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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 prostrate* 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).

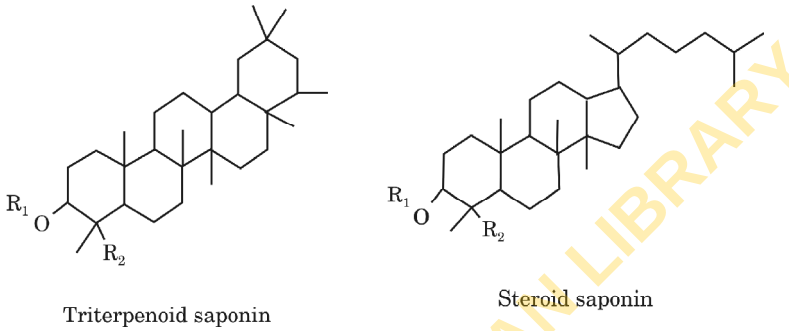

Fig. 1: Structure of triterpenoid and steroid saponins *(Source*: Francis *et al.,* 2002)

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 hypothetic 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)

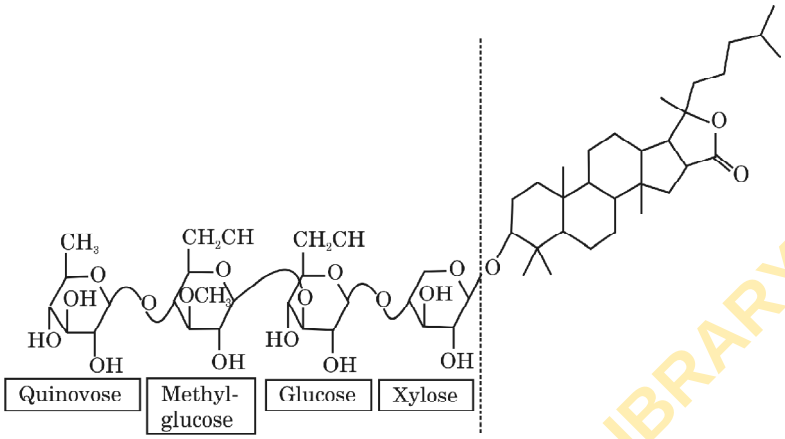

Fig. 2: Molecular structure of a hypothetic saponin composed of an aglycone and a linear glycosidic chain constituted by the four most frequent monosaccharides (Glucose, xylose, Methyl-glucose, quinovose) found in saponins *Source*: (Caulier *et al*., 2011).

(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_{450} 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. (Osbourn, 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 (Parkhust *et al*., 1973; Hosttetman, 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 $mgkg^{-1}$ 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 gatrointestinal 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 ginsenocides 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 3β-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^+-K^+-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 ug/ml saponin greatly reduced the SR Ca^{2+} 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 sg/ml as well as increased exposure time up to 30 min did not further reduce the SR Ca^{2+} loading ability of the SR, which

indicates that the inhibitory action of 10-150 jug/ml saponin is not dose dependent (Launikonis and Stephenson, 1997). Relatively low concentrations of saponin cause inhibition of the skeletal SR Ca^{2+} loading ability in a species dependent manner, probably by increasing the Ca^{2+} loss through SR Ca^{2+} 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_{23} (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 NH_4-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 fruitcescens* 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_{12} and B_2 as main components, was obtained from dried powder of *Pittosporum* plant (Yamaguchi, 1993).

Saponns as anti-ulcer agents

Carbenoxolone, the succinic acid derivative of glycyrrhetinic 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^{8+} 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^{th} day of culture, caused a decrease in the growth (62.3%), decreases in aflatoxin B_1 production (38.7%) and in aflatoxin G_1 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_1, 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_{29} would reduce the hepatoprotective activity while the OH group at C_{21} 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_3 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^{3+} 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-γ, interleukin-2 and tumour necrosis factor-α in T cells stimulated by concanavalin A. Saikosaponin is also arrested by the G_0/G_1 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 $p27^{kip}$ 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_{50} 13.1, 5.2, and 4.8 µg 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-amnesis 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.

REFERENCES

Abe, I., Rohmer, M. and Prestwich, G.C. (1993). Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. *Chem. Rev*., 93: 2189–2206.

Abreu, A., Carulla, J.E., Lascano, C.E., Diaz, T.E., Kreuzer, M. and Hess, H.D. (2004). Effects of *Sapindus saponaria* fruits on rumen fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. *J. Anim. Sci*., 82: 1392–1400.

Abd-El-Wahab, R.H., Zaghloul, M.S., Kamel, W.M. and Moustafa, A.R.A. (2008). Diversity and distribution of medicinal plants in North Sinai, Egypt. *Afri. J. Env. Sci. Tech.,* 2(7): 157–171.

Acharya, D., Mitaine-Offer, A.C. and Kaushik, N. (2009). Cytotoxic spirostane type saponins from the roots of *Chlorophytum borivilianum*. *J. Nat. Prod*., 72(1): 177–181.

Agarwal, S.K. and Rastogi, R.P. (1974). Triterpenoid saponins and their genins. *Phytochemistry*, 13: 2623.

Aguwa, C.N. and Okonji, C.O. 1986. Antifungal effects of extracts of some Nigerian herbal plants. *J. Ethnopharmacol*., 15: 45.

Arletti, R., Benelli, A., Cavazzuti, E., Scarpetta, G. and Bertolini, A. (1999). Stimulating property of *Turnera diffusa* and *Pfaffia paniculata* extracts on the sexual behavior of male rats. *Psychopharmacology,* 143: 15–19.

Amoros, M., Fauconnier, B. and Girre, R.L. (1987). *In vitro* antiviral activity of a saponin from *Anagallis arvensis*, Primulaceae, against herpes simplex virus and poliovirus. *Antivir. Res*., 8(1): 13–25.

Anisimov, M.M., Prokofieva, N.G. and Strigma, L.T. (1978). Comparative study antioxidant activity of Vietnamese Ginseng saponin and its components. *Biol. Pharm. Bull.*, 21(9): 978–981.

Applebaum, S.W., Marco, S. and Birk, Y. (1969). Saponins as possible factors of resistance of legume seeds to attack of insects. *J. Agric. Food Chem*., 17: 618.

Arao, T., Udayama, M., Kinjo, J. and Nohara, T. (1998). Preventive effects of saponins from the *Pueraria lobata* root on *in vitro* immunological liver injury of rat primary hepatocyte culture. *Planta Medica*, 64(5): 413–416.

Armah, C.N., Mackie, A.R., Roy, C., Price, K., Osbourn, A.E., Bowyer, P. and Ladha, S. (1999). The membrane-permeabilizing effect of avenacin A-1 involves the reorganization of bilayer cholesterol. *Biophys. J*., 76: 281–290.

Augustin, J.M., Kuzina, V., Andersen, S.B. and Bak, S. (2011). Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry*, 72: 435–457.

Avakadian, E.V., Sugimoto, R.B. and Taguchi, S. (1984). Effects of *Panax ginseng* extract on energy metabolism during exercise in rats. *Planta Medica,* 50: 151.

Berrang, B., Davies, K.H. and Wall, M.E. (1974). Saponins of two Alfalfa cultivars. *Phytochemistry,* 13: 2253.

Bachran, C., Heisler, I. and Bachran, D. (2008). Chimeric toxins inhibit growth of primary oral squamous cell carcinoma cells. *Cancer Biol. Ther*., 7(2): 237–242.

Baumann, E., Stoya, G., Volkner, A., Richter, W., Lemke, C. and Linss, W. (2000). Hemolysis of human erythrocytes with saponin affects the membrane structure. *Acta Histochem*., 102: 21–35.

Chaieb, I. (2010). Saponins as insecticides: A review. *Tunisian Journal of Plant Protection*, 5(1): 39–50.

Chang, H.M. and But, P.P. (1986). Pharmacology and Applications of Chinese Materia Medica, Vol. 1. Singapore: World Scientific, pp. 10–41.

Chao, A.C., Nguyen, J.V., Broughall, M., Rechia, J., Kensil, C.R., Daddona, P.E. and Fix, J.A. (1998). Enhancement of intestinal model compound transport by DS-1, a modified Quillaia Saponin. *J. Pharm. Sci.*, 87(11): 1395–1399.

Cheeke, P.R. (1996). Biological effects of feed and forage saponins and their impact on animal production. *In*: Saponins used in food and agriculture. [Waller, G.R. and Yamasaki, Y. *Eds*.,] New York: Plenum Press, pp. 377–386.

Cheeke, P.R. (2000). Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. *Proc. Am. Soc. Anim. Sci.* http://www.asas.org/ JAS/symposia/proceeding/0909.pdf.

Cheeke, P.R. (1971). Nutritional and physiological implications of saponins. *A review Canad. J. Ani. Sci*., 51: 621–632.

Chen, Y.P. and Zhu, H.Y. (1998). Studies on the anti-mutation effect of saponin from the seed of *Camellia oleifera* Fujian*. J. Trad. Chin. Med*., 3: 19–20.

Chwalek, M., Lalun, N., Bobichon, H., Ple, K. and Voutquenne-Nazabadioko, L. (2006). Structure–activity relationships of some hederagenin diglycosides: Haemolysis, cytotoxicity and apoptosis induction. *Biochim. Biophys. Acta*, 1760: 1418–1427.

Coulter, A., Wong, T.Y., Drane, D., Bates, J., Macfarlan, R. and Cox, J. (1998). Studies on experimental adjuvanted influenza vaccines: Comparison of immune stimulating complexes (Iscoms (TM)) and oil-in-water vaccines. *Vaccine*, 16: 1243–1253.

Clement-Kruzel, S., Hwang, S.A., Kruzel, M.C., Dasgupta, A. and Actor, J.K. (2008). Immune modulation of macrophage pro-inflammatory response by golden seal and *Astragalus* extracts. *J. Med. Food*, 11(3): 493–8.

Das, M.C. and Mahato, S.B. (1983). Triterpenoids review. *Phytochemistry*, 22: 1071.

de Oliveira, C.A.C., Perez, A.C., Merino, G., Prieto, J.G. and Alvarez, A.I. (2001). Protective effects of *Panax ginseng* on muscle injury and inflammation after eccentric exercise. *Comp. Biochem. Physio.,* 130: 369–377.

Delmas, F., Di Giorgio, C., Elias, R., Gasquet, M., Azas, N., Mshvildadze, V., Dekanosidze, G., Kemertelidze, E. and Timon-David, P. (2000). Antileishmanial activity of three saponins isolated from ivy, alpha-hederin, beta-hederin and hederacolchiside A(1), as compared with their action on mammalian cells cultured *in vitro*. *Planta Medica*, 66: 343–347.

Diaz, A., Avendano, M. and Escobar, A. (1993). Evaluation of *Sapindus saponaria* as a defaunating agent and its effects on different rumen digestion parameters. *Livest. Res. Rural De*v., 5: 1–6.

Dinda, B., Debnath, S., Mohanta, B.C. and Harigaya, Y. (2010). Naturally occurring triterpenoid saponins. *Chem. Biodivers*., 7(10): 2327–2580.

Dollahite, J.W., Shaver, T. and Camp, B.J. (1962). Injected saponins as abortifacients. *Am. J. Vet. Res*., 23: 1261–1263.

Dorsaz, A.C., Hostettmann, M. and Hostettmann, K. (1988). Molluscicidal saponins from *Sesbania sesban*. *Planta Medica*, 54: 225–227.

Dourmashkin, R.R., Dougherty, R.M. and Harris, R.J.C. (1962). Electron microscopic observations on Rous sarcoma virus and cell membranes. *Nature,* 194: 1116–1119.

Elgamah, M.H.A., Shaker, K.H., Pollman, K. and Seifert, K. (1995). *Phytochemistry*, 40(4): 1233–1236.

Endo, M. and Kitazawa, T. (1978). E-C coupling studies on skinned cardiac fibers. *In*: Biophysical aspects of cardiac muscle, *Ed*., Morad, M. Academic Press, New York, pp. 307–327.

Fenwick, G.R., Price, K.R. and Tsukamoto, C. (1991). Saponins: *In*: Mello, F.J., Duffus, C.M. and Duffus, J.H. (1991). Saponins in toxic substances in crop plants. Cambridge: The Royal Society of Chemistry, pp. 285–327.

Francis, G., Kerem, Z., Makkar, H.P. and Becker, K. (2002). The biological action of saponins in animal systems: A review. *Br. J. Nutr*., 88(6): 587–605.

Fuchs, H., Bachran, D. and Panjideh, H. (2009). Saponins as tool for improved targeted tumor therapies. *Curr. Drug Targets*, 10(2): 140–151.

Gauthier, C., Legault, J., Girard-Lalancette, K., Mshvildadze, V. and Pichette, A. (2009). Haemolytic activity, cytotoxicity and membrane cell permeabilization of semisynthetic and natural lupane- and oleanane-type saponins. *Bioorg. Med. Chem*., 17: 2002–2008.

Gee, J.M., Price, K.R., Ridout, C.L., Wortley, G.M., Hurrell, R.F. and Johnson, I.T. (1993). Saponins of quinoa (*Chenopodium quinoa*): Effects of processing on their abundance in quinoa products and their biological effects on intestinal mucosal tissue. *J. Sci. Food and Agric*., 63: 201–209.

Gee, J.M., Wortley, G.M., Johnson, I.T, Price, K.R., Rutten, A.A., Houben, G.F. and Penninks, A.H. (1996). Effect of saponins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and on the integrity of tissue preparations *in vitro*. *Toxico. In Vitro*, 10: 117–128.

Gee, J.M. and Johnson, I.T. (1988). Interraction between haemolytic saponins, bile salts and small intestinal mucosa in the rat. *J. Nutri*., 118: 1391–1397.

Grayson, T.H., Williams, R.J., Wrathmell, A.B., Munn, C.B. and Harris, J.E. (1987). Effects of immunopotentiating agents on the immune response of rainbow trout, *Salmo gairdneri* Richardson, to ERM vaccine. *J. Fish Biol*., 31: 195–202.

Grininger, P. and Fisher, H. (1958). Dietary saponin and plasma cholesterol in the chicken. *Proc. Soc. Exp. Biol. and Med*., 99: 424–426.

Guo, S., Falk, E., Kenne, L., Ronnberg, B. and Sundquist, B.G. (2000). Triterpenoid saponin-containing an acetylated branched D-fucosyl residue from *Quillaja saponaria* Molina. *Phytochemistry*, 53: 861-868.

Haralampidis, K., Trojanowska, M. and Osbourn, A. (2002). Biosynthesis of triterpenoid saponins in plants. *Adv. Biochem. Eng*., 75: 31–49.

Haridas, V., Arntzen, C.J. and Gutterman, J.U. (2001). Avicins, a family of triterpenoid saponins from *Acacia victoria* (Bentham), inhibit activation of nuclear factor-kappa B by inhibiting both its nuclear localization and ability to bind DNA. *Proc. Nat. Acad. Sci*., 98: 11557–11562.

Heisler, I., Sutherland, M. and Bachran, C. (2005). Combined application of saponin and chimeric toxins drastically enhances the targeted cytotoxicity on tumor cells. *J. Control. Release*, 106(1–2): 123–137.

Hess, H.D., Beuret, R.A., Lo¨tscher, M., Hindrichsen, I.K., Machmu¨ller, A., Carulla, J.E., Lascano, C.E. and Kreuzer, M. (2004). Rumen fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with *Sapindus saponaria* fruits and *Cratylia argentea* foliage. *Anim. Sci*., 79: 177–189.

Hess, H.D., Monsalve, L.M., Lascano, C.E., Carulla, J.E., Diaz, T.E. and Kreuzer, M. (2003). Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: Effects on *in vitro* rumen nitrogen turnover and methanogenesis. *Aust. J. Agric. Res*., 54: 703–713.

Hiai, S., Oura, H., Tsuhada, T. and Hirai, J. (1971). Stimulating effect of *Panax ginseng* extract on RNA polymerase activity in Rat liver nuclei. *Chem. Pharm. Bull*., 19: 1956.

Holstein, S.A. and Hohl, R.J. (2004). Isoprenoids: Remarkable diversity of form and function. *Lipids*, 39: 293–309.

Hongping, P., Ling, H., Zhan, Z., Gang, L., Xijun, H. and Zhiming, H. (1993). Experimental studies on anti-aging effect of total saponin from stalk and leaf sanchi (*Panax natoginseng*). *Chem. Abs*., 120(11): 95.

Hostetmann, K. (1987). Toxicity of *Phytolacca dodecandra* to the watersnail *Biophalaria glabrata*. *Bull. Sci. Frib. Sc. Nat*., 76: 51.

Hu, X.Y., Zhang, W.Q., Fang, J.Y., Cai, W.M. and Tang, Z.C. (2002). Chitosan treatment raises the accumulation of saponin and the transcriptional level of genes encoding the key enzymes of saponin synthesis in cultured Panax ginseng cells. *Journal of Plant Physiology and Molecular Biology*, 28: 485–490.

Hu, K., Berenjian, S. and Larsson, R. (2010). Nanoparticulate *Quillaja* saponin induces apoptosis in human leukemia cell lines with a high therapeutic index. *Int. J. Nanomedicine*, 5: 51–62.

Huong, N.T., Matsumoto, K., Kasai, R., Yamasaki, K. and Watanabe, H. (1998). *In vitro* antioxidant activity of Vietnamese Ginseng saponin and its components. *Biol. Pharm. Bull.,* 21(9): 978–81.

Ishaaya, I. and Birk, Y. (1965). Soybean saponins: The soybean saponins on certain enzymes. *J. Food Sci*., 30: 118.

Ivan, M., Koenig, K.M., Teferedegne, B., Newbold, C.J., Entz, T., Rode, L.M. and Ibrahim, M. (2004). Effect of the dietary *Enterolobium cyclocarpum* foliage on the population dynamics of rumen ciliate protozoa in sheep. *Small Rum. Res.,* 52: 81–91.

Jackson, K. and Shaw, T. (1954). Chemical and biological properties of a respiratory inhibitor from alfalfa saponin. *Arch. Biochem. Biophys*., 84: 411.

Jenkins, K.J. and Atwal, A.S. (1994). Effects of dietary saponins on fecal bile acids and neutral sterols and availability of vitamins A and E in the chick. *J. Nutri. Biochem*., 5: 134–138.

Jiang, T., Huang, B.K., Zhang, Q.Y., Han, T., Zheng, H.C. and Qin, L.P. (2007). Effect of *Liriope* platyphylla total saponin on learning, memory and metabolites in aging mice induced by D-galactose *Zhong Xi Yi Jie He Xue Bao*, 5(6): 670–4.

Jie, Y.H., Cammisuli, S. and Baggiolini, M. (1984). Immunomodulatory effects of Panax ginseng in the mouse. *Agents and Actions*, 15: 386–391.

Johnson, I.T., Gee, J.M., Price, K., Curl, C. and Fenwick, G.R. (1986). Influence of saponin on gut permeability and active nutrient transport *in vitro*. *Journal of Nutrition*, 116: 2270–2277.

Jones, T. and Elliot, G. (1969). Two rapid assays for saponin in individual alfalfa plants. *Crop Science*, 9: 688.

Jun, H.K., Park, K.Y. and Jo, J.B. (1989)). Inhibitory effects of Ginseng saponins on Aflatoxin production in culture. *Chem. Abstr*., pp. 116–199.

Just, M.J., Recsio, M.C., Giner, R.M., Cuellar, M.J., Marez, S., Bilia, A.R. and Rios, J. (1998). Anti-inflammatory activity of unusual Lupane saponins from *Buleurum fruiticescens*. *Planta Medica*, 64(5): 404–407.

Kang, Y.J., Chung, H.J. and Nam, J.W. (2011). Cytotoxic and antineoplastic activity of timosaponin A-III for human colon cancer cells. *J. Nat. Prod.,* 74(4): 701–706.

Kanjanapruthipong, J. and Leng, R.A. (1998). The effects of dietary urea on microbial populations in the rumen of sheep. *AJAS*, 11(6): 661–672.

Kensil, C.R. (1996). Saponins as vaccine adjuvants. *Crit. Rev. Ther. Drug Carrier Syst*., 13(1–2): 1–55.

Keukens, E.A.J., de Vrije, T., van den Boom, C., de Waard, P., Plasman, H.H., Thiel, F., Chupin, V., Jongen, W.M.F. and de Kruijff, B. (1995). Molecular basis of glycoalkaloid induced membrane disruption. *Biochim. Biophys. Acta*, 1240: 216–228.

Kinch, J., Purcell, S., Uthicke, S. and Friedman, K. (2008). Population status, fisheries and trade of sea cucumbers in the Western Central Pacific. *In*: Toral-Granda, V., Lovatelli, A. and Vasconcellos, M. Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper. No. 516. Rome, FAO, pp. 7–55.

Kinjo, J., Udayama, M., Okawa, M. and Nohara, T. (1999). Study of structure hepatoprotective relationships of oleanene-type triterpenoidal glucuronides obtained from several fabaceous plants on rat primary hepatocyte cultures. *Biol. Pharm. Bull*., 22(2): 203–6.

Knight, A.P. and Walter, R.G. (2003). Plants affecting the skin and liver. *In*: A Guide to Plant Poisoning of Animals in North America, Teton New Media, Jackson WY.

Lacaille-Dubois, M.A., Hanquet, B., Cui, Z.H., Lou, Z.C. and Wagner, H. (1999). A new biologically active acylated triterpene saponin from *Silene fortunei*. *J. Nat. Prod*., 62: 133–136.

Lasisi, O.T., Soetan, K.O., Fafunso, M.A. and Aiyelaagbe, O.O. (2003). Effect of condensed saponins on egg hatching of bovine gastrointestinal nematodes *in vitro*. *Nig. Vet. J*., 24(3): 30–34.

Lasztity, R., Hidvegi, M. and Bata, A. (1998). Saponins in food. *Food Re*v. *Int.*, 14: 371–390.

Launikonis, B.S. and Stephenson, D.G. (1997). Effect of saponin treatment on the sarcoplasmic reticulum of rat, cane toad and crustacean (yabby) skeletal muscle. *J. Physiol*., 504(2): 425–37.

Lee, S.J., Son, K.H., Chang, H.W., Kang, S.S. and Kim, H.P. (1998). Anti-inflammatory activity of *Lonicera japonica*. *Phytoth. Res*., 12: 445–447.

Lee, S.W., Lee, J.S., Kim, Y.H. and Jim, K.D. (1986). Effect of Ginseng saponin on the Na+/K+ ATPase of dog cardiac sarcolemma. *Arch. Pharm. Res*., 9: 29.

Lee, K.T., Sohn, I.C., Kim, D.H., Choi, J.W. and Kwon, S.H. (2000). Hypo-glycaemic and hypolipidemic effects of tectorigenin and kaika-saponin III in the streptozotocin-induced diabetic rat and their antioxidant activity *in vitro*. *Arch. Pharma. Research,* 23: 461–466

Lindahl, I.L., Davis, R.E. and Tertel, R.T. (1957). Alfalfa saponins: Studies of their chemical, pharmacological and physiological properties in relation to ruminant bloat. *US. Dept. Agric. Tech. Bull*., p. 1161.

Liu, S.J., and Zhou, S.W. (2000). *Panax notoginseng* saponins attenuated cisplatin induced nephrotoxicity. *Acta Pharmacol. Sin*., 21(3): 257–260.

Makkar, H.P.S., Aregheore, E.M. and Becker, K. (1999). Effects of saponins and plant extracts containing saponins on the recovery of ammonia during urea ammoniation of wheat straw and fermentation kinetics of the treated straw. *J. Agri. Sci.*, 132: 313–321.

Malinow, M.R., McNaughty, A.L. and Kohler, G.O. (1985). Effects of synthetic glycosides on cholesterol absorption. *Ann. N.Y. Acad. Sci*., 23: 454.

Marhuenda, M.M.J. and De La, C. (1993). Antiulcerogenic activity of aescine in different experimental models. *Phytoth. Res*., 7(1): 13–16.

Menin, L., Panchichkina, M., Keriel, C., Olivares, J., Braun, U., Seppert, E.K. and Saks, V.A. (2001). Macrocompartmentation of total creatine in cardiomyocytes revisited. *Molec. Cell. Biochem.*, 220: 149–159.

Messina, M.J. (1999). Legumes and soybeans: Overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr*., 70(3): 439–450.

Milgate, J. and Roberts, D.C.K. (1995). The nutritional and biological significance of saponins. *Nutri. Res.*, 15(8): 1223–1249.

Mohamad, T., Hussin, K. and Surif, S. (2001). Tumbuhan beracun di sekelilingkita. Kuala Lumpur: Dewan Bahasa dan Pustaka, pp. 1–12.

Mowat, A.M., Smith, R.E., Donachie, A.M., Furrie, E., Grdic, D. and Lycke, N. (1999). Oral vaccination with immune stimulating complexes. *Immuno. Let.*, 65: 133–140.

Muetzel, S., Hoffmann, E.M. and Becker, K. (2003). Supplementation of barley straw with *Sesbania pachycarpa* leaves *in vitro*: Effects on fermentation variables and rumen microbial concentration structure quantified by ribosomal RNA-targeted probes. *Br. J. Nutr*., 89: 445–453.

Murthy, E.N., Chiranjibi, P., Reddy, C.S. and Raju, V.S. (2010). Piscicidal plants used by Gond tribe of Kawal wildlife sanctuary, Andhra Pradesh, India. *Ind. J. Nat. Prod. Resour*., 1(1): 97–101.

Ndukwe, I.G., Amupitan, J.O., Isah, Y. and Adegoke, K.S. (2007). Phytochemical and antimicrobial screening of the crude extracts from the root, stem, bark and leaves of *Vitellaria paradoxa*. *Afri. J. Biotech*., 6(16): 1905–1909.

Newbold, C.J., El Hassan, S.M., Wang, J., Ortega, M.E. and Wallace, R.J. (1997). Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. *Br. J. Nutr*., 78: 237–249.

Newman, H.A.I., Kummerow, F.A. and Scott, H.M. (1958). Dietary saponins, a factor which may reduce liver and serum cholesterol levels. *Poultry Sci*., 37: 42–46.

Nishikawa, M., Nojim, S., Akiyama, T., Sankawa, U. and Inove, K. (1984). Interaction of digitonin and its analogues with membrane cholesterol. *J. Biochem*., 96: 1231.

Oakenfull, D.G. (1981). Saponins in foods-A review. *Food Chem.,* 6(19): 21–23.

Oakenfull, D.G. (1986). Aggregation of saponins and bile acids in aqueous solution. *Austra. J. Chem*., 39: 1671.

Oakenfull, D.G., Topping, D.L., Illman, R.J. and Fenwick, D.E. (1984). Prevention of dietary hypercholesterolaemia in the rat by soya bean and *Quillaja* saponins. *Nutri. Repo. Inter.,* 29: 1039–1045.

Oda, K., Matsuda, H., Murakami, T., Katayama, S., Ohgitani, T. and Yoshikawa, M. (2000). Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. *Bio. Chem*., 381: 67–74.

Odenyo, A.A., Osuji, P.O., Karanfil, O. and Adine, W.K. (1997). Microbiological evaluation of *Acacia angustissima* as a protein supplement for sheep. *Anim. Feed Sci. Tech*., 65: 99–112.

Ohtsuki, T., Koyano, T. and Kowithayakorn, T. (2004). New chlorogenin hexasaccharide isolated from *Agave fourcroydes* with cytotoxic and cell cycle inhibitory activities. *Bioorg. Med. Chem*., 12: 3841–3845.

Okubo, K., Kudou, S., Uchida, T., Yoshiki, Y., Yoshikoshi, M. and Tonomura, M. (1994). Soyabean saponin and isoflavonoids: Structure and antiviral activity against human immunodeficiency virus *in vitro*. *ACS Symp. Ser.*, 546. *Phytochem. Canc. Prev*.m 1: 330–339.

Ong, H.C. (2004). *Tumbuhan liar. Khasiat ubatan dan kegunaan lain.* Kuala Lumpur: Utusan Publication and Distributor. pp. 6–9.

Onning, G. and Asp, N. (1994). Effect of oat saponins on plasma and liver lipids in gerbils *(Meriones unguiculatus)* and rats. *Br. J. Nutri.,* 73: 275–286.

Osbourn, A.E. (2003). Saponin in cereals. *Phytochem.,* 62: 1–4

Oteake, N., Seto, H., Ra, S., Zeni, F. and Kobayashi, E. (1987). Cytotoxic preparations from *Glycyrrhiza glaba*. Japans patent 62240696. *Chem. Abstr*., 109: 11715.

Peterson, D.W. (1950). Growth of chicks fed high Alfalfa diet or a diet containing *Phytolacca dodecandra*; Oleanoglycotoxin-A. *Phytochem*., 12: 1437–1442.

Pithayanukul, P., Laovachirasuwan, S., Bavovada, R., Pakmanee, N. and Suttisri, R. (2004). Anti-venom potential of butanolic extract of *Eclipta prostrata* against Malayan pit viper venom. *J. Ethnopharmacol*., 90: 347–352.

Plock, A., Sokolowska-Kohler, W. and Presber, W. (2001). Application of flow cytometry and microscopical methods to characterize the effect of herbal drugs on *Leishmania* spp. *Experi. Parasitol*., 97: 141–153.

Podolak, I., Galanty, A. and Sobolewska, D. (2010). Saponins as cytotoxic agents: A review. *Phytochem. Rev*., 9(3): 425–474.

Potter, S.M., Jimenez-Flores, R., Pollack, J., Lone, T.A. and Berber-Jimenez, M.D. (1993). Protein saponin interaction and its influence on blood lipids. *J. Agric. and Food Chem*., 41: 1287–1291.

Potter, J.D., Illman, R.J., Calvert, G.D., Oakenfull, D.G. and Topping, D.L. (1980). Soya saponins, plasma lipids, lipoproteins and faecal bile acids: A double blind cross-over study. *Nutri. Rep. Inter.,* 22: 521–528.

Potter, J.D., Topping, D. and Oakenfull, D. (1979). Soya saponins and plasma *Quilaia* saponin. *J. Nutri*., 42: 597

Price, K.R. and Fenwick, D.E. (1990). The chemistry and biological significance of saponins in foods and feeding stuffs. *Int. Rev. Food Sci. Nutr*., 157: 62.

Quin, G.W. and Xu, R.S. (1998). Recent advances in bioactive natural products from Chinese medicinal plants. *Medical Research Reviews*, 18: 375–382.

Rajput, Z.I., Song-hua, H., Chen-wen, X. and Abdullah, G.A. (2007). Adjuvant effects of saponins on animal immune responses. *J. Zhejiang Univ. Sci*., 8(3): 153–161

Ripsin, C.M. and Keenan, J.M. (1992). The effects of dietary oat products on blood cholesterol.*Trends in Food Science and Technology,* 3: 137–141.

Rizvi, H., Shoeb, A., Kapil, R.S. and Popli, S.P. (1980). Two diuretic triterpenoids from *Antiderma* menasu. *Phytochemistry*, 19(11): 2409–2410

Rosales, M., Laredo, M., Cuesta, A., Anzola, H. and Hernandez, L. (1989). Use of tree foliages in the control of rumen protozoa. *Livest. Res. Rural Dev.,* 1: 79–85.

Sawangjaroen, N., Subhadhirasakul, S., Phongpaichit, S., Siripanth, C., Jamjaroen, K. and Sawangjaroen, K. (2005). The *in vitro* anti-giardial activity of extracts from plants that are used for self - medication by AIDS patients in southern Thailand. *Parasitol. Res*., 95: 17–21.

Scaglione, F., Ferrara, F., Dugnani, S., Falchi, M., Santoro, G. and Fraschini, F. (1990). Immunomodulatory effects of two extracts of *Panax ginseng* C.A. Meyer. *Drugs Exp. Clin. Res*., 16(10): 537–42.

Schopke, T.H. and Hiller, K. (1990). Triterpenoid Saponins. Part 6. *Die Pharmazie*, 45**:** 313–342.

Seeman, P. (1974). Ultrastructure of membrane lesions in immune lysis, osmotic lysis and drug-induced lysis. *Fed. Proc*., 33(10): 2116–24.

Shinn-Pyng, Y., Chun-Hung, L., Tzeng-Gan, S., Pai-Po, L. and Winton, C. (2006). Effect of saponin on hematological and immunological parameters of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture.

Shinnick, F.L., Ink, S.L. and Marlett, J.A. (1990). Dose response to a dietary oat bran fraction in cholesterol-fed rats. *J.Nutri.,* 120: 561–568.

Sidhu, G.S. and Oakenfull, D.G. (1986). A mechanism for the Hypocholesterolaemic activity of saponins. *Br. J. Nutri*., 55: 643–649.

Smolina, T.P., Solovéva, T.F. and Besednova, N.N. (2001). Immunotropic activity of panaxansbioglycans isolated from ginseng. *Antibiot Khimioter*, 46(7): 19–22.

Soetan, K.O. and Lasisi, O.T. (2008). Studies on the anthelmintic activities of saponins in Pearl millet (*Pennisetum glaucum*). *Trop. Vet*., 26(1 and 2): 14–19.

Soetan, K.O., Oyekunle, M.A., Aiyelaagbe, O.O. and Fafunso, M.A. (2006). Evaluation of the antimicrobial activity of saponins extract of *Sorghum bicolor L.* Moench. *African Journal of Biotechnology,* 5(23): 2405–2407.

Southon, S., Johnson, I.T., Gee, J.M. and Price, K.R. (1988). The effect of *Gypsophila* saponins in the diet on mineral status and plasma cholesterol concentration in the rat. *British J. Nutri.,* 59: 49–55.

Stine, K.J., Hercules, R.K., Duff, J.D. and Walker, B.W. (2006). Interaction of the glycoalkaloid tomatine with DMPC and sterol monolayers studied by surface pressure measurements and Brewster angle microscopy. *J. Phys. Chem*., 110: 22220–22229.

Stolzenberg, S.J. and Parkhurst, R.M. (1976). Blastocidal and contraceptive actions by an extract and compounds from endod (*Phytolacca dodecandra*). *Contraception,* 14: 39–51.

Sun, Y., Cai, T.T. and Zhou, X.B. (2009). Saikosaponin a inhibits the proliferation and activation of T cells through cell cycle arrest and induction of apoptosis. *Int. Immunopharmacol*., 9: 978–983

Takechi, M. and Tanaka, Y. (1995). Structure-activity relationship of synthetic methyl urosylate glycosides. *Phytochemistry,* 34: 675.

Takechi, M., Doi, K. and Wakayama, Y. (2003). Biological activities of synthetic saponins and cardiac glycosides. *Phytother. Res*., 17: 83–85.

Tchesche, R. and Wulff, G. (1973). Chemie und Biologie der saponine. *In*: Herz, W., Grisebach, H. and Kirby, G.W. *Eds*., Fortschritte der Chemie Organischer Naturstoffe/ Progress in the Chemistry of organic Natural Products, 30, Springer-Verlag, Wien-New York, pp. 461–606.

Tewary, P.V., Chaturvedi, C. and Pandey, V.B. (1973). Antifertility activity of costus speciosus Sm. *Indian J. Pharmaco.*, 35: 114–115.

Thalib, A., Widiawati, Y., Hamid, H., Suherman, D. and Sabrani, M. (1996). The effects of saponin from *Sapindus rarak* fruit on rumen microbes and performance of sheep. *J. Ilmu Ternak dan Veteriner,* 2: 17–20.

Tong, Q.Y., Qing, Y. and Shu D. (2011). Deltonin, a steroidal saponin, inhibits colon cancer cell growth *in vitro* and tumor growth *in vivo via* induction of apoptosis and antiangiogenesis. *Cell Physiol. Biochem*., 27(3–4): 233–242.

Topping, D.L., Storer, G.B., Calbert, G.D., Illman, R.J., Oakenfull, D.G. and Weller, R.A. (1980). Effects of dietary saponins on faecal bile acids and neutral sterols, plasma lipids, and lipoprotein turnover in the pig. *American J. Clin. Nutri.,* 33: 783–786.

Vincken, J.P., Heng, L., de Groot, A. and Gruppen, H. (2007). Saponins, classification and occurrence in the plant kingdom. *Phytochemistry*, 68(3): 275–297.

Voces, J., Cabral de Oliveira, A.C., Prieto, J.G., Vila, L., Perez, A.C., Duarte, I.D.G. and Alvarez, A.I. (2004). Ginseng administration protects skeletal muscle from oxidative stress induced by acute exercise in rats. *Braz. J. Med. Biol. Res*., 37(12): 1863–1871.

Voutquenne, L., Lavaud, C., Massiot, G. and le Men-Olivier, L. (2002). Structure–activity relationships of haemolytic saponins. *Pharm. Biol*., 40: 253–262.

Wakabayashi, C., Hasegawa, H., Murata, C., Ichiyama, M. and Saiki, I. (1997). Expression of *in vivo* Antimetastatic effect of ginseng protopanaxtriol saponins is mediated by their intestinal bacterial metabolites after oral administration. *Chem. Abstr*., 129(7): 44.

Walker, B.W., Manhanke, N. and Stine, K.J. (2008). Comparison of the interaction of tomatine with mixed monolayers containing phospholipid, egg sphingomyelin and sterol. *Biochim. Biophys. Acta*, 1778: 2244–2257.

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B. and Newbold, C.J. (2002). Natural products as manipulators of rumen fermentation. *Asian-Australas. J. Anim. Sci.*, 15: 1458–1468.

Wang, J.K., Ye, J.A. and Liu, J.X. (2012). Effects of tea saponins on rumen microbiota, rumen fermentation, methane production and growth performance — A review. *Trop. Anim. Health. Prod.*, 44: 697–706.

Wiart, C., Mogana S. Khalifah, S., Mahan, M. and Ismail, S. (2004). Animicrobial screening of plants used for traditional medicine in the Stat of Perak, Peninsular *Malaysia. J. Fitotera*, 75: 68–73.

Woldemichael, G.M. and Wink, M. (2001). Identification and biological activities of triterpenoid saponins from *Chenopodium quinoa. J. Agric. Food Chem*., 49: 2327–2332.

Wu, S., Hua, Z.J., Xio, Y.L. and Wang, Y. (1991). Effect of Ginseng polypeptide on the 3H-TdR integration of human blood lymphocyte. *Chin. Med. J.,* 104(5): 399–401.

Yamaguchi, A. (1993). Antidiabetics containing aqueous extract of *Pittosporum* plants. Jpn. Kokai. Tokyo. Koho. JP 05,271,086. *Chem. Abst*., p. 129.

Yesilada, E., Bedir, E., Calis, I., Takaishi, Y. and Ohmoto, Y. (2005). Effects of triterpene saponins from *Astragalus* species on *in vitro* cytokine release. *J. Ethnopharmacol*., 96(1-2): 71–77.

Yoshida, Y., Wang, M.Q., Shan, B.E. and Yamashita, U. (1997). Immunomodulating activity of Chinese medical herbs and *Oldenlandia diffusa* in particular. *Int. J. Immunopharmacol*., 19(7): 359–370.

Yoshiki, Y. and Okubo, K. (1995). Active oxygen scavenging activity of DDMP saponin in soyabean seed. *Biosci. Biotechn. Biochem*., 59(8): 1556–1557.

Yoshiki, Y., Kudou, S. and Okubo, K. (1998). Relationship between chemical structures and biological activities of triterpenoid saponins from soybean (Review). *Bioscience Biotechnology and Biochemistry*, 62: 2291–2299.

Yoshikawa, M., Murakami, T., Kishi, A., Kageura, T. and Matsuda, H. (2001). Medicinal flowers. III. Marigold (1): Hypoglycaemic, gastric emptying inhibitory and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasapo- nins A, B, C and D, from Egyptian *Calendula officinalis*. *Chemical and Pharmaceutical Bulletin*, 49: 863–870.

Yui, S., Ubukata, K., Hodono, K., Kitahara, M., Mimaki, Y., Kuroda, M., Sashida, Y. and Yamazaki, M. (2001). Macrophage-oriented cytotoxic activity of novel triterpene saponins extracted from roots of *Securidaca inappendiculata*. *International Immunopharmacology*, 1: 1989–2000.

Zhang, S. and Hu, Z. (1985). Anti-ulcerogenic effects of Ginseng flower saponins in the rat. *Zhongyao Tongbao,* 10: 331. *Chem. Abstr*., 104: 512.