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Saponins: Extraction, bio-medicinal properties and way forward to anti-viral representatives

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ABSTRACT

Medicinal or herbal plants are widely used for their many favourable properties and are generally safe without any side effects. Saponins are sugar conjugated natural compounds which possess a multitude of biological activities such as medicinal properties, antimicrobial activity, antiviral activity, etc. Saponin production is a part of the normal growth and development process in a lot of plants and plant extracts such as liquorice and ginseng which are exploited as potential drug sources. Herbal compounds have shown a great potential against a wide variety of infectious agents, including viruses such as the SARS-CoV; these are all-natural products and do not show any adverse side effects. This article reviews the various aspects of saponin biosynthesis and extraction, the need for their integration into more mainstream medicinal therapies and how they could be potentially useful in treating viral diseases such as COVID-19, HIV, HSV, rotavirus etc. The literature presents a close review on the saponin efficacy in targeting mentioned viral diseases that occupy a high mortality rate worldwide. This manuscript indicates the role of saponins as a source of dynamic plant based anti-viral remedies and their various methods for extraction from different sources.

1. Introduction

Around 8% people are admitted to hospitals as a result of side effects or reactions to synthetic drugs in the United States of America and as much as 100,000 people die every year due to the toxicities caused by these drugs (Oleszek, 2002). On the other hand, the cases of side effects or reactions due to herbs or herbal drugs are very rare and are not even categorized in databases of the National Poison Control Centre of the United States (Hostettmann and Marston, 1995; Oleszek, 2002).

The term "saponin" finds its origin in the Latin word 'Sapo' which means soap as saponin molecules, upon being shaken with water, form soap like foams (Oleszek, 2002). Saponins can interact with plasma membranes due to their lyobipolar property which, for aqueous solutions, results in a decrease in the surface tension (Melzig et al., 2001). Saponins are synthesized in a variety of plant species as secondary metabolites. In plant cells, saponins accumulates as precursors in their non-functional form. On encountering a pathogen, saponins are transformed into active antibiotics with the action of plant enzymes. The three classes of saponins are 1) Triterpenoid, 2) Steroid, 3) Glycoalkaloids all have different chemical structure and bio medicinal properties (Mert-Türk, 2006).

Lately, there has been a growing trend seen in people to move towards plant-based remedies as these are considered to be free from side effects (Van Dyck et al., 2010). Synthetic drugs, essentially, only minimize the symptoms caused by specific diseases, whereas herbal drugs allow the body to generate a healing process of its own (Liu et al., 2008;

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Vincken et al., 2007). The herbal mechanisms of action are not yet fully established. Several phytochemicals are present in plants such as alkaloids, tannins, carbohydrates, proteins and saponins. These phytochemicals can potentially carry a lot of medicinal properties and may be one of the underlying factors on which herbal medicines and therapies are based. Most of these plants extracts, including saponins, are found to exhibit antioxidant (Augustin et al., 2011; Francis et al., 2002; Sparg et al., 2004; Wina et al., 2005), anti-cancer (Kwon et al., 2003; Vongsangnak et al., 2004;. Wu, Lin and Chau, 2001) and antiviral properties (Apers et al., 2001; Gosse et al., 2002) among others. Glycyrrhiza is a plant expressing powerful medicinal properties due to production of triterpene saponins as their secondary metabolites. Triperpene saponins have key biological activities like anti-inflammatory, anti-viral, anti-tumor (Li et al., 2020). The investigated pharmaceutical and bio-medicinal importance of saponins has molded the research towards extraction of saponins from different plant species (Faizal and Geelen, 2013). The extraction of saponins for treating virus specific diseases is an emerging field with potential results. The present study reveals the bio-medicinal aspects of saponins with special emphasis on viral diseases and how saponins can combat against those virus dependent diseases. The study aimed at summarizing the anti-viral, bio-medicinal importance of different saponin compounds and its methods of extractions.

2. Saponins and their biosynthesis

Saponins are chemically described as glycosides of steroids and triterpenes. They are made up of nonpolar aglycones attached to varying numbers of monosaccharide moieties (Oleszek, 2002). The soap-like behaviour of saponin in water is due to their molecules possessing combinations of non-polar and polar structural moieties. Saponins, a surface-active and non-volatile in nature, are found to occur naturally across a variety of organisms; however, they are most extensively present in the plant kingdom (Hostettmann and Marston, 1995). Mostly identified saponins are secondary metabolites derived from plants, however, they can also be found in starfish (Asteroidea) (Liu et al., 2008) and sea cucumbers (Holothuroidea) (Van Dyck et al., 2010), among other marine animals. Magnoliophyta, which includes both dicotyledons and monocotyledons are capable of synthesizing saponins in which dicotyledons are generally their major producers. Saponins are produced in plants as a sort of defense mechanism due to their fungicidal, molluscicidal, antimicrobial and allelopathic properties (Augustin et al., 2011; Francis et al., 2002; Sparg et al., 2004). Saponins are also present in plant tissues that are less prone to bacterial attack, insect predation, or fungal attack (Wina et al., 2005), providing an account of the occurrence of saponins in plant kingdom (Vincken et al., 2007). Apart from plants, fungi such as *Penicillium brasilianum* is also the source of the saponins (J. Zhang et al., 2018).

Saponins are classified into two classes, known as steroid glycosides and triterpenoids. This classification is done on the basis of structural features of where the sugar units are attached (Hostettmann and Marston, 1995). The glycosides of cucurbitacins and steroidal alkaloids are also included in the chemical classification of saponins (Vincken et al., 2007) (Fig. 1). Sugar moieties, with for example, glucuronic acid, glucose, galactose, rhamnose, xylose or methylpentose are linked to a hydrophobic aglycone via a glycosidic bond, giving rise to either a triterpenoid or a steroid (Haralampidis et al., 2002). The two classes of saponins are basically differentiated based on the fact that in the case of steroidal saponins, three methyl groups are removed from the 30 carbon atom-containing precursor molecule, oxidosqualene, leaving behind a 27 C atom molecule; while in triterpenoids all 30 C atoms remain (Abe et al., 2004; Sparg et al., 2004). Glycosteroid alkaloids are amongst the few other classes of compounds that have also been categorized as saponins (Haralampidis et al., 2002). Plant steroidal saponins form two core structures known as: spirostan (16β, 22:22α, 26-diepoxy-cholestan) and furostan (16β, 22-epoxycholestan) (Vincken et al., 2007) (Fig. 1). Naturally occurring saponins are mostly made up of (25*R*)-spirostan derivatives, (25*S*)-spirostan derivatives and (25*R*)- and (25*S*)-furostan derivatives (Hostettmann and Marston, 1995). Similarly, triterpenoidal saponins have tetracyclic dammaran and pentacyclicoleanans as their core structures (Fig. 1). Hopan, ursan and lupan are some other aglycones of triterpenoid saponins (Thakur et al., 2011).

There are lots of plants and plant materials from which saponins are extracted and used for several purposes. Few of the most widely studied among these are, Ginseng (Kwon et al., 2003; J. Wu et al., 2001; Y. Zhang, Liu, Qi, Li and Wang, 2013), Alfafa (Van Atta, Guggolz and Thompson, 1961), soymilk (Lai et al., 2013), sugarbeet (Ridout et al., 1994), soy and chickpea (Serventi et al., 2013), asparagus (Vázquez-Castilla et al., 2013), and plum fruits (Yoon and Wrolstad, 1984). Saponin accumulation by plants is an important part of their normal developmental process. Environmental factors like availability of water, light and other nutrients, or combined effects of these are known to influence this accumulation (Szakiel et al., 2011). Varying distributions of saponins has been found during seasonal fluctuations or ontogenesis in tissues or organs of different plants. The variations observed in these distributions depend on the requirements of the particular species such as targeting their respective pests and other herbivores who feed on them. According to Ndamba et al. (1994), in *Phytolaccadodecandra* (soapberry), the accumulation of saponins occurs early on to prevent loss of fruits and assist the maturation process of seeds (Ndamba et al., 1994). Lin et al. (2009) explained that *Dioscoreapseudojaponica* Yamamoto (yam) mostly accumulates saponins in their tubers so as to protect these reproductive organs (Lin, Chen, Liu and Yang, 2009). Papadopoulou et al. (1999) stated that *Avenae spp.* (oats) also accumulate saponins to counteract soil-borne fungi in the epidermis of their roots (Papadopoulou et al., 1999).

Enhancers like derivatives of jasmonate or yeast extracts have been found to increase the level of saponins (Yendo et al., 2010). Jasmonates also activate defense responses in plants against herbivores and therefore, this further validates the hypothesis of involvement of saponins in plant defense mechanisms (Howe and Jander, 2008). The process of accumulation or biosynthesis of saponins in plants has been broken down in four different steps 1) Formation of 2,3-oxidosqualene from acetyl-CoA (nonspecific synthesis along with phytosterols), 2)

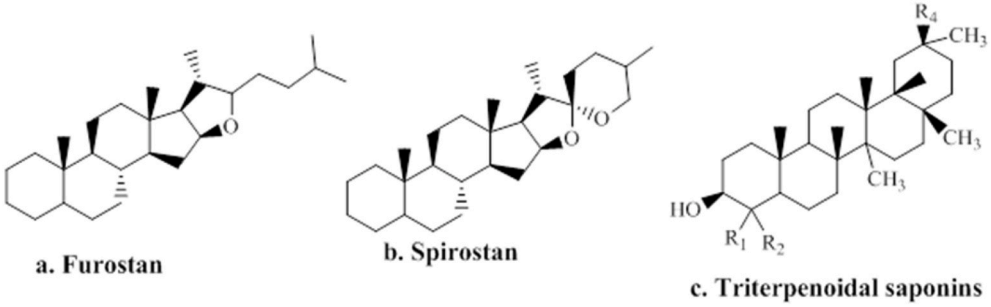


Fig. 1. Types of saponins: (a) Furostan (b) Spirostan and (c) Triterpenoidal saponin.

Cyclization of 2,3-oxidosqualene (saponin variety), 3) Decoration of the cyclization product (elaboration of sapogenin heterogeneity) and 4) sapogenin glycosylation (bestowing respective biological activity).

Azmir et al. (2013) and Wang and Weller (2006) have reviewed the advancements made in the extraction processes of active metabolites from plant materials (Azmir et al., 2013; Wang and Weller, 2006). The advancements result from higher consumption of plant-derived extracts, due to growing public interest and awareness about positive impacts of herbal and natural products in health care. Generally, the methods for extraction of saponins are broadly divided into two categories: conventional methods and green technologies. Green technologies include methods like Microwave-assisted extraction, accelerated solvent extraction and Ultrasound-assisted extraction, whereas the conventional extraction processes are Soxhlet, Reflux extraction and Maceration (Heng et al., 2013) (Table 1).

3. Biomedicinal properties

Plant extracts containing saponins are widely used in medical or pharmaceutical sectors. They are also considered important constituents of various traditional folk drugs such as an extract of Panax sp. (ginseng) or Glycyrrhiza (liquorice). Clinical studies have not yet been able to confirm the nature of the effects of these herbal extracts (Asl and Hosseinzadeh, 2008; Xiang et al., 2008). The consumption of saponins has been shown to have positive effects on plasma cholesterol in different animal models but similar studies in humans have failed to produce any significant inferences in this regard (Milgate and Roberts, 1995). Apart from them possessing various pharmaceutical properties such as immunological adjuvant activities (Estrada et al., 2000; Sun et al., 2012; Verza et al., 2012), hemolytic activities (Hassan et al., 2010; Sun et al., 2011) and antioxidant properties (Chan et al., 2013; Dini et al., 2009), saponins are also shown to demonstrate anti-cancer effects (T.-C. Cheng et al., 2011; Man et al., 2010; Waheed et al., 2012) and thus attract a lot of attention as potential targets for drug discovery and development (Table 2, Fig. 2).

4. Antimicrobial properties of saponins

The antimicrobial properties, playing both beneficial as well as detrimental roles in human health and welfare, include antibacterial and antifungal effects (Haralampidis et al., 2002) (Fig. 3).

Table 1
Methods for saponin extraction and purification.

Methods	Extraction/ Purification	Source	Saponin extract	Reference
HPLC	Purification	*Bupleurum falcatum*	Saikosaponin c, a and d	Park et al. (2000)
HPLC and TLC	Purification	Alfalfa (*Medicago sativa L*)	Tritepene glycosides (alfalfa saponin)	Tava & Odoardi (1996)
Alkali and Acid solution	Extraction	*Camellia oleifera*	Total saponins	(Y. Liu, Li, Xu and Han, 2016)
Microwave assisted solvent extraction (MASE)	Extraction	Safed musli (*Chlorophytum borivilianum*)	Total saponin	Deore et al. (2015)
Macreation	Extraction	*Quillaja Saponaria*	Triterpene	Sarkhel (2016)
Soxhlet	Extraction	*Tribulus terrestris*	Steroidal saponin	Sarvin et al. (2018)
Heat reflux	Extraction	*Panax notoginseng*	Notoginseng saponin	Vongsangnak et al. (2004)
Ultrasound assisted extraction	Extraction	*Tribulus terrestris*	Steroidal saponin	Sarvin et al. (2018)

Table 2
Plant extracted saponins with their medicinal properties.

Plant	Saponin	Properties	Reference
Glycyrrhiza uralensis	Licorice saponin A3 (985.1 g/mol) (Fig. 2)	Anti-cancer	Xue et al. (2012)
Panax ginseng (roots)	Ginsenoside Ro (957.1 g/mol) (Fig. 2)	Remedy for weakness and diabetes mellitus, decreases plasma triacylglycerol levels 3	(Hu, 1977; Masuno et al., 1996)
Aralia elata	Aralia-saponin II (945.1 g/mol) (Fig. 2)	Anti-tumor	(Li et al., 2015; Y. Sun et al., 2017)
Soybeans	Soyasaponin I (943.1 g/mol) (Fig. 2)	Anti-carcinogenic, plasma cholesterol lowering, anti-viral, hepatoprotective, antioxidant and anti-mutagenic	(Lee et al., 2005; Xiao et al., 2007; W. Zhang and Popovich, 2008)
Dipsacus asper	Akebia saponin D (929.1 g/mol) (Fig. 2)	To prevent neurological disorder such as Alzheimer's disease	(T. Zhou, Deng and Qiu, 2012)
Kalopanax Pictus (stem bark)	alpha-Hederin (751 g/mol)	Anti-inflammatory	Da et al. (2003)
Bolbostemma paniculatum	Tubeimoside I (1347.5 g/mol)	Anti-inflammatory, antitumor	Liang et al. (2007)
Phytolacca americana	Phytolaccoside (827 g/mol)	Enhances absorption of heparin and heparin disaccharide	Cho et al. (2003)
Centella asiatica L.	Asiaticoside (959.1 g/mol)	Enhances wound repair	Kimura et al. (2008)
Phytolacca esculenta (roots)	Esculentoside L (973.1 g/mol) (Fig. 2)	Treatment of edema, bronchitis, and tumors	Yang-Hua (1990)
P. fruticosa (leaves)	3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester	It possesses α-Amylase (27.1 ± 2.05 μg/mL) and α-Glucosidase (440.5 ± 12.7 μg/mL) inhibitory activity.	Hanh et al. (2016)

5. Antibacterial & antifungal properties

Saponin mediated cell lyses occurs at saponin-cholesterol rich domains. The surface activity of saponin enables it to penetrate into the lipid bilayer where it binds to cholesterol (Baumann et al., 2000) (Fig. 3). A lack of cholesterol in the membranes will prevent formation of such pores by saponins (Segal et al., 1974). Saponin disturbs the permeability of outer membrane of bacterial cells. In 90% of Gram-negative bacteria, the outer layers of the cell envelope are covered by lipopolysaccharides (LPS). It is thought that perhaps the cell permeability increases due to saponin-Lipid A interaction, which allows the uptake of antibiotics (colistin, ampicillin) even in resistant bacterial cells thereby causing their death (Arabski et al., 2009).

Saponin also possesses potential antifungal property (Mary et al., 1986) (Fig. 3). It forms a complex with sterols present in fungal membranes leading to formation of pores and loss of membrane integrity (Morrissey and Osbourn, 1999). A study based on visualization of the membranes using electron microscopy showed formation of lesions after the fungi were treated with saponins (Seeman et al., 1973). This permeabilization affects the fluidity of membranes and makes for an effective antifungal agent (Armah et al., 1999).

18-β-glycyrrhetinic acid, a triterpenoid saponin obtained from the *Glycyrrhiza uralensis* Fischer, has shown antibiotic effect towards *S. aureus*. It decreases the expression of SaeR and Hla, crucial genes causing virulence, of methicillin-resistant *Staphylococcus aureus* (MRSA). This compound also acts like a TH1-immunological adjuvant, thus

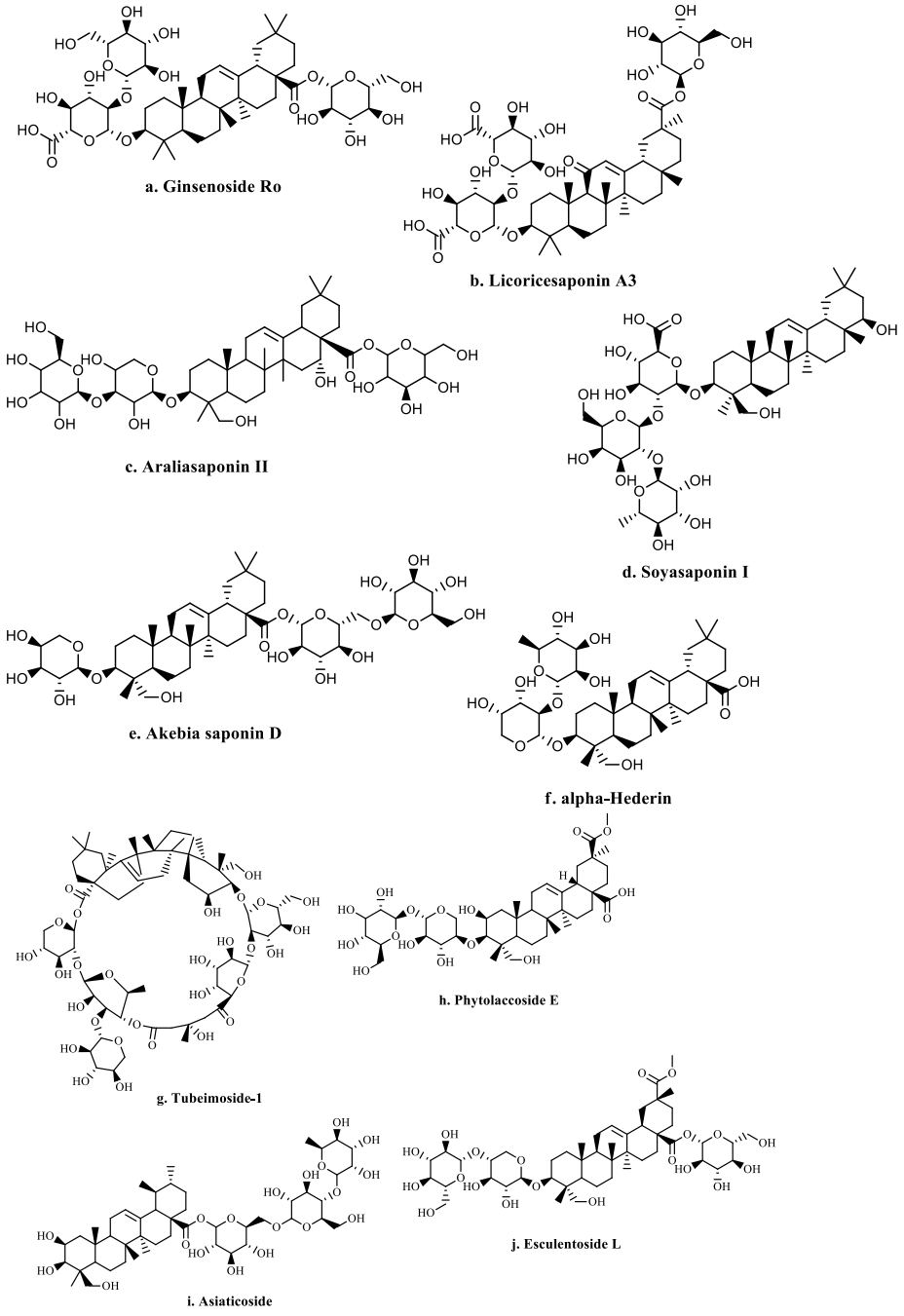


Fig. 2. Plant extracted saponins which display bio-medicinal properties.

showing antimicrobial activity against *C. albicans* (- Kim, Joo, Kim and Han, 2013). Glabridin prevents yeast-hyphal transition to express antibacterial activity against *C. albicans* (Messier and Grenier, 2011). *Lichochalcone E* reduces the production of α-toxin to kill *S. aureus* (- Zhou et al., 2012). *Liquiritigenin* decreases the production of α-hemolysin, displaying antibacterial tendency against *S. aureus* (Dai et al., 2013). *Licochalcone A* inhibits formation of biofilms and transition of yeast hyphae to manifest antibacterial action against *C. albicans* (Messier and Grenier, 2011).

6. Antiviral properties

Antiviral agents inhibit or block replication of viruses by interfering with the process of attachment of the virus to cells and delaying its genomic replication by interfering with viral enzymes. An effective antiviral compound essentially needs to target only the viral process and have no effect on the ongoing cellular process during viral replication. As a result, the virus will not be able to reach its receptor, which are the functional host cellular receptors commandeered by the virus. Antiviral compounds do not interfere with the normal function of uninfected cells; hence they are effective and safe. Interaction with viral envelope proteins and its destruction, preventing binding of the virus to the host cells by damaging the virus binding sites, and coating of cells are some of the mechanisms by which saponins exert their antiviral action (Amoros et al., 1987; Apers et al., 2001; Gosse et al., 2002; Verma and Raychaudhuri, 1970).

Amoros et al. (1987) found that the triterpene saponin extracted from *Anagallis arvensis* shows a potential inhibitory effect on poliovirus

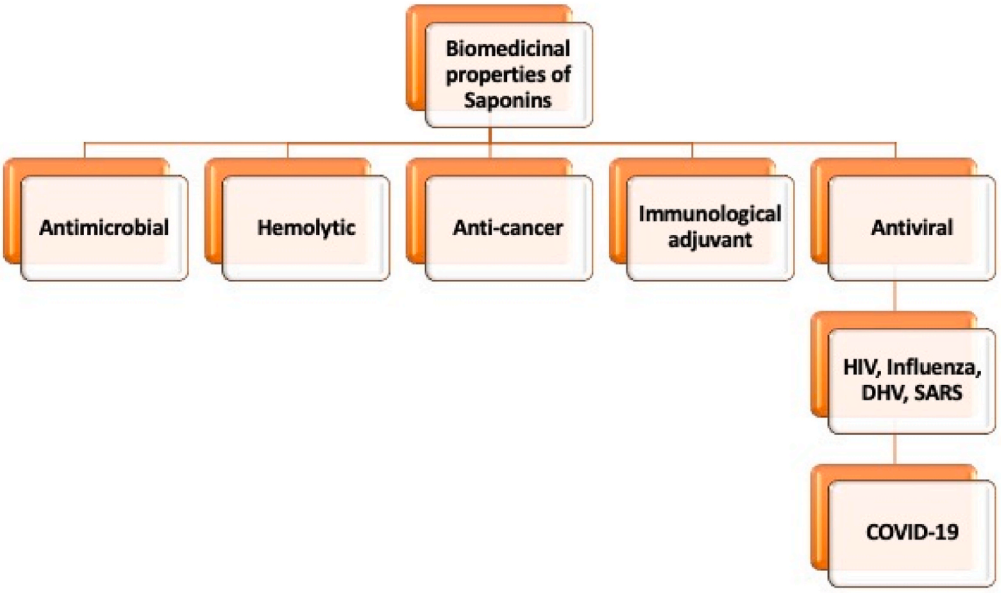


Fig. 3. Biomedicinal properties of saponins.

type 2 and herpes simplex virus (HSV) type 1 by reducing the viral yield (Fig. 3). Saponins extracted from tea seeds play an important role in inactivating the human type A and B influenza virus (Hayashi et al., 2000) as well as Procrine reproductive and respiratory syndrome virus (PRRSV), which is a type of swine pathogen (Neumann et al., 2005; Tian et al., 2007) (Table 3, Fig. 4). The PRRSV consists of ssRNA based genome that causes respiratory syndrome in piglets, immune suppression, reproductive failures and secondary infections in pregnant swine (Rossow, 1998). The viral "N protein" is conserved in various PRRSV subtypes and it down-regulates interferons while activating NF-kB pathway (Luo et al., 2011). PABP (Poly A binding protein), PCBP1 (Cellular poly (c) binding protein-1), and PCBP2 (Cellular poly (c) binding protein-2) are host cell proteins that play an important role in the replication process. Saponins, in this case, decrease the RNA level as well as N protein level and silence PABP, PCBP1 (Cellular poly (c) binding protein-1) and PCBP2 (Cellular poly (c) binding protein-2) lowering the replication and transcription rate of PRRSV (Beura et al., 2011; - Wang et al., 2012).

Jassim and Naji (2003) found that the naturally extracted triterpenoid saponin from *Maesa chisia, Maesa lanceolata*, and *Maesa indica* exhibits direct virucidal activity against viruses such as vaccinia virus, HSV and Newcastle disease virus (Jassim and Naji, 2003) (Table 3, Fig. 4).

Licorice is a herb and is considered a part of traditional Chinese medicine (TCM). More than 20 triterpenoids fall under licorice; where glycyrrhizin, extracted from the roots of Licorice, and 18-β-glycyrrhetinic acid have potential antiviral properties listed in (Table 3, Fig. 4).

According to Matsumoto et al., (2013), Glycyrrhizin targets the release of infectious hepatitis C virus (HCV) particles, thereby acting as an effective anti-viral agent (Matsumoto et al., 2013). According to another study, it was found that almost a 50% reduction in the viral load of HCV is seen upon treatment with 1472 μg/mL of Glycyrrhizin (Ashfaq et al., 2011). Huang et al., (2012), revealed that during the HSV infection, stress and adhesion forces between polymorphonuclear (PMN) leukocytes and cerebral capillary vessel endothelial cells (CCEC) are increased but on perfusion of glycyrrhizin, there is a decrease in the stress and adhesion forces between CCEC and PMN (Huang et al., 2012).

Glycyrrhizin also improves myocarditis caused by coxsackievirus B3 (CVB3) by minimizing the serological levels of cardiac enzymes, improving weight loss profile and enhancing the cellular survival rate. This takes place by the reduction in the expression of inflammatory cytokines such as interleukin-6, interleukin-1β and NF–κB. The degradation of NF–κB inhibitor, IκBκ, is halted by glycyrrhizin leading to inhibition of NF–κB (induced by CVB3). This comes as a new therapy for combating viral myocarditis . Soufy et al., (2012), observed glycyrrhizin as exceptional immune stimulant (Soufy et al., 2012). Glycyrrhizin has activated T lymphocyte proliferation thereby enhances the effect of the duck hepatitis virus (DHV) vaccine. Treatments with glycyrrhizin alone or in combination with DHV vaccine lead to the formation of higher anti-DHV antibody numbers than with DHV vaccine alone.

According to various studies it was also found that Glycyrrhizin may prove useful in combating some of the effects of influenza virus (Table 3, Fig. 4). 100 μg/mL of Glycyrrhizin weakens the H5N1-induced production of interleukin-6 (IL-6), ligand5 (CCL5), chemokine (C-X-C motif), ligand10 (CXCL10), and chemokine (C–C motif) and also suppresses apoptotic pathways induced by H5N1 (Michaelis et al., 2010). The DNA binding site known as high-mobility-group box1 (HMGB1) is responsible for enhancing the replication of the Influenza virus. Glycyrrhizin decreases the binding of HMGB1 to DNA, which inhibits the polymerase activity of the influenza virus (Moisy et al., 2012; Smirnov et al., 2012).

Wang's study (Wang et al., 2013) revealed that glycyrrhizin could also be used as an antiviral component in licorice against the infection caused by enterovirus 71 (EV71) and coxsackievirus A16 (CVA16) (J. Wang et al., 2013). It inhibits viral entry into the host cells in the case of EV71 and directly inhibits CVA16. Glycyrrhizin also acts as a strong inducer of the autophagy-activator, Beclin1. It plays a vital role in the production of Beclin1 and was found to induce the process by more than two folds higher than rapamycin (the reference compound for Beclin1 synthesis). It also inhibits the replication of HSV1 (Laconi et al., 2014).

As compared to glycyrrhizin, 18-β-glycyrrhetinic acid expresses limited antiviral activity. It inhibits rotavirus-replication which occurs right after viral entry into the host cell. The viral yield was reduced by 99% after adding 18-β-glycyrrhetinic acid to the cultures after viral adsorption had occurred. Viral proteins (VP2,VP6, and NSP2) were also found to reduce (Hardy et al., 2012). 18-β-glycyrrhetinic acid also targets human respiratory syncytial virus (HRSV) by inhibiting the internalization-stimulating interferon secretion and prevents the attachment of virus (Yeh et al., 2013).

Glycyrrhizin isolated from another Chinese medicinal herb *Glycyrrhiza radix* roots has been used for the treatment of SARS (Patel et al., 2020) (Table 3, Fig. 4). According to an *in vitro* study, glycyrrhizin has

Table 3
Anti-viral properties and mode of action of some plant extracted saponins.

Saponin & Plant name	Virus	Mode of action	$IC_{50}/EC_{50}/SI$	References
Saikosaponin a (SSa) extracted from *Radix bupleuri* (Fig. 4)	Human coronavirus HCoV-229E	SSa intervenes in viral absorption, penetration into the host cell and other early stage steps of viral replication	EC_{50} = 8.6 ± 0.3 μM SI = 26.6 μM	(Cheng et al., 2006)
	Influenza A virus infected A549	SSa diminishes replication of the virus, production of aberrant pro-inflammatory cytokines and histopathology of lungs.	IC 50 = 1.98, 2.21 and 2.07 μM for H1N1 PR8, H9N2 and H5N1 strains of Influenza A virus	(Chen et al., 2015)
Saikosaponin B2 extracted from *Scrophularia scorodonia* (Fig. 4)	Human coronavirus HCoV-229E	Inhibits the viral attachment, penetration, and replication	EC 50 = 1.7 ± 0.1 μM	(Cheng et al., 2006)
Betulinic acid extracted from heartwood of *Juniperus formosana* (Fig. 4)	SARS CoV	Inhibits replication. Inhibits the SARS CoV CL protease.	IC 50 = 10 μM	Wen et al. (2007)
Celastrol, extracted from the bark of *Tripterygium regelii* (*T. regelii*) (Fig. 4)	SARS-CoV	SARS-CoV $3CL^{pro}$ inhibitory activity	IC 50 = 10.3 ± 0.2 μM	(Ryu, Park, et al., 2010b)
Pristimerin (*T. regelii*)			IC 50 = 5.5 ± 0.7 μM	
Tingenone (*T. regelii*) (Fig. 4)			IC 50 = 9.9 ± 0.1 μM	
Ig uesterin (*T. regelii*) (Fig. 4)			IC 50 = 2.6 ± 0.3 μM	
18-hydroxyferruginol extracted from *Torreya nucifera (T. nucifera)* (Fig. 4)	SARS-CoV	Act as SARS-CoV $3CL^{pro}$ inhibitors	220.8 ± 10.4 μmol/L	Ryu, Jeong, et al. (2010)
Hinokiol extracted from *Torreya nucifera (T. nucifera)* (Fig. 4)			233.4 ± 22.2 μmol/L	
Ferruginol extracted from *Torreya nucifera* (Fig. 4)			49.6 ± 1.5 μmol/L	
18-oxoferruginol extracted from *TTorreya nucifera (T. nucifera)* (Fig. 4)			163.2 ± 13.8 μmol/L	
O-acetyl-18-hydroxyferruginol extracted from *Torreya nucifera* (Fig. 4)			128.9 ± 25.2 μmol/L	
Methyl dehydroabietate extracted from *Torreya nucifera (T. nucifera)* (Fig. 4)			207.0 ± 14.3 μmol/L	
Isopimaric acid extracted from *Torreya nucifera (T. nucifera)* (Fig. 4)			283.5 ± 18.4 μmol/L	
Kayadiol extracted from *Torreya nucifera (T. nucifera)* (Fig. 4)			137.7 ± 12.5 μmol/L	
Glycyrrhizin, extracted from roots of *Glycyrrhiza glabra* (Fig. 4)	SARS CoV	Inhibits the viral penetration and adsorption	SI = ≥ 65	Hoever et al. (2005)
	2019-nCoV		ND	
	HCV	Targets the step where the viral particles are infecting cells and causes inhibition of core genes as well as full length particles	14 ± 2 μg/mL	Ashfaq et al. (2011)
	HSV	It reduces the adhesion force and stress between CCEC and PMN.	ND	Huang et al. (2012)
	CVB3	Preventing degradation of IκBκ to induce NF–κB activity via CVB3.	ND	(. Zhang et al., 2012)
	DHV	T lymphocyte proliferation activation	10^5 dilution	Soufy et al. (2012)
	H5N1	It weakens the H5N1-induced production of CXCL10, IL-6 and CCL5, and suppresses the H5N1-induced apoptosis.	200 μg/mL	Michaelis et al. (2010)
	Influenza virus	It minimizes HMGB1 binding to DNA and inhibits the influenza virus polymerase activity.	ND	Moisy et al. (2012)
	CVA16 EV71	CVA16 is inactivated directly, while anti-EV71 effects can be seen on event(s) during the entry of the virus into the cell.	3 mM	(. Wang et al., 2013)
	HSV1	HSV1 replication resistance.	2 mM	Laconi et al. (2014)
18β-glycyrrhetinic acid extracted from roots of Licorice (Fig. 4)	Rotavirus	It minimizes the levels of viral proteins (VP2, VP6, NSP2) after the viral entry.	25 μg/mL	Hardy et al. (2012)
	HRSV	Prevents the virus from attaching, and also internalizes and stimulates IFN secretion.	300 μg/mL	Yeh et al. (2013)
Arganine C extracted from *Tieghemella heckelii* (Fig. 4)	HIV	Strongly inhibits HIV entry into cells	20 μM	Gosse et al. (2002)
Soyasaponins extracted from soyabean seeds (Fig. 4)	Human immunodeficiency virus (HIV) Epstein-Barr virus (EBV)	Inhibits HIV-induced cytopathic effects and virus -specific antigen expression 6 days post infection. Also inhibits HIV-induced cell fusion in the MOLT-4 cell system	ND	Okubo et al. (1994)
Quillaja saponins extracted from *Quillaja saponaria* (Fig. 4)	Rhesus rotavirus (RRV)	Inhibits the virus–host attachment through disruption of cellular membrane proteins and/or viral receptors.	Therapeutic index = 2.95	Tam & Roner (2011)

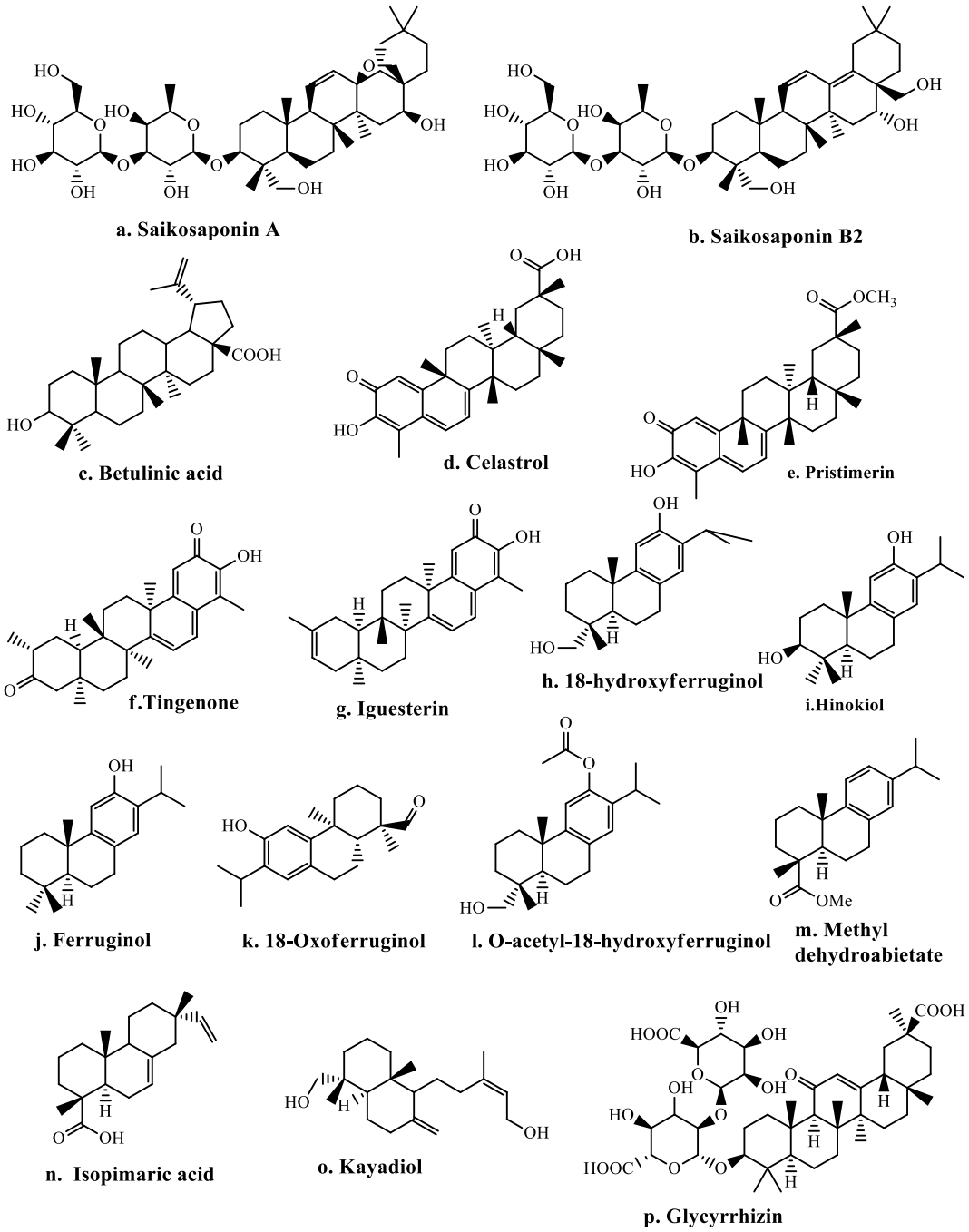
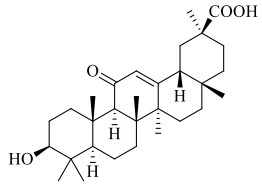


Fig. 4. Chemical structures of some anti-viral saponins.

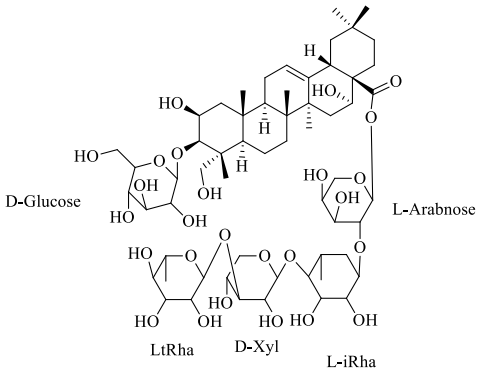
been demonstrated to cause anti-SARS-CoV effects. It inhibits the viral penetration and adsorption and was most active when administered both during and after the adsorption-period of the virus (Cinatl et al., 2003). The antiviral property of glycyrrhizin was enhanced by chemical modifications, but it also increased the cytotoxicity levels. Hence the ratio measuring the window between cytotoxicity and antiviral activity (selectivity index) was minimized compared to glycyrrhizin (selectivity index: $\geq$ 65) (Hoever et al., 2005).

Glycyrrhizin has been used for many years as anti-HIV-1, anti-duck hepatitis virus (DHV) drug (Bailly and Vergoten, 2020). Cinatl and group investigated the efficacy of glycyrrhizin against SARS-associated coronavirus (FFM-1 and FFM-2) (Cinatl et al., 2003). The study was performed on Vero cells where glycyrrhizin as a drug (EC_{50}) was found to cause cytotoxicity on viral cells, inhibit their replication, and penetration into the host cell. The 15 derivatives of glycyrrhizin possessed anti- SARS CoV activity as reported by Hoever (Hoever et al., 2005). The addition of 2-acetamido-β-D-glucopyranosylamine to glycyrrhizin showed an enhanced anti- SARS CoV activity as compared to glycyrrhizin alone. Other glycyrrhizin derivatives like glycyrrhizin amides and their conjugates seemed to have enhanced anti- SARS-CoV activity up to as much as 70 times with negative cytotoxic effects (Hoever et al., 2005).

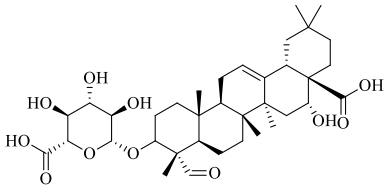
Although 2019-nCoV belongs to the SARS-CoV family, whether glycyrrhizin has any anti-2019- nCoV effects needs further investigation. Molecular docking studies indicated that glycyrrhizin has a possible biding to human ACE2 molecules, which act as receptors for the S-protein present on the membrane of 2019-nCoV (- Zhou and Huang,



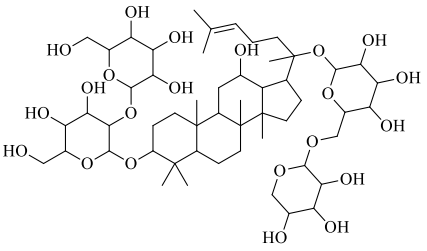
q. 18-Beta-glycyrrhetinic acid



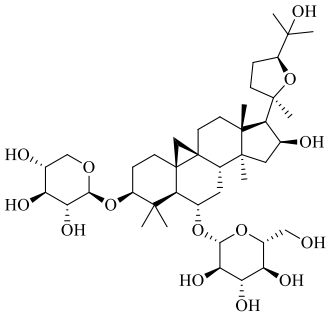
r. Arganine C



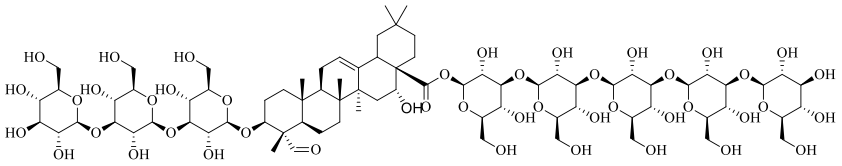
s. Quillaja saponin



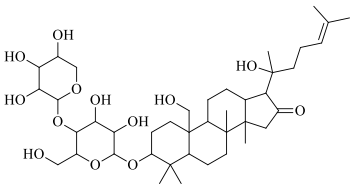
t. Gingenosides Rb2



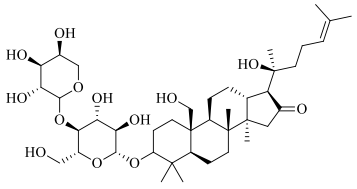
u. Astragaloside IV



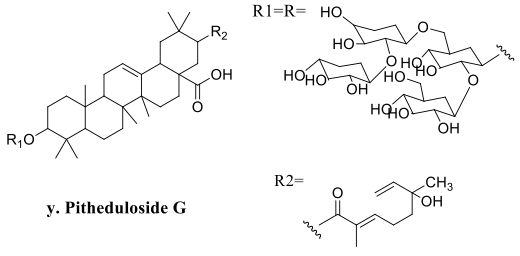
v. Quil A



w. Bacoside-A



x. Bacoside-B



y. Pitheduloside G

Fig. 4. (*continued*).

2020). The ΔG of the binding reaction was estimated to be -9 kcal/mol for ARG-559, GLN-388, ARG-393, and ASP-30 sites (Feng et al., 2020). The expected binding site of glycyrrhizin is thought to be located near a hydrophobic site based on hydropathy index of ACE2. Moreover, the sites ARG-393 and GLN-388 are proximal to a zinc metallopeptidase, which is speculated to regulate the activity of ACE2 in cells (Feng et al., 2020). Therefore, properties such as its anti-viral effects on SARS-CoV, low cytotoxicity and potential interaction with ACE2 makes glycyrrhizin a strong candidate to be tested for its efficiency against the 2019-nCoV infection.

Saikosaponin extracted from *Radix bupleuri,* also belonging to the Traditional Chinese Medicine (TCM), is another important saponin-compound to exhibit anti-viral properties. Saikosaponin (SSa) is one of the various types of triterpenoid saponins present in *Radix bupleuri* (Yuan et al., 2017) (Table 3, Fig. 4). Pei-Win Cheng et al. (2006) performed the *in vitro* antiviral screening of Saikosaponin A, B2, C, and D against human coronavirus HCoV-229E by using the 2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino) carbonyl-2H-tetrazolium hydroxide] (XTT) assay, and found that Saikosaponin B2 shows some amount of an anti-coronaviral activity (P. W. Cheng, Ng, Chiang and Lin, 2006). Stachybonoid A (1) from *Stachybotrys chartarum* shows inhibitory activity against the replication of dengue virus.

It is mostly observed that addition of adjuvant to vaccines enhances their immunogenicity, thereby inducing a strong immune response against viral diseases. For developing vaccine candidates against SARS CoV, the idea of saponin based microemulsion adjuvants producing antigen specific antibodies is being explored (Sharma et al., 2020).

7. Role of saponins against viral diseases

Saponins have been extensively studied and known for its anti-microbial properties. Saponins are found to be beneficial since they have rich biomedical properties which include anti-bacterial, anti-fungal properties. The role of saponins extended towards viral diseases which are posing threat to human health and well-being. These viral diseases are targeted by saponins through their saponin specific interaction with viral particle and likewise inhibition. Following diseases present a closer picture of the role of saponins against viral infections.

1. Influenza A Virus: Influenza virus infection has been the most widespread respiratory illness since 2009 when H1N1 breaks out with high mortality rate over worldwide (Dong et al., 2017). The mammals including humans and birds are infected with virus as their host and in turns effects the respiratory symptoms with mild to severe symptoms. A number of subtypes of influenza virus are known to cause lethal disease in humans like H7N7, H5N1, H7N9, H10N8, H3N2, H1N1. Amongst them all, H1N1 and H3N2 are supposed to be the most potent subtypes in causing serious illness in the human population (- Chen et al., 2014; Farooqui et al., 2011; Guan et al., 2013; Subbarao et al., 1998). Previous studies reveal the beneficial function of saponin ginsenosides in treating influenza virus A symptom. Ginsenosides, majorly obtained from ginseng, is a steroidal saponin present in a variety of plants. The chemical structure of ginseng is a carbon skeleton consisting of four rings in trans geometry, with structural differences in the position of OH group and number and type of sugar molecules attached to the skeleton (Escobar-Sánchez et al., 2015). Ginsenosides Ro is an exclusive oleanane type ginsenoside. Ginsenoside Ro is a non-steroidal categorized in oleanolic acid group which is different from other two groups of ginsenosides that are protopanaxadiol (PPD) and protopanaxatriol (PPT) (Rokot et al., 2016). Ginsenosides contain the potential of anti-inflammatory, anti-oxidative and anti-cancer properties. Ginsenosides as anti-inflammatory agents lower down the production of inflammatory enzymes such as iNOS and COX2 via regulating NF-kB signaling pathways. Ginsenosides Rb 1also regulates the TNF-α production in macrophages cells through NF-kB signaling pathway. Ginsenosides Rb1 synthesis a metabolic compound K (CK) which also exerts anti-inflammatory effects and reducing the pro-inflammatory cytokines (J. H. Kim, Yi, Kim and Cho, 2017; M.-Y. Kim, Yoo and Cho, 2014). Ginsenosides typically interact with the virus life cycle of H1N1 in inhibiting the virus as found in experimental reports *in vitro* and *in vivo* conditions. The antiviral properties were seen in ginsenosides Rb which interferes with viral glycoprotein hemagglutinin thereby inhibiting the attachment of virus with receptor α 2–3' sialic receptor. The virus thus loses the interaction with the cell and is unable to penetrate into the host body which reduces its activity (Dong et al., 2017). Another saponin called saikosaponin (SSa) is also effective against influenza virus. SSa diminishes the replication of the virus, production of aberrant pro-inflammatory cytokines and histopathology of lungs (J. Chen et al., 2015). Glycyrrhizin inhibits influenza virus by acting as a barrierH5N1 by slowing down the production of H5N1 induced CXCL10, IL-6, and CCL5 and inhibiting H5N1 induced apoptosis (Michaelis et al., 2010).

2. HIV: Saponins are potent anti-HIV compounds which inhibit the virus. Soya saponin interferes with the cytopathic effects triggered by HIV virus. It acts on virus by blocking the HIV induced fusion in the MOLT-4 cell system (Okubo et al., 1994). The HIV specific antigen expression is also suppressed by soya saponin within 6 days of infection. Arganine C serves at viral entry and blocks the entry of virus into the cell (Gosse et al., 2002). Terpenoids is a metabolic compound or secondary metabolite produced by diversified species of plants either during growth or developmental stages (X.-W. Yang et al., 1999). Legumes and oats are included as examples of a few plant species. Terpenoids hold significant medicinal, antimicrobial properties and identified as compounds which bear importance in developing disease resistance plants. Terpenoids are formed by cyclization of 2,3 oxidosqualene through isoprenoid pathway which forms oleanane as the primary compound. The terpenoid chain then undergoes various chemical changes including substitution, glycosylation and dehydrogenation, catalyzed by enzymes like glycosylase, cytochrome P-450 mediated monooxygenase etc (Haralampidis et al., 2002). Terpenoid present in *Ganoderma lucidum* (Reishi) commonly referred to as medicinal mushroom, has many medicinal properties such as anti-inflammatory, anti-microbial, anti-tumor, antioxidant and anti-histaminic. The terpenoids isolated from *G. lucidum* were found to inhibit HIV as the terpenoids show anti-HIV-1 protease activity which suppresses HIV progression (Cör et al., 2018). These compounds are ganoderic acid beta, lucidumol B, ganodermanondiol, ganodermanontriol and ganolucidic acid A and their IC50 values were recorded in the range of 20–90 mM(Min, Nakamura, Miyashiro, BAE, & Hattori, 1998). The terpenoids, IVc, IVd, IVe, and IVf were isolated from *Aesculus chinensis* and all four had anti-HIV protease activity (X.-W. Yang et al., 1999). A new saponin compound was isolated from Acacia pennata called as 21β-O-[(2E)-6-hydroxyl-2,6-dimethyl-2,7-octadienoyl] pitheduloside G (Fig. 4), which was found to have an anti-HIV-1 PR activity with IC50 value of 2.0 ± 0.2 μM. Another saponin was also isolated from the same plant was pitheduloside G with much less inhibitory effect against HIV-1 PR activity. The IC50 value of pitheduloside G was recorded in the range of 18 ± 0.5 μM (Nguyen et al., 2018).

3. Hepatitis B virus (HBV): Hepatitis B virus affects the liver cell causing both acute and chronic hepatitis. Significant amounts of research are made in the area of developing antiviral drugs for HBV. The anti-hepatitis medicinal effects were seen in saponin astragaloside IV isolated from Chinese herb *Radix Astragali.* Experimental evidence on astragaloside saponin confirmed its ability in acting as anti-hepatitis by reducing the hepatitis B virus secreting $HepG_2$ 2.2.15 cells (Wang et al., 2009). Total saponins (TSTA) isolated from *Taraphochlamys affinis* has inhibitory effects on HB surface antigen and HBeAg secreted by human hepatoma 2.2.15 cells. However, it is also shown that TSTA has no cytotoxic role on the cells (Lin et al., 2013). Other saponin, bacosides A & B obtained from ayurvedic plant brahmi showed a dose dependent reduction in viable cells of human liver carcinoma cell lines (Fig. 4). This reduction in viability of cell is due to DNA fragmentation which led the cells to undergo apoptosis. Their IC50 values were recorded as 0.625 μg/mL and 9.8 μg/mL for bacoside A & B, respectively (Kalachaveedu

et al., 2014).

4. Chikungunya: Quil A and fraction B are two saponins derived from Quillaja Sp. which show potent results against chikungunya virus (Fig. 4). The anti-viral activity of the two saponins inhibited the viral DNA replication which makes both the saponin strong pharmacological properties in fighting against the virus (Mahomoodally and Gurib-Fakim, 2013; Metz et al., 2013).

5. Rotavirus: Infants are mainly prone to diarrhoeal diseases caused by rotaviruses. Research has found beneficial role of Quillaja saponin (isolated from Chilean soapbark tree) against rhesus rotavirus (RRV). The suggested pathway involved in the inhibition of virus is possibly by hindering the interaction between virus and its host (Tam and Roner, 2011). Ginsenoside-Rb2 through oral administration has proved to be useful in protecting against the severity of rotavirus. The positive results of oral administration were seen in newborn mice with 75 mg/kg of ginsenoside-Rb2 (H. Yang, Oh, Kim, Cho and Yoo, 2018).

5. Herpes simplex virus (HSV): A saponin obtained *in vitro* from *Anagallis arvensis*, Primulaceae shows potent anti-viral results by interfering with the cytopathic effect of HSV virus type 1 (Amoros et al., 1987). Chemically, the saponin is 3-O-glucose-(1–3 or 4)- [arabinose (1–4 or 3)]-glucose (1–2)-xyloside of 23-hydroxyprotoprimulagenin A.

8. Hemolytic activity

The clinical use of saponins is limited due to their haemolytic activity mediating toxicity in animals as well as humans. The mechanism of erythrocyte-membrane destruction with the aid of saponins (haemolysis) is not yet completely understood and only a little is elucidated in several scientific investigations conducted (Gauthier et al., 2009). Baumann et al. (2000) have reported that saponins interact with the sterols present in membranes of erythrocytes and produce hemolytic reactions (Baumann et al., 2000). This leads to rupture of the erythrocyte membrane leading to increased cell permeability and loss of haemoglobin. An alternate mechanism involved in haemolysis has also been explored (Baumann et al., 2000) wherein the extensive interaction between saponins and water channels, aquaporins, mediates an increase in membrane permeability due to entry of water molecules into the cells causing erythrocytes' rupture and apparent haemolysis. Additionally, forty-seven food and medicinal plants were studied to explore the presence of any correlations between the hemolytic and adjuvant-like activities of saponins and it was found that no such interconnections exist (Oda et al., 2000).

9. Immunological adjuvant activity

Guo and Kenne (2000) found that many different active triterpenoid saponins are present in *Quillaja saponaria* Molina (Chilean soapbark tree) (Guo and Kenne, 2000). These saponins show a strong adjuvant activity which is used for developing many human and animal vaccines (Dalsgaard, 1987; Kensil, 1996; S. Wu et al., 1991). Saponin-based adjuvants show a tendency to control cell-mediated immune system and enhance production of antibodies (Oda et al., 2000) (Fig. 5). The adjuvant effect is similar in the case of T-independent as well as T-dependent antigens and also causes cytotoxic $CD8^{+}$ lymphocytic responses along with expressing it against mucosal antigens (Kensil, 1996).

Saponins show stimulatory effects on constituents of specific

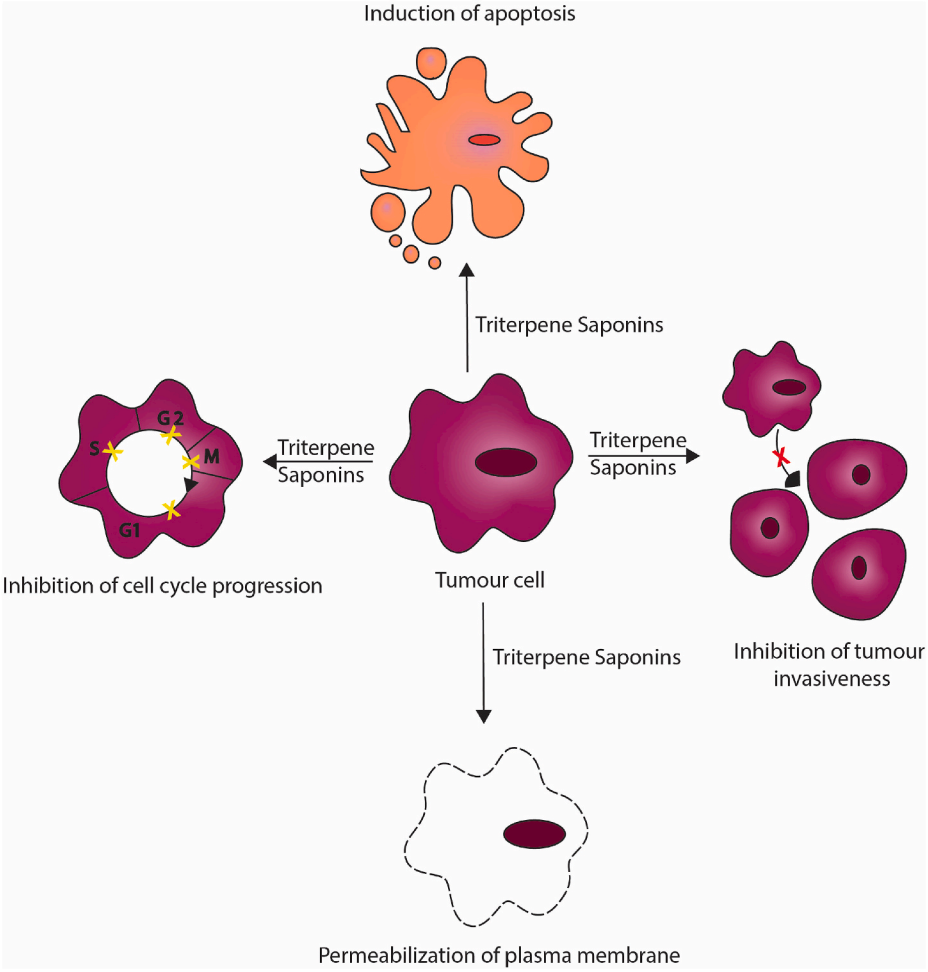


Fig. 5. Effects of saponins on tumor cells.

immunity such as cytotoxic T-lymphocytes and also express certain non-specific immune reactions such as monocyte proliferation (Delmas et al., 2000; Yui et al., 2001) and inflammation (de Oliveira et al., 2001; Haridas et al., 2004).

The mechanism of action of saponins towards immunological stimulations has not yet been clearly elucidated. Jie et al. (1984) and Kensil (1996) reported that saponins are responsible for the production of cytokines like interferons and interleukins which enhance its properties of immunostimulant (Jie et al., 1984; Kensil, 1996). Saponins also interact with antigens to generate several responses and help in antigen-presentation to cytosolic proteases (Barr et al., 1998).

10. Anti-tumor activity

Ebbesen et al. (1976) reported for the first time about an antitumor activity of saponins (Ebbesen et al., 1976). The first *in vivo* study in this regard was performed on a mouse model for checking the anti-tumor activity of triterpenoid saponins (Fig. 5). Yu et al. (1995) proved that the saponins extracted from *Bolbostemma paniculatum* (tubeimoside) were able to obstruct melanoma growth in mice. Saikosaponin A has the potential to stop the proliferation of ovarian cancer cells (Yu et al., 1995). The effects of saponins on tumor cells are classified into three broad categories: (1) cytostatic (2) pro-apoptotic and (3) anti-metastatic effects (Bachran et al., 2008) (Fig. 5).

11. Other properties

11.1. Pharmaceutical applications

The discovery of biological activities of saponins is not only limited to use in traditional medicine and remedies but, more recently, also in pharmaceutical applications (Güçlü-Üstündağ et al., 2007; Sparg et al., 2004). It is used as a starting compound for the semi-synthesis of steroidal drugs in the pharmaceutical industry. Sheng and Sun (2011) reviewed the clinical significance of triterpene saponins in the prevention and treatment of metabolic and vascular diseases (Sheng and Sun, 2011). All these studies, especially the discovery about the anti-cancerous properties of saponins have intensified efforts for efficient extraction of saponins from plant materials.

11.2. Industrial sectors

Apart from the various anti-microbial and anti-viral roles of saponins, these plant-based compounds are known for their emerging industrial and commercial applications based on their extraordinary physico-chemical properties. Steroidal saponins and tritepenoids have gained importance in industrial sector as raw materials, additives, surfactants etc (Balandrin, 1996). They have also been used for many years in preparations of herbal medicines, hormones, cosmetics etc (Roopashree and Naik., 2019; Hawley et al., 1971). As mentioned before, saponins form foam on mixing with water; this and other chemical properties of saponins render them useful as additives in food and cosmetics. They can further be exploited in other cases such as in food preservation, flavoring agents and to aid in removing cholesterol present in dairy products (Balandrin., 1996). Production of tea oil also generates byproducts rich in saponins which have been considered as vermicides to manage earthworm casts found in sports fields and golf courses (Guclu-Ustundg et al., 2007).

12. Conclusion and future perspectives

Saponins show various medicinal properties and are used for curing a multitude of diseases such as bacterial, fungal as well as viral infections along with different kinds of cancers as part of traditional Chinese medicine. They also find use in various industrial settings due to their diverse chemical properties. Plant-based products such as saponins have always been attractive in terms of medicinal and therapeutic uses due to their minimal to zero side effects as opposed to synthetic drugs, some of which lead to serious toxicities and reactions in the body. Due to the difficult and less explored nature of their extraction processes (making them more expensive) and properties (elusive mechanisms of action), the goodness of saponins have not been entirely exploited in the field of healthcare and medicine. However, recently an increased urge is seen towards the use of saponins and other herbs for preparing remedies. Progress is being made towards understanding how saponins carry out their cellular actions or affect the pathways they target.

With the outbreak of the 2019-nCoV pandemic, the search for effective therapeutic strategies as well as vaccines for the virus has become extremely crucial. Saponin such as glycyrrhizin has shown a good amount of preliminary evidence for their potential against the 2019 novel coronavirus and thus, carrying out further research in this regard may prove beneficial for the world battling a deadly disease right now. Moreover, exploring utilization of saponins in other key areas of human health and medicine such as neurodegenerative disorders-for which ample amount of general as well as clinical evidence exists-also needs to be taken up more proactively. The role of saponins has also been elucidated in other virus specific diseases that have a high rate of infection spread and reported deaths globally. The bio-medicinal and anti-viral characteristics of various saponins present its extended approach in preparing effective and safe plant-based remedies offering an effective alternative to synthetic drugs.

Authors' contributions

PS, AT, and Al wrote and edited the manuscript; PB, and SP contributed to the chemical structures of the compounds; VS, RM and NKP edited and made the final version of the manuscript. The author(s) read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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