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## Saponins – A Ubiquitous Phytochemical: A Review of Its Biochemical, Physiological and Pharmacological Effects

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### ABSTRACT

*Saponins are ubiquitous phytochemicals widely reported to be present in many species of plants and animals. Some saponin containing plants, mainly legumes, have been used as animal feed, but others are toxic. In this review, the biochemical, physiological and pharmacological effects of saponins in humans and animals have been highlighted. Physiological and biochemical effects examined include the effects on biological membranes, gastrointestinal absorption, blood and liver cholesterol, enzymes and general body metabolism, reproductive, antioxidant and free-radical scavenging effect, smooth muscle activity and effects on ruminant digestion. Pharmacological effects of saponins such as anti-inflammatory, diuretic, hypoglycaemic, antidiabetic, antiulcer and anti-ageing effects are also examined. From the literature reviewed, it can be concluded that saponins, in general, are toxic when administered intravenously but possess a lot of therapeutic potentials especially as cytotoxic agents.*

**Key words:** Saponins, Bioactive, Cytotoxic, Ubiquitous, Phytochemical

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## INTRODUCTION

Saponins are defined as triterpenoid or steroid glycosides produced by some bacteria, lower marine animals and plants (Yoshiki *et al.*, 1998). The most known of these saponins are secondary metabolites produced in Magnoliophyta, consisting of both monocotyledons and dicotyledons. However, the majority of saponin-producing plants are dicotyledons (Vincken *et al.*, 2007). Saponins are found widely in the plant kingdom with the triterpenoid saponins predominant mostly in cultivated crops, while steroid saponins are common in medicinal plants (Fenwick *et al.*, 1991). Triterpenoid saponins have been detected in many legumes, ginseng, sunflower, horse chestnut, liquorices, spinach, tea, quinoa, sugar beet and alliums. Steroid saponins are found in oats, yucca, tomato seed, yam, fenugreek, ginseng, asparagus, aubergine and capsicum peppers. Saponins have also been reported to be tensoactive glycosides containing a hydrophobic nucleus of triterpenoid structure with carbohydrate chains linked to the nucleus (Kensil, 1996).

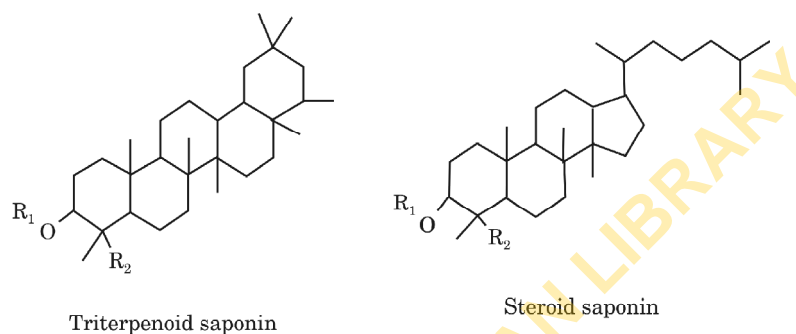
Oral ingestion of saponins by humans and animals has been associated with a wide spectrum of biological activity. For example, recent reports showed that the triterpenoid saponins isolated from *Eclipta prostrata* have antimicrobial, immunosuppressant, anti-guardian and anti-venom potentials (Pithayanukul *et al.*, 2004; Wiart *et al.*, 2004; Sawangjaroen *et al.*, 2005). Further, Ndukwe *et al.* (2007) reported that the presence of saponins in *Vitellaria paradoxa* could be responsible for the traditional use of its kernel fat extract (shea butter) as a muscle relaxant and in the treatment of sprains, wounds and colds as generally practiced in Nigeria. In addition, some saponins exhibit antiviral activity probably due to their effect on surface tension. Majority of the medicinal plants, screened so far, for photochemical contents, have been reported to contain high amounts of saponins.

Medicinal plants refer to species having medicinal (including veterinary), aromatic, and culinary importance (Abd-El-Wahab *et al.*, 2008). According to the World Health Organisation (WHO), these medicinal plants contain compounds or secondary metabolites that can be used for therapeutic purposes (WHO, 2008). Saponins are secondary metabolites of plants and have been used in human diets for controlling cholesterol. However, some (including those produced by the soapberry) are very poisonous if swallowed, are able to lyse red blood cells and cause urticaria or skin rash in many people (Mohamad *et al.*, 2001).

## Classification and Biosynthesis of Saponins

Chemically, saponins generally occur as glycosides of steroids or polycyclic triterpenes (Fig. 1), and are classified according to the chemical character of the aglycone (known as sapogenin) into steroid and triterpenoid saponins.

Two main types of steroid aglycones are known, spirostan and furostan derivatives. The main triterpene aglycone is a derivative of oleanane. Saponins vary widely structurally, depending on the aglycone, the nature of the side chains and the position of attachment of these moieties on the aglycone (Francis *et al.*, 2002).



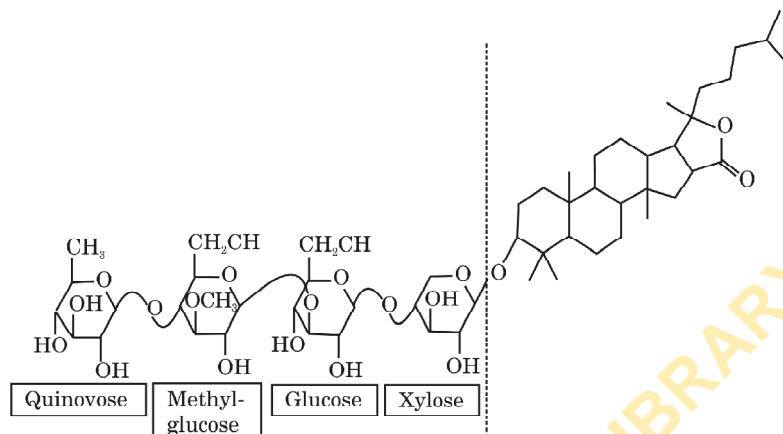
**Fig. 1:** Structure of triterpenoid and steroid saponins (Source: Francis *et al.*, 2002)

Both triterpenoid and steroid saponins originate from the precursor molecule-squalene which is oxidized to oxidosqualene that is subsequently converted to cyclic derivatives. The carbohydrate component of saponins consist of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid. Saponins that have one sugar molecule attached at the C3 position are classified as monodesmoside saponins, and those that have a minimum of two sugars, are classified as bidesmoside saponins. There are two main types of triterpenoid saponins: (1) neutral triterpenoid saponins that have a normal sugar attached to sapogenin, and (2) acidic triterpenoid saponins with sugar moiety containing uronic acid or one or more carboxylic groups attached to the sapogenin (Lasztity *et al.*, 1998).

The molecular structure of a hypothetical saponin is found in Fig. 2.

A second more systematic mode of classification has also been proposed for saponins based on their carbon skeletons, the formation of which follows the main pathways for the biosynthesis of triterpenes and steroids. In this way, saponins were classified into 11 main classes: lupanes, hopanes, dammaranes, oleananes, taraxasteranes, ursanes, cucurbitanes, cycloartanes, lanostanes, steroids and tirucallanes (Vicken *et al.*, 2007).

Normally, saponin biosynthesis proceeds *via* the isoprenoid pathway in which 3 isoprene units (molecules containing 5-carbon atoms) are first linked in a head-to-tail manner to each other, to form farnesyl pyrophosphate (a 15-carbon atom molecule). Subsequently, two farnesyl pyrophosphates are linked in a tail-to-tail manner to give squalene (a 30-carbon atom molecule)



**Fig. 2:** Molecular structure of a hypothetical saponin composed of an aglycone and a linear glycosidic chain constituted by the four most frequent monosaccharides (Glucose, xylose, Methyl-glucose, quinovose) found in saponins. Source: (Caulier *et al.*, 2011).

(Holstein and Hohl, 2004). Oxidation of squalene gives oxidosqualene, which is the common starting point for cyclization reactions in triterpenoid biosynthesis (Abe *et al.*, 1993; Haralampidis *et al.*, 2002). There are three main enzymes involved in the biosynthesis of saponins; these are; oxidosqualene cyclases, which forms the basic triterpenoid skeletons; cytochrome P<sub>450</sub> monooxygenases, which catalyze oxidations; and uridine diphosphate-dependent glycosyltransferases, which catalyze glycosylations (Vicken *et al.*, 2007; Augustin *et al.*, 2011).

### Toxicity of Saponins

Although saponins are generally believed to be harmless when taken orally, they can be highly toxic when administered intravenously (Trease and Evans, 1989). The reason for the innocuous nature of orally ingested saponins may be attributable to the fact that saponins generally are very poorly absorbed following oral administration to humans and animals (Podolak *et al.*, 2010). Despite the sometimes negative biological actions of saponins on animal and humans, they do occur in a wide variety of crops and edible plants. However, most edible cereals and grasses generally appear to be deficient in saponins, with a few exceptions such as *Panicum virgatum*, *Avena* spp. (Osborn, 2003).

Saponins have been shown to be toxic to insects and this has led to the suggestion that they might provide protection from insect predation (Applebaum *et al.*, 1969). Further, saponins and their aglycones have been implicated in providing resistance of certain ancient woods in temples against attacks from termites (Tschesche and Wulff, 1970). Good examples are

*Quillaja saponaria* from South America and *Melaleuca spp.* from Australia. Several species of locust have shown increased mortality when fed on alfalfa or other saponin-containing legumes. Their larvae developed more slowly and the adults were smaller than when they were fed saponin-free herbage (Applebaum *et al.*, 1969). However, not all saponins are toxic to insects, for example, the saponins of *Cicer arietinum* and *Lens culinaris* are much less toxic than those in alfalfa or soybeans, when fed to azuki beetles (Chaieb, 2010).

Severe toxicity to fish is an inherent property of saponins and this has been known and exploited for centuries, especially in the fishing industry (Kinch *et al.*, 2008). Saponins added to water rapidly causes paralysis and death of fish (Murthy *et al.*, 2010). Small fish, tadpoles, fresh water snails *etc.* have been used as bioassay for saponin toxicity and saponins extracted from *Phytolacca dodecandra* have been suggested as a means of controlling the water snail *Biomphalaria glabrata* which transmits the endemic disease Schistosomiasis (Parkhurst *et al.*, 1973; Hosttettman, 1987). The toxicity of saponins to fish is directly related to damage to the delicate gills consequently leading to asphyxiation and deaths of the fish. Saponin at as low as  $0.9 \text{ mgkg}^{-1}$  decreases the respiratory protein level and acid-base balance, and modulates the immune system of Giant freshwater prawns, *Macrobrachium rosenbergii* (Shinn-Pyng *et al.*, 2006).

### Biochemical and Physiological Effects of Saponins

Most saponins are highly surface-active, and many form addition-complexes with sterols, including those associated with the plasma membranes of fungal, plant and animal cells (Johnson *et al.*, 1986). In recent past, saponins were treated exclusively as anti-nutritional or toxic constituents of plants. However, it has subsequently been established that saponins have both advantageous and disadvantageous effects on animals and humans (Cheeke, 1971; Oakenfull, 1981). High levels of saponin-containing meals in diets for monogastric animals have growth depressing effects beyond those that may be accounted for by effects of fibre on caloric intake or on endogenous nitrogen intake. Parts of the growth inhibition caused by saponins have been suggested to be due to their anorexia-inducing effects. Quillaia saponins have in fact been employed as an anorexic agent in studies on amino acid metabolism (Peterson, 1950).

Berrang *et al.* (1974) found that alfalfa saponin at levels of 0.2% or more in the diet of chicks induced a reduction in growth, feed intake and feed-conversion efficiency. Later studies also showed a depressant effect of alfalfa saponins on egg production (Jenkins and Atwal, 1994). However, there are species differences in the responses by monogastric animals to dietary saponin; poultry being much more sensitive than the others. In contrast to the monogastrics, saponins generally have no growth depressing effects on

ruminants (Lindahl *et al.*, 1957). However, saponin toxicity in ruminants leads to photosensitization followed by liver and kidney degeneration and gastrointestinal complications (Knight and Walter, 2003). Important biochemical and physiological effects of saponins include:

### ***Alteration of membrane integrity***

The most extensively studied effect of several saponins is their ability to cause membrane perturbation (Augustin *et al.*, 2011). This property has been referred to as the 'hemolytic activity of saponins', because it is thought to be the molecular basis for the often observed ability of saponins to cause lysis of mammalian erythrocytes (Baumann *et al.*, 2000; Chwalek *et al.*, 2006). Saponins have long been known to have pronounced haemolytic properties on erythrocyte membranes and this property has been used for their detection (Gee *et al.*, 1988). However, saponins differ greatly in their haemolytic activities and there is a wide variation among different animal species in the susceptibility of their erythrocytes to haemolysis by saponins. Among different animal species, the order of susceptibility is guinea pig, horse, dog, rat, rabbit, goat, sheep and cattle (Francis *et al.*, 2002).

The mechanism by which saponins cause haemolysis has been much investigated. Initially, it was thought that a reaction of the saponin with cholesterol in the erythrocyte wall, resulting in permeability changes may be responsible for the haemolytic activity (Jones and Elliot, 1969). Moreover, there are indications that the haemolytic activity of saponins could not strictly be ascribed to their detergent-like properties but must be related to an ability to interact with some constituents of the cell membrane (Seeman, 1974; Nishikawa *et al.*, 1984).

Alteration of membrane integrity by saponins was first described by Dourmashkin *et al.* (1962) when they investigated saponin-inactivated Rous sarcoma virus particles by electron microscopy. Subsequently, a staggering number of the biological effects of saponins have been ascribed to their specific ability to form pores in cellular membranes and this has contributed to their common use in physiological research (Menin *et al.*, 2001; Plock *et al.*, 2001).

Alterations in cell membrane integrity brought about by saponins are believed to proceed through the following steps (Seeman, 1974; Nishikawa *et al.*, 1984): (i) the hydrophobic aglycone of the saponin molecule penetrates the lipid bilayer and may specifically interact with other membrane components such as cholesterol; (ii) the presence of the saponin induces cholesterol-rich domains, producing conducting channels and making the membrane leaky; (iii) the increased cell permeability allows the efflux of potassium ion and influx of water, sodium and other ions; (iv) finally, complete cellular disintegration occurs with leakage of cellular constituent into the extracellular fluid.

The composition of membranes, with regards to concentration levels as well as the structure of incorporated membrane sterols, has been suggested to influence the ability of saponins to cause membrane perturbation (Armah *et al.*, 1999; Keukens *et al.*, 1995; Stine *et al.*, 2006; Walker *et al.*, 2008). Also, different aspects of the chemical composition of saponins themselves such as the structure of the aglycone (Voutquenne *et al.*, 2002; Takechi *et al.*, 2003; Gauthier *et al.*, 2009), the number and length of saccharide side chains (Woldemichael and Wink, 2001; Voutquenne *et al.*, 2002; Chwalek *et al.*, 2006) as well as the types and linkage variants of the incorporated saccharides (Takechi *et al.*, 2003) contribute to the overall cellular membrane lysing activity of saponins.

The agglutination of erythrocytes and of phospholipid residues is another important property of saponins, a phenomenon related to their interactions with biological membranes. Saponins such as ophiopogonins and ginsenosides have been reported to haemagglutinate human, rabbit, and sheep erythrocytes but were not haemolytic (Takechi and Tanaka, 1995).

#### ***Effects of saponins on gastrointestinal activity***

Some saponins increase the permeability of intestinal mucosal cells *in vitro*, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed (Johnson *et al.*, 1986). An increase in the apparent permeability of the intestinal brush border at sublethal levels of saponins has been reported and this may have important implications for the uptake of macromolecules, such as allergens, whose passage through the epithelium is normally somewhat restricted (Gee *et al.*, 1996). Feeding of rats with bitter quinoa diets (containing saponins) resulted in serious growth impairment probably caused by decreased food conversion efficiency (Gee *et al.*, 1993). Saponins have also been shown to obstruct the absorption of micronutrients through the intestinal mucosa. For instance, *Gypsophila* saponins in the diet depressed mean liver iron concentrations and total liver iron by impairing intestinal absorption (Southon *et al.*, 1988). Similarly, triterpenoid saponins from *Gypsophila* and *Quillaja* included in the diet appeared to interfere with the absorption of vitamins A and E in chicks (Jenkins and Atwal, 1994).

Saponins reduce protein digestibility probably by the formation of sparingly digestible saponin–protein complexes (Potter *et al.*, 1993) and endogenous saponins affected the chymotrypsic hydrolysis of soyabean protein, particularly glycinin (Shimoyamada *et al.*, 1998).

#### ***Effects of saponin on blood and liver cholesterol levels***

Dietary saponins have been shown to cause significant lowering of plasma cholesterol concentrations in various animal species (Grininger and Fisher, 1958; Newman *et al.*, 1958). Consequently, it has been proposed that saponin-

containing foods might be therapeutically useful for reduction of the risk of heart disease in hypercholesterolaemic human subjects (Malinow *et al.*, 1978; Potter *et al.*, 1979). The hypocholesterolaemic activity of dietary saponins may be due to the formation of some complexes with dietary cholesterol or their bile salt precursors which can then be made unavailable for absorption. Most saponins form insoluble complexes with 3 $\beta$ -hydroxysteroids and are known to form large, mixed micelles with bile acids and cholesterol (Messina, 1999). Formation of mixed micelles blocks reabsorption of bile acids from the small intestine in the rat (Oakenfull, 1986; Sidhu and Oakenfull, 1986). Further, in animal feeding trials, increased faecal excretion of bile acids has been observed in response to dietary saponins (Oakenfull, 1986).

In humans, saponins are reported to assist in the prevention of cardiovascular diseases by lowering plasma cholesterol concentrations through the excretion of cholesterol (Johnson *et al.*, 1986). Saponins cause a depletion of body cholesterol by reducing its reabsorption, thus increasing its excretion in much the same way as other cholesterol-lowering drugs, such as cholestyramine (Malinow *et al.*, 1985).

#### ***Effects of saponins on enzymes and general body metabolism***

Saponins have been shown to have direct effects on enzyme activities. The alfalfa saponins, for example, have been shown to inhibit respiration in the isolated rat diaphragm which has been explained by the inhibition of succinate oxidase in the liver (Jackson and Shaw, 1954). In addition, alfalfa saponins cause inhibition of trypsin and chymotrypsin *in vitro*, although the physiological significance of this *in vitro* inhibition has been questioned (Ishaaya and Birk, 1965). Similarly, ginseng saponins have been reported to inhibit the Na<sup>+</sup>-K<sup>+</sup>-ATPase from dog cardiac sarcolemma, apparently by direct interaction with a membrane bound enzyme (Lee *et al.*, 1986). Crude ginseng root saponins have been found to have powerful effects on carbohydrate and lipid metabolism (Avakadian *et al.*, 1984).

Saponins have been acclaimed for their ability to reduce skeletal muscle fatigue from exercise (Voces *et al.*, 2004). This action is probably due to the conservation of endogenous carbohydrate stores with lower production of lactate and pyruvate coupled with increased oxidation of fatty acids (Southon *et al.*, 1988). In most instances, saponins interact with cholesterol molecules in the sarcolemma and the transverse-tubular system of muscle fibres causing perforations in the membranes (Endo and Kitazawa, 1978). Treatment with 10  $\mu$ g/ml saponin greatly reduced the SR Ca<sup>2+</sup> loading ability of skinned fibres from the extensor *digitorum longus* muscle of rat with a rate constant of 0.24/min (Launikonis and Stephenson, 1997). Saponin concentrations up to 150  $\mu$ g/ml as well as increased exposure time up to 30 min did not further reduce the SR Ca<sup>2+</sup> loading ability of the SR, which

indicates that the inhibitory action of 10-150 µg/ml saponin is not dose dependent (Launikonis and Stephenson, 1997). Relatively low concentrations of saponin cause inhibition of the skeletal SR Ca<sup>2+</sup> loading ability in a species dependent manner, probably by increasing the Ca<sup>2+</sup> loss through SR Ca<sup>2+</sup> release channels (Launikonis and Stephenson, 1997).

Ginseng saponin is also reported to increase mitotic activity in the bone marrow and can thus protect animals against radiation injury (Milgate, 1995). Further, ginseng saponin may protect against liver damage from carbon tetrachloride, galactosamine or ethanol (Hiai *et al.*, 1971). However, there are other saponins such as cauloside A and C, plasticodoside C and hederagenin that have been demonstrated to strongly inhibit protein synthesis in bone marrow (Anisimov *et al.*, 1978).

#### ***Effects of saponins on smooth muscle activity***

Lindahl *et al.* (1957) examined the effects of alfalfa saponin on the motility of smooth muscle and reported that intraruminal and *intravenous* administration of saponins to sheep resulted in a pronounced reduction in ruminal motility. Alfalfa saponins were also found to inhibit eructation, with evidence that direct effects on the central nervous system were involved. The significance of these observations lies in their relationship to the bloat situation in addition to a possible role in stable foam formation (Cheeke, 1971). Saponins could interfere with loss of gas from the rumen by inhibiting eructation (Cheeke, 1971).

#### ***Reproductive effects of saponins***

Saponins have abortifacient, antizygotic and antiimplantation properties (Tewary *et al.*, 1973). Consequently, saponins from various plants have untoward effects on animal reproduction with attending significant reduction in animal productivity (Stolzenberg and Parkhurst, 1976). Saponins from *Gutierrezia* sp. and *Agave lechuguilla* have been reported to cause abortion in rabbits, goats and cows when administered intravenously at concentrations above 2.3 mg/kg body weight (Dollahite *et al.*, 1962). Extract of *Mussaenda pubescens* is capable of terminating pregnancy in rats and is used traditionally as contraceptive in the Fujian province of China (Quin and Xu, 1998). Saponins have been shown to have both positive and negative effects on the viability of human sperm cells *in vitro* with some ginseng saponins increasing motility as well as progression of sperm (Chen and Zhu, 1998) while *Sesbania sesban* saponins were spermicidal at 1.0–1.3 mg/ml (Dorsaz *et al.*, 1988). Saponin-rich extracts of *Turnera diffusa* and *Pfaffia paniculata* have been reported to improve the copulatory performance of sexually sluggish or impotent rats, but are apparently ineffective in sexually potent rats (Arletti *et al.*, 1999).

### ***Antioxidant effects of saponins***

A number of saponins have been found to have antioxidant effects. For instance, certain soyasaponins, have been reported to contain an antioxidant moiety attached at C<sub>23</sub> (Yoshiki *et al.*, 1998). This moiety, allows saponins to scavenge superoxides by forming hydroperoxide intermediates, thus preventing tissue destruction by free radicals (Hu *et al.*, 2002). The antioxidant action of Vietnamese ginseng saponin against free-radical mediated cellular damage was examined *in vitro* and the Vietnamese ginseng was suggested to be protective against free-radical-induced injury (Huong *et al.*, 1998). Yoshiki and Okubo (1995) have also reported active oxygen-scavenging activity of saponins.

### ***Effects of saponins on ruminant digestion***

A large number of plants containing saponins are forages eaten by ruminants and produce both beneficial and deleterious effects on ruminant digestive physiology with profound consequences on production (Francis *et al.*, 2002). In these ruminants, and other domestic animals, the dietary saponins have significant effects on all phases of metabolism, from the ingestion of feed to the excretion of wastes (Cheeke, 1996). Usually the plants, containing saponins, themselves are not used commercially as animal feed; rather, the saponins are extracted from plant parts and used as feed additives (Francis *et al.*, 2002). For example, extract of *Yucca schidigera* a desert plant native to the southwestern United States and Mexico (Cheeke, 2000) contains 4.4% of steroid saponins and has been used widely as feed additives (Guo *et al.*, 2000).

Saponins kill rumen protozoa by forming complexes with sterols in the protozoal membrane surface (Wang *et al.*, 2012). This is advantageous because rumen protozoa such as *Epidinium caudatum* and *Eudiplodinium maggii* have been observed to engulf bacteria *in vitro* and generally cause rapid intrarumen nitrogen cycling, and excess ammonia excretion in the urine (Kanjanapruthipong and Leng, 1998). The addition of *Enterolobium cyclocarpum*, a plant with high saponin content to sheep feed has been reported to decrease protozoal counts in sheep rumen without changing the composition of protozoa community; *Entodinium* is the most predominant species (92%), followed by *Dasytricha* (3%), *Polyplastron* + *Eremoplastron* (2.2%), and *Isotricha* (2%) (Ivan *et al.*, 2004). Rosales *et al.* (1989) earlier reported a similar occurrence in a Holstein cow fed *Enterolobium cyclocarpum* where the predominant protozoa was *Entodinium* sp. (80%) followed by *Diplodinium* sp. (18%) and *Eudiplodinium* sp., and *Isotricha prostoma* were only 1% of the total protozoa.

Saponin from *Sapindus saponaria* fruit has been reported to have a negative effect on rumen protozoa (Hess *et al.*, 2003); depressing protozoal numbers in the rumen of Swiss White Hill sheep (Hess *et al.*, 2004) but not in that of African sheep (Abreu *et al.*, 2004). This variability may be due to

an adaptation of the microbial population to saponin, the previous experience of the animal to saponin, or both (Wallace *et al.*, 2002). Several other saponin-containing plants such as *Acacia auriculiformis*, *Camellia sinensis*, *Phytolacca dodecandra*, *Samanea saman*, and *Sesbania pachycarpa* exerted considerably high antiprotozoal activity in the *in vitro* rumen fermentation (Muetzel *et al.*, 2003).

A positive effect of *Yucca* saponins in ruminant nutrition has been attributed to the enhancement of the entrapment of  $\text{NH}_4\text{N}$  from urea-supplemented straw (Makkar *et al.*, 1999). This increases the availability of nutrients to rumen bacteria and reduces environmental damage by decreasing losses of ammonium ion to the air. Moreover, supplementation of feed with leaves of *Sesbania sesban*, known for its high saponin content, has been found to have the potential to improve protein flow from the rumen by suppressing protozoal action (Newbold *et al.*, 1997). A significant increase in cellulolytic and total bacteria population in the rumens of sheep fed with *Sapindus saponaria* fruit has been reported (Diaz *et al.*, 1993). Similarly, Thalib *et al.* (1996) reported that total cellulolytic bacteria increased when sheep were orally ingested with a methanol extract of *Sapindus rarak*. However, the positive effects of saponins were more pronounced when administered directly into the rumen than when added as feed supplement (Odenyo *et al.*, 1997).

### **Pharmacological Effects of Saponins**

Saponins have been ascribed a number of pharmacological actions (Fuchs *et al.*, 2009), the important ones being, hypocholesterolaemic and abortifacient effects (Francis *et al.*, 2002), immunomodulatory potential, anticancer effect, adjuvant properties for vaccines as immunostimulatory complexes, and synergistic enhancement of the toxicity of immunotoxins (Heisler *et al.*, 2005; Bachran *et al.*, 2008). Interestingly, the wide range of chemical and physical properties of saponins is related to the extent and range of their pharmacological effects (Price and Fenwick, 1990; Just *et al.*, 1998; Chao *et al.*, 1998).

#### ***Saponins as anti-inflammatory agents***

Saponins from *Bupleurum fruitescens* have been shown to exhibit anti-inflammatory activity. These saponins are active against carrageenan and tetradecanoyl phorbol acetate-induced acute oedemas (Just *et al.*, 1998). Accordingly, Ong (2004) suggested that saponins can be used as anti-inflammatory agents and in the treatment for tuberculosis.

#### ***Saponins as diuretic agents***

Saponins of *Vigna radiata*, *Vigna mungo*, *Vigna sinensis* and *Antidesma menasu* have been shown to stimulate diuresis (Rizvi *et al.*, 1980).

### ***Saponins as hypoglycaemic and antidiabetic agents***

Saponins isolated from plants such as *Pueraria thunbergiana* (Lee *et al.*, 2000), and *Calendula officinalis* (Yoshikawa *et al.*, 2001) have been reported to have hypoglycaemic effects. An anti-diabetic, which contains saponins, tannins, vitamins B<sub>12</sub> and B<sub>2</sub> as main components, was obtained from dried powder of *Pittosporum* plant (Yamaguchi, 1993).

### ***Saponins as anti-ulcer agents***

Carbenoxolone, the succinic acid derivative of glycyrrhetic acid is effective in the management of peptic ulcer patients and was also found to be effective against gastric ulcers (Zhang and Hu, 1985). Triterpenoid saponins from the leaves of *Pyrenacantha standtii* was shown to exhibit dose dependent anti-ulcerogenic properties comparable to the anti-ulcer drug cimetidine (Aguwa and Okonji, 1986). Aescine, the acid triterpene glycoside isolated from the dried seeds of the horse chestnut, was demonstrated to be anti-ulcerogenic to rats with cold restraint-induced and ethanol-induced ulcers (Marhuenda *et al.*, 2003).

### ***Saponins as Immunomodulatory agents and role in vaccine production***

Saponin-based adjuvants have the unique ability to stimulate the cell-mediated arm of the immune system, as well as enhance antibody production (Oda *et al.*, 2000). Amoros *et al.* (1987) reported that saponins inhibit virus attachment to cells and suggested that this active biological compound can be used to compete with virus for the binding site at the cell's receptor. Saponins induce a strong adjuvant effect to T-dependent as well as T-independent antigens. Moreover, saponins induce strong cytotoxic CD<sup>8+</sup> lymphocyte responses and potentiate the response to mucosal antigens (Kensil, 1996).

Saponins have stimulatory effects on the components of specific immunity and some non-specific immune reactions such as inflammation (de Oliveira *et al.*, 2001; Haridas *et al.*, 2001) and monocyte proliferation (Delmas *et al.*, 2000; Yui *et al.*, 2001). Quillaja and other saponins have been reported to increase immune-cell proliferation *in vitro* (Lacaille-Dubois *et al.*, 1999). Moreover, immune-stimulating complexes formulated with Quillaja saponin preparations induced specific cytotoxic T-lymphocyte responses (Coulter *et al.*, 1998) and have been reported to induce antibody responses and/or protective immunity in cat, cow, dog, horse, turkey, monkeys, pig, sheep, rabbit, guinea-pig, turkey and seal (Mowat *et al.*, 1999). Oral administration of Panax ginseng (Jie *et al.*, 1984) and *Lonicera japonica* (Lee *et al.*, 1998) have also been reported to stimulate immune responses *in vivo*. Further, ginseng extract are reported to significantly increase the blood polymorphonuclear leukocyte phagocytosis (Scaglione *et al.*, 1990), lymphocyte

proliferation (Wu *et al.*, 1991), interferon-gamma and tumour necrosis factor production (Smolina *et al.*, 2001).

Astragalus, a Chinese traditional herb, containing triterpene saponins is thought to strengthen and boost the immune system by potentiating macrophage activity (Clement-Kruze *et al.*, 2008). Laboratory studies have found Astragalus to increase number and phagocytic activity of macrophages, phagocytosis, natural killer cell activity, interferon production and transformation of T-cells (Chang and But, 1986). Astragalus saponin is believed to induce the cellular and humoral immune responses with slight haemolytic activity (Rajput *et al.*, 2007). Yang *et al.* (2005) reported very low haemolytic effect (0.66%) with 500 µg/ml concentration of *Astragalus membranaceus* saponin (AMS). Yesilada *et al.* (2005) investigated the effect of 13 cycloartane- and 1 oleanan-type triterpene saponins isolated from Turkish species including *Astragalus brachypterus*, *A. cephalotes*, *A. microcephalus*, and *A. trojanus*, as well as methanol extracts from the roots of *A. cephalotes*, *A. oleifolius* and *A. trojanus* on *in vitro* cytokine release. All the triterpene saponins evaluated showed a significant interleukin-2 (IL-2) inducing activity between 35.9% and 139.6%. Of all the extracts tested, *Astragalus oleifolius* showed the highest IL-2 inducing potentials. In addition, Astragalus herbal mixture stimulated macrophages to produce interleukin-6 and tumor necrosis factor (Yoshida *et al.*, 1997).

Saponins have been widely used as adjuvants for many years and have been included in several veterinary vaccines (Rajput *et al.*, 2007). The adjuvant action of saponins was, however, not so pronounced in some of the non-Fmammalian species tested (Grayson *et al.*, 1987).

### ***Antimicrobial activities***

The first systemic investigations into the antimicrobial activity of saponins were reported by Tschesche and Wulff (1973). These investigations confirmed the weak antibacterial and fungistatic effects of the majority of saponins. Based on thorough investigations, saponins were classified into three groups according to their antibiotic activity. The monodesmosidic spirostanol saponins belonging to the first group have strong fungistatic activity but the antibacterial activity is practically absent. Ginseng saponins showed strong inhibitory activities against the aflatoxin-producing fungus *Aspergillus parasiticus*. Addition of 0.3% saponins of red ginseng to *A. parasiticus* on the 9<sup>th</sup> day of culture, caused a decrease in the growth (62.3%), decreases in aflatoxin B<sub>1</sub> production (38.7%) and in aflatoxin G<sub>1</sub> production (22.9%) while an addition of 0.05% ginseng saponins to the culture medium resulted in a significant decrease in aflatoxin production, the addition of 5% of it produced complete inhibition of aflatoxin production (Jun *et al.*, 1989).

Saponins from soybean were reported to have antiviral activity against Human Immunodeficiency Virus (HIV) *in vitro* (Okubo *et al.*, 1994). These

saponins were divided into 3 groups based on their respective type of aglycone, A<sub>1</sub>, B and E. Bb, a major constituent of group B saponins, completely inhibited HIV-induced cytopathic effects and virus-specific antigen expression 6 days after infection at concentrations greater than 0.25 mg/ml, but it exhibited no direct effect on HIV reverse transcriptase activity (Okubo *et al.*, 1994). Soetan *et al.* (2006) evaluated the antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench and reported that the saponins extract had inhibitory action on gram positive organism but no inhibitory action on gram negative organism and fungi tested.

#### ***Saponins as hepatoprotective agents***

Saponins from the roots of *Pueraria lobata* showed hepatoprotective action *in vitro* (Arao *et al.*, 1998). Structure-activity relationships for the sapogenol moiety suggested that the OH group at C<sub>29</sub> would reduce the hepatoprotective activity while the OH group at C<sub>21</sub> could enhance the protective activity (Kinjo *et al.*, 1999). Furthermore, structure-activity relationships for the sugar moiety suggested that the oxygen-bearing group at C5 would enhance the hepatoprotective activity though the configuration of the OH group at C<sub>3</sub> could be less important for hepatoprotective activity (Kinjo *et al.*, 1999).

#### ***Saponins as blood lipid lowering agents***

Oats have well-documented blood-cholesterol-lowering effects (Ripsin and Keenan, 1992). There is evidence that the soluble fibre in oats is responsible for this effect (Shinnick *et al.*, 1990). However, another constituent in oats which could affect the cholesterol concentration in blood is the saponins. Onning and Asp (1994) investigated the effects of oat saponins on plasma and liver lipids in gerbils and rats, and reported that oat saponins had minor effects on lipid metabolism in these animals. Bile acid excretion is increased and the blood cholesterol level reduced when rats, pigs and humans are fed saponin-rich diets (Potter *et al.*, 1980; Topping *et al.* 1980; Oakenfull *et al.*, 1984)

#### ***Saponins as cytotoxic and antitumour agents***

Cytotoxic activity has been described for saponins and a large number of saponins have long been established as cytotoxic agents (Das and Mahato, 1983; Podolak *et al.*, 2010). These saponins were observed to inhibit the growth of cells *in vitro* without destroying them, and their cytotoxic potency is structure-dependent (Agarwal and Rastogi, 1974). Many antitumour pharmaceutical preparations used for the chemotherapeutic management of various types of cancer contain saponins in their chemical formulations. Chief among these are ginseng, quillaia and gypsophila saponins (Oteake *et al.*, 1987) that have been shown to inhibit the growth of both benign and malignant tumours (Agarwal and Rastogi, 1974; Schopke and Hiller, 1990). Ginseng protopanaxatriol saponins were found to have anti-metastatic effect

in mice that were transplanted with melanoma cells following oral administration (Wakabayashi *et al.*, 1997). Similarly, ginsenosides saponins, isolated from *Panax notoginseng* have been found to be valuable in preventing cisplatin-induced nephrotoxicity (Liu and Zhou, 2000).

Several molecular mechanisms of anticancer activity for ginsenosides exist and these mechanisms appear to collectively converge on various signaling pathways. The pathways include induction of apoptosis, regulation of cell cycle, prohibition of invasion, reduction of inflammatory response and inhibition of angiogenesis (Hu *et al.*, 2010). A number of cell cycle proteins and apoptosis-related proteins, protein kinases, transcription factors and growth factors are affected by ginsenosides (Dinda *et al.*, 2010). For example, Rh2 and Rg3 inhibit cancer cell proliferation by inducing gene and protein expression of the cell cycle regulatory protein p21, thereby arresting tumor cell cycle progression by inducing cancer cell apoptosis through activation of caspase-3 protease and by sensitizing multidrug-resistant tumor cells to chemotherapy (Tong *et al.*, 2011; Kang *et al.*, 2011).

Evaluation of the immunosuppressive activity of a triterpene saikosaponin a from *Bupleurum falcatum* on the concanavalin A revealed that saikosaponin a stimulated mouse CD<sup>3+</sup> T cells, isolated from the lymph node of BALB/c mice (Sun *et al.*, 2009). Triterpene saikosaponin also had a potent antiproliferative action, and also significantly inhibited cell activation in a concentration-dependent manner. Moreover, saikosaponin also actively suppressed the productions of interferon- $\gamma$ , interleukin-2 and tumour necrosis factor- $\alpha$  in T cells stimulated by concanavalin A. Saikosaponin is also arrested by the G<sub>0</sub>/G<sub>1</sub> phase of the activated T cells, and these effect was due to the down-regulation of CDK6 and cyclin D3 protein level and up-regulation of p27<sup>kip</sup> protein level (Sun *et al.*, 2009).

The importance of the number of sugar moieties in a sugar chain and the structure of aglycone in relation to the cytotoxic activity of saponins was confirmed by the studies on saponins isolated from *Agave fourcroydes*. Chlorogenin, hecogenin and tigogenin hexasaccharides (with the same sequence of sugar chains) exhibited cytotoxic activities against HeLa cells (IC<sub>50</sub> 13.1, 5.2, and 4.8  $\mu\text{g ml}^{-1}$ , respectively), while diglucosides were inactive (Ohtsuki *et al.*, 2004).

One main problem associated with the use of saponins as antitumor agents is their high toxicity, which is accompanied with a misleading correlation between *in vitro* and *in vivo* data, and complicates the possible use of saponins as cytotoxic agents in the clinical setting (Gauthier *et al.*, 2008). However, there have been sporadic reports on the structure-activity relationship, suggesting a correlation between the cytotoxic effects and certain structural features (Thakur *et al.*, 2011). For example, the monodesmosidic saponins are usually hemolytic and more cytotoxic than the bisdesmosidic saponins (Acharya *et al.*, 2009).

### ***Saponins as anti-amnestic agents***

Ginseng saponins have been shown to demonstrate anti-amnesia in mice. These saponins improved learning processes and memory retention in either passive or conditioned avoidance-tasks in experimental animals (Zhang and Hu, 1985).

### ***Saponins as anti-aging agents***

Saponins from the stalk and leaf of *Panax notoginseng* given to *Drosophila melanogaster* prolonged the lifespan and the flying capability, but lowered the lipofuscin content in the head of mice (Jiang *et al.*, 2007). The saponins inhibited the lipid peroxide formation in tissues and elevated the blood and brain superoxide dismutase activity (Hongping *et al.*, 1993). These results suggest that anti-aging activity of saponins is related to their free-radical scavenging action (Hongping *et al.*, 1993).

### ***Saponins as anthelmintic agents***

Triterpenoid saponins from *Zygophyllum* are used in traditional medicine as an anthelmintic agent (Elgamas *et al.*, 1995). Saponins of *Pennisetum galucum* inhibited the hatching of the bovine nematode eggs *in vitro* (Soetan and Lasisi, 2008). Lasisi *et al.* (2003) earlier reported the anthelmintic effect of condensed saponins from *Sorghum bicolor* on the hatching of bovine nematodes *in vitro*.

## **CONCLUSIONS**

In view of the numerous biological effects of saponins in humans and animals, it is recommended that more studies should be carried out on the screening of saponins from food and herbal plants for their biological and beneficial effects for the use of humans and animals. Studies on the structural characterization of saponins from plants is also recommended.

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