T. Muranaka. Combinatorial biosynthesis of legume natural and rare triterpenoids in engineered yeast. Crit Rev Plant Sci 1996; 15: 235-84.

31. E. Al-Sayed, O. A. Eldahshan, D. M. Bahgat, A. N. B. Singab. Cytotoxic Oleanane-Type Saponins from the Leaves of Albizia anthelmintica Brongn. Chem. Biodivers.2006;13:1666-1673

32. D. Sobolewska, A. Galanty, K. Grabowska, J. Makowska-Wąs, D. Wróbel-Biedrawa, I. Podolak. Saponins as cytotoxic agents: an update (2010–2018). Part I—steroidal saponins. Phytochem. Rev. 2020; 19: 139-89

33. E. Beit-Yannai, S. Ben-Shabat, N.

Goldschmidt, B. P. Chapagain, R. H. Liu, Z. Wiesman. Antiproliferative activity of steroidal saponins from Balanites aegyptiaca—an in vitro study. Phytochem. Lett. 2011; 4: 43-47

34. X. Wu, N.-H. Chen, Y.-B. Zhang, G.-C. Wang, Y.-F. Feng, Y.-L. Li. A New Steroid Saponin from the Rhizomes of Paris polyphylla var. yunnanensis. Chem. Nat. Comp. 2017; 53: 93-98
35. M. Arabski, S. Wąsik, K. Dworecki, W. Kaca. Laser interferometric and cultivation methods for measurement of colistin/ampicilin and saponin interactions with smooth and rough of Proteus mirabilis lipopolysaccharides and cells. J. Microbiol. Methods 2009; 77:178-183
36. M. Karabaliev, V. Kochev. Interaction of solid supported thin lipid films with saponin. Sens. Actuators B Chem. 2003;88:101-105
37. S. Sparg, M. Light, J. Van Staden. Biological activities and distribution of plant saponins. J ethnopharmacol 2004; 94: 219-43
38. D. B. Ahmed, I. Chaieb, K. B. Salah, H. Boukamcha, H. B. Jannet, Z. Mighri, M. Daami-Remadi, 'Antibacterial and antifungal activities of Cestrum parqui saponins: possible interaction with membrane sterols. Int. J. Plant Sci. 2012; 3: 1-7
39. K. Souad, S. Ali, A. Mounir, T. M. Mounir. Spermicidal activity of extract from Cestrum parqui. Contraception 2006; 75, 152-156.
40. M. S. Mohammed, W. J. Osman, E. A. Garelnabi, Z. Osman, B. Osman, H. S. Khalid, M. A. Mohamed. Secondary metabolites as anti-inflammatory agents. J Phytopharmacol 2014; 3, 275-285
41. S. S. Patel, J. K. Savjani. Systematic review of plant steroids as potential antiinflammatory agents: Current status and future perspectives. J Phytopharmacol 2015; 4: 121-25
42. G. Yuan, M. L. Wahlqvist, G. He, M. Yang, D. Li. Natural products and anti-inflammatory activity. Asia Pac. J. Clin. Nutr. 2006; 15: 143-52
43. M. Kawano, M. Otsuka, K. Umeyama, M. Yamazaki, T. Shiota, M. Satake, E. Okuyama. Anti-inflammatory and analgesic components from "hierba santa," a traditional medicine in Peru. J. Nat. Med. 2009; 63: 147-58
44. D. M. Bahgat, H. S. Mossalem, E. Al-Sayed, O. A. Eldahshan, A. N. B Singab, H. M Abu El Einin. Influence of saponin fraction from Albizia anthelmintica on Biomphalaria alexandrina snail; the intermediate host of Schistosoma mansoni in Egypt. Egypt. J. Aquat. Biol. Fish. 2018; 22:231-240
45. S. K. Okwute. Plants as potential sources of pesticidal agents: a review. In Pesticides–Advances in chemical and botanical pesticides: Intech Janiza Trdine, 2012, 207-32
46. R. Najet, B. A. Dorsaf, D. Mejda, B. H. M. Habib. Pesticidal potentialities of Cestrum parqui saponins. Int. J. Agric. Res. 2007; 2: 275-81
47. I. Chaieb, W. Tayeb. Comparison of the molluscicidal activity of Cestrum parqui (Solanaceae) and Quillaja saponaria (Quillajaceae) saponins. Tuni. J. Med. Plants Nat. Prod 2009, 2, 31-35
48. W. H. Organization, UNICEF, 'Global vector control response 2017-2030', 2017
49. I. Chaieb, A. Ben Hamouda, M. Trabelsi, M. Ben Halima, M. Ben Hamouda.Toxicity investigation of Cestrum parqui saponins to Culex pipiens larvae. Pest Tech 2009;3: 73-75

50. C. Huanquilef, J. Espinoza, A. Mutis, L. Bardehle, E. Hormazábal, A. Urzúa, A. Quiroz. Antifeedant Activities of Organic Fractions from Cestrum parqui Leaves on the Red-Haired Bark Beetle Hylurgus ligniperda. J. Soil Sci. Plant Nutr.2021; 21:13-21

51. M. A. Fouad, K. M. Mohamed, M. S. Kamel, K. Matsunami, H. Otsuka, Cesdiurins I–III, steroidal saponins from Cestrum diurnum L. J. Nat. Med. 2008; 62: 168-73

52. P. R. Ribeiro, R. Braz-Filho, A. J. Araújo, L. V. Costa-Lotufo, L. G. Souza, H. V. Nobre Junior, C. R. d. Silva, J. B. d. Andrade Neto, E. R. Silveira, M. A. S. Lima. New Epimeric Spirostanol and Furostanol-Type Steroidal Saponins from Cestrum laevigatum L. J Braz Chem Soc 2016; 27: 2170-2180

53. V. U. Ahmad, F. T. Baqai, I. Fatima, R. Ahmad. A spirostanol glycoside from Cestrum nocturnum. Phytochemistry 1991, 30, 3057-3061.

54. V. U. Ahmad, F. T. Baqai, R. Ahmad. A diosgenin tetrasaccharide from Cestrum nocturnum. Z. Naturforsch. B 1995; 50: 1104-10.

55. R. R. Mosad, M. H. Ali, M. T. Ibrahim, H. M. Shaaban, M. Emara, A. E. Wahba. New cytotoxic steroidal saponins from Cestrum parqui. Phytochem. Lett. 2017; 22: 167-173

56. E. Galarraga, A. C. Mitaine-Offer, J. M. Amaro-Luis, T. Miyamoto, C. Tanaka, L. Pouységu, S. Quideau, L. B. Rojas, M.-A. Lacaille-Dubois. Steroidal saponins from the fruits of Cestrum ruizteranianum. Nat. Prod. Commun. 2011; 6: 1825-1836

57. C. A. K. Ta, J. A. Guerrero-Analco, E. Roberts, R. Liu, C. D. Mogg, A. Saleem, M. Otárola-Rojas, L. Poveda, P. Sanchez-Vindas, V. Cal. Antifungal saponins from the maya medicinal plant Cestrum schlechtendahlii G. Don (Solanaceae). Phytother Res 2016; 30: 439-46

58. M. Haraguchi, M. Motidome, H. Morita, K. Takeya, H. Itokawa, Y. Mimaki, Y. Sashida. New polyhydroxylated steroidal sapogenin and saponin from the leaves of Cestrum sendtenerianum. Chem. Pharm. Bull 1999; 47: 582-84.

59. M. Haraguchi, Y. Mimaki, M. Motidome, H. Morita, K. Takeya, H. Itokawa, A. Yokosuka, Y. Sashida. Steroidal saponins from the leaves of Cestrum sendtenerianum. Phytochemistry 2000, 55, 715-720