POTENT ANTIFUNGAL ACTIVITY OF SAPONINS FROM MEDICINAL PLANTS AGAINST CANDIDA ALBICANS.

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Abstract

Candida albicans, is usually a harmless fungus in healthy individuals, but life-threatening when found in large quantities or in inappropriate parts of the body. It developed resistance against the antifungal drugs fluconazole and amphotericin B, used in an abusive way, by adopting different mechanisms. Medicinal plants, which offer a wide variety of secondary metabolites, especially saponins, have been reported for their large biological activities. These phytochemicals are composed of a hydrophobic part (aglycone) and a hydrophilic one (glycone), which, based on their aglycone part, are classified into either triterpenoid, or steroidal or alkaloidal saponins.

The aim of this study is to investigate the role of saponins as an effective treatment against drug-resistant *C. albicans*.

Broth microdilution and disk diffusion techniques were employed to determine the efficacy of the triterpenoid and steroidal saponins against *C. albicans*. Eventually, these phytochemicals, produced by different medicinal plants, have been found to have potent antifungal activities against *C. albicans*. These properties depend mainly on the type and number of sugars in the hydrophilic part. Also, some important aspects make saponins potent and very effective. The following are: the types and position of different functional groups, in the aglycone, which are essential for the proper functioning of the saponins against *C. albicans*. These functional groups may include hydroxyl, carboxyl, aldehyde, ketone and acetoxy groups. However, their positions on the sapogenin, and/or genin, and the effect they have on the compound differs among saponins. Each saponin has its own properties that enhance its antifungal effect. Also, monodesmosidic saponins are better antifungal agents than bidesmosidic ones. The triterpenoid saponin Anagallisin C with an oleanolic acid aglycone was found to be the most potent compound against *C. albicans* with an MIC of 1 µg/mL. Also, two steroidal saponins: TG-I,

from *Trillium grandiflorum* and TTS-12 from *Tribulus terrestris* were able to exert great fungistatic activities against *C. albicans*. The former exhibited an MIC value of 1.56 µg/mL, as for the latter, it produced an MIC_{80} of 1 µg/mL. Thus, these three compounds together with other potent ones, could be used as alternatives to treat fungal infections caused by *C. albicans*. Eventually, circumventing the fast increase of antifungal resistance against the substantial use of the antifungal drugs fluconazole and amphotericin B.

Keywords: Medicinal plants, phytochemicals, saponins, triterpenoid, steroidal, *Candida albicans*, Fluconazole, Amphotericin B, antifungal, antifungal resistance, MIC, MFC, fungicide.

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1. Introduction

Candida albicans, is generally considered a harmless commensal fungus present in the gut, reproductive tracts, and oral cavities of humans (Basmaciyan et al., 2019). However, it becomes pathogenic once it travels to other parts of the body where it colonizes its new host and eventually causes further infections (Nobile & Johnson, 2015b; Tadec et al., 2016). It is known to be lethal in immunocompromised patients suffering from AIDS and those undergoing chemotherapy (Maheshwari et al., 2016). Treatment of *C. albicans* infections include the administration of azoles such as fluconazole and polyenes such as amphotericin B (Lewis et al., 1998). However, due to the extensive use of these antifungal drugs, *C. albicans* has developed pathogenic strains that are resistant to these medications.

It has been known for centuries that most plants have medicinal properties due to the broad spectrum of phytochemicals produced (Bennett & Wallsgrove, 1994; L. Yang et al., 2018). An important example are saponins, that insure the protection of these plants from invaders such as bacteria, fungi, viruses and even herbivores (Wink, 2001). In addition, these phytochemicals have been reported to have positive impact on human health, especially their abilities to combat viral, bacterial and fungal infections (Probst et al., 2017; Upadhyay & Dixit, 2015).

Saponins are an excellent example of phytochemicals that have many uses. They are traditionally used as cosmetics, fragrances, and pharmaceuticals (Güçlü-Üstündağ & Mazza, 2007). Saponins in nature include a large structurally related group of compounds that have a triterpenoid, steroid, alkaloid hydrophobic aglycone (sapogenin) that is connected, at carbon C-3, to one, two or three hydrophilic oligosaccharides glycone moieties (Moses et al., 2014; Natori et

al., 1981; Singh & Sharma, 2019). Triterpenoids are the most dominant amongst all three types of saponins (Kareru et al., 2008). Also, each aglycone type is glycosylated with the proper number of sugar moieties (either at C-3, C-28 or both positions), resulting in the relevant type of saponin (Friedman, 2006; Vincken et al., 2007).

The aim of this study is to investigate the role of saponins as an effective treatment against drug-resistant *C. albicans*.

2. Literature review

In this literature review, an overview of the biology and pathogenicity of *Candida albicans* is discussed. In addition, the emergence of antifungal resistance and the underlying mechanisms, will be highlighted. Light will be shed on phytochemicals, especially saponins, along with their specific modes of actions, structural properties and biosynthetic pathway. Moreover, an extensive focus and discussion on the importance of saponins as alternative ways of treatment of fungal infections and in particular drug resistant *C. albicans*.

2.1. Biology and pathogenicity of the fungus *Candida albicans*

Candida albicans is a single-celled fungus usually present as a harmless commensal microorganism in healthy individuals as part of their normal flora (Nobile & Johnson, 2015b) , mainly in their gastrointestinal (GI) tract (Kennedy & Volz, 1985; Kumamoto, 2011), skin (Ca, 2002), genitourinary tract (Achkar & Fries, 2010) and oral cavities (Ganguly & Mitchell, 2011). *C. albicans* may become an opportunistic pathogen if found in large quantities in its initial residence or once it migrates to new locations in the body. Factors contributing to the pathogenicity of *C. albicans* include changes in the pH, stress or administration of therapeutic agents (Nobile & Johnson, 2015b). Some examples of *C. albicans* infections include

vulvovaginal candidiasis, an infection confined to the mucosa of the female genital tract that is colonized by a huge amount of yeasts (Consolaro et al., 2005) and which is one of the leading causes of vaginitis (Sobel et al., 1998). Also, candidemia, a fatal condition where the fungus disseminates into the bloodstream (Wenzel, 1995). In addition, infections with *C. albicans* in immunocompromised individuals, such as patients undergoing chemotherapy, those suffering from AIDS and infections resulting from chronic-indwelling medical devices are life threatening (Fox et al., 2013; Kojic & Darouiche, 2004; Weig et al., 1998).

The invasion of this pathogenic fungus is also facilitated and enhanced by means of biotic factors (humans, animals, predators, pathogens) (H. E. Roy & Lawson Handley, 2012), abiotic factors (climate change: temperature, light, CO_2 levels, moisture…) (Fones et al., 2017) and sometimes it could be autonomously (Rahel & Olden, 2008). These opportunistic pathogens had deleterious effects on human health, in means of giving rise to lethal diseases such as those caused by *Candida* species (Brown et al., 2012) and being the main factors in causing death in immunocompromised patients at a mortality rate of 40% (M. Pfaller & Diekema, 2007). In addition, resistant fungal strains have negatively impacted and threatened the food industry through their release of toxins that caused fruits and vegetables spoilage (Fisher et al., 2012). Agriculture has also been badly affected due to the invasion of field crops by resistant fungi causing massive damage to them (Fisher et al., 2012). All of these factors contributed to a major threat to the biodiversity and lead to large economic losses on the expense of the planet (Mooney, 2005).

Moreover, a notable feature of *C. albicans* and which is one of its most dangerous virulence factors, is its ability to form biofilms, with extremely complex structures, on solid surfaces (Feng et al., 2015).

As opposed to planktonic *C. albicans* which are free-roaming cells, a biofilm is a group of fungal cells that can have the following morphologies: round yeast cells, pseudohyphal cells, hyphal cells (Chandra et al., 2001; Davey & O'toole, 2000; Kolter & Greenberg, 2006; Nobile & Johnson, 2015b). They can exist and grow in biotic environments (botanical, aquatic, human mucosa, oral cavities, vagina) and abiotic environments, such as chronic indwelling devices that are enclosed in an extracellular matrix (Davey & O'toole, 2000; Hall-Stoodley et al., 2004; Kolter & Greenberg, 2006). In addition, *C. albicans* biofilms do not share the same properties with those of their planktonic cells (Davey & O'toole, 2000; Kolter & Greenberg, 2006; Nobile & Johnson, 2015b).

There are four stages of *C. albicans* biofilm development: the very first stage of the cycle is called the planktonic stage, this is where *C. albicans* yeast cells are in their free roaming stage and not attached to any surface. Then, the second stage which is called the initial adherence stage, is when the yeast cells gain access to a solid surface and they adhere to it. The latter stage is followed by biofilm development and maturation, which indicates the development of extracellular polymeric substances (EPS) (that increase the virulence), and the formation of pseudohyphal and hyphal cells. Finally, the last and most dangerous phase, is the dispersion and migration of the yeast cells yeast cells to other surfaces, where they will colonize them and form new biofilms (Gulati & Nobile, 2016).

C. albicans biofilm develop powerful resistance to different forms of antifungal drugs and host immune system which makes it hard to treat infections. Thus, causing a threat to human health, especially in clinical settings (Fox et al., 2013; Hall-Stoodley et al., 2004). Noteworthy, biofilms in general account for 80% of the overall infections caused by pathogenic microbes (Fox & Nobile, 2012; Nobile & Johnson, 2015a).

Furthermore, it is important to note that these biofilms associated with chronic indwelling devices, can lead to systemic infections caused by disseminated candidemia (Fox et al., 2013).

2.2. Antifungal drugs for the treatment of *C. albicans* and their modes of action

Antifungal drugs such as fluconazole and amphotericin B (AMB) have been used for the treatment of different fungal infections and diseases such as vulvovaginal candidiasis (Qin et al., 2018). These drugs share a common key feature, their ability to gain access to the fungal cell membrane and eventually altering its permeability (Qin et al., 2018). The mode of action of these drugs will be discussed in this section.

2.2.1. Fluconazole and its mode of action

Fluconazole, first discovered and administered between the years 1980s and 1990s (Larsen et al., 2004), is a triazole/ pyrrole ring antifungal drug. It is also a water-soluble medicine that exhibits its effect by blocking the synthesis of the sterol ergosterol (Pasanen et al., 1999), the equivalent of cholesterol in humans and animals, and which is mainly found in the cell membrane of fungal microorganism, and eventually lysing the membrane (Lalla & Dongari-Bagtzoglou, 2014). First, it gains access to the enzyme lanosterol demethylase, which converts lanosterol into ergosterol, then blocks the production of the latter, leading to a diminished level of this sterol. However, toxic intermediates of sterol will assemble and pile up (Robbins et al., 2017) causing a drastic stress to the cell membrane and eventually impeding fungal growth (figure 1a) (Kanafani & Perfect, 2008; White et al., 1998). This drug has been widely used due to its positive effect in treating infections caused by *C. albicans* (Eschenauer et al., 2015), *Trichophyton* species, *Malassezia spp.* and many others (Qin et al., 2018). In addition, studies have shown the huge effective pharmacokinetic properties that fluconazole offers, some of which are: its low toxic

effect on the liver, extensive antimicrobial spectrum, high bioavailability and its relatively satisfactory oral intake (Peyton et al., 2015; Wildfeuer et al., 1997).

2.2.2. Amphotericin B and its mode of action

Amphotericin B (AMB), first administered in the late 19510s, is a water- insoluble polyene anti-fungal drug (Qin et al., 2018). It is highly toxic to humans , but it has strong antifungal properties (Schöffski et al., 1998). The mode of action of this drug is as follows: once it attaches to the ergosterol present in the fungal cell membrane, it removes it through the formation of extramembranous sterol aggregates. The formation of pores will diminish the proton gradient which leads to ion leakage and disturbance of the cell membrane and eventually impairing fungal growth (figure 1b) (Kreft & Jetz, 2007; Sychantha et al., 2018).

AMB is usually used for treating infections caused by *C. albicans* which are the leading cause of immunocompromised individual (Fridkin & Jarvis, 1996) and this is because of its wide antifungal spectrum (Ellis, 2002). Due to its high toxicity, AMB represents some side effects such as, kidney damage (Sabra & Branch, 1990), hypotension and nausea (Khoo et al., 1994). Therefore, it is important to note that this drug should be administered in appropriate dosages.

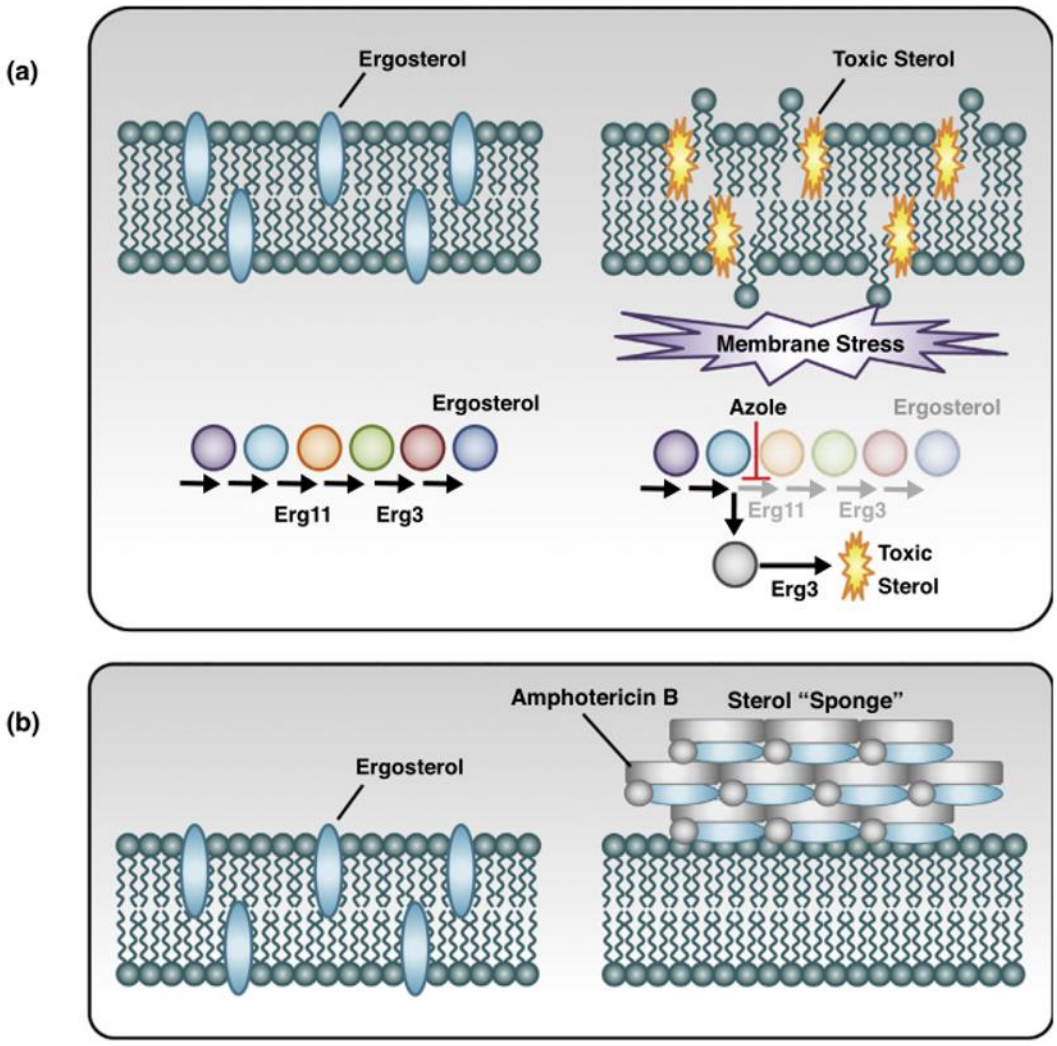


Figure 1: Representational illustration of the different mechanisms of the antifungal drugs (a) azoles and (b) polyenes (Revie et al., 2018). (a) Azoles block the synthesis of ergosterol by inhibiting lanosterol demethylase. Then, the accumulation of toxic intermediates of sterols will block and stop fungal growth through their elicitation of a strong stress to the cell membrane. (b) Polyenes remove the ergosterol from the bilayer by forming extramembranous aggregates of sterol and causing damage to the cell membrane due to ion leakage.

2.3. Antifungal drug resistance mechanisms

Even though the above-mentioned antifungal drugs had a major role in the treatment of several fungal diseases and infections, their massive and abusive use have contributed to the increasing emergence of resistant fungal strains (Revie et al., 2018). Resistant fungi have adopted new ways and mechanisms to evade antifungal drugs through the use of their virulence factors, phenotypic plasticity in addition to other mechanisms (Pigliucci et al., 2006).

2.3.1. Resistance to polyenes

Fungi have adopted mechanisms of resistance against polyenes (AMB), such as making it difficult for these drugs to attack their target host.

It is known that white blood cells such as macrophages and neutrophils, are produced by the immune system as a defense mechanism to attack and deteriorate pathogens such as *C. albicans*. These leukocytes exert their effects by exposing these pathogens to different Reactive Oxygen Species that in turn will destruct the surrounding tissue of these pathogens and even expose them to apoptosis. However, *C. albicans* can escape these defensive mechanisms by the overexpression of the antioxidant cytoplasmic enzyme catalase that it produces. Catalase that is encoded by CAT1 *C. albicans* gene, has contributed to the resistance of polyenes. This cytoplasmic enzyme regulates and reduces the levels of oxidative stress by decomposing hydrogen peroxide into oxygen and water. This mechanism will pave the way for *C. albicans* to further resist these drugs (Chakravarti et al., 2017; Dahlgren & Karlsson, 1999; Dantas et al., 2015; Pemán et al., 2009). For further clarification, a study performed by (Wysong et al., 1998) demonstrated, *in vitro*, that the absence of CAT1 in *C. albicans* will weaken and almost diminish the power of this fungus to evade the white blood cells produced by the human immune system.

Also, in the same study, the *in vivo* effect of the deletion of this gene was expressed by the significant depletion of *C. albicans* infection.

Another mechanism of polyene resistance is associated with the changes made to the ergosterol content in the fungal cell membrane, where ergosterol is replaced with other molecules such as 14α- methyl fecosterol which is a sterol intermediate (Ghannoum & Rice, 1999; Kanafani & Perfect, 2008; Pemán et al., 2009). These two mechanisms will culminate to a reduced oxidative damage and as a result, AMB will fail to attack its target host (Pemán et al., 2009).

2.3.2. Resistance to azoles

Resistance to azoles (fluconazole), mainly in *Candida* species, has been strongly reported (Kanafani & Perfect, 2008). What follows are mechanisms the resistant fungi acquired to adapt and survive stress: a) introduction of efflux pumps encoded by specific genes, blocked the access and even reduced the affinity of the drugs to the target enzyme present in the fungal cell membrane (Morschhäuser et al., 2007; Pfaller, 2012; Posteraro et al., 2003; Slaven et al., 2002). This is due to the overexpression of transporter proteins, such as the ATP-binding cassette (ABC) proteins, that work mainly on pumping the drug outside of the fungal cell membrane. As a result, the total concentration of azole inside the cell will drop (Katiyar & Edlind, 2001; Whaley et al., 2018). b) point mutations in specific genes that encoded for the target enzyme, lanosterol demethylase, that is responsible for the synthesis of the fungal ergosterol in the cell membrane. This leads to the inability of the drug to bind appropriately to its target host (Pfaller, 2012).

In view of the development of resistance to these drugs by *C. albicans*, alternative sources from phytochemicals are explored to combat this drug resistance. In the following section, we shed

light on the use of phytochemicals as effective alternative to the commonly used antifungal drugs.

2.4. Different types of phytochemicals produced by medicinal plants

Trees provide a wide range of secondary metabolites, such as fats, sugars, flavonoid alkaloids, proteins and saponins , which are also called phytochemicals that are not crucial for the plant's survival and proper functioning (L. Yang et al., 2018); however, they are associated with the plant's appearance or color, taste, and aroma (Bennett & Wallsgrove, 1994). They offer several useful characteristics through the elicitation of defensive mechanisms against herbivores, snails or even competing plants (Mitchell-Olds et al., 1998). Additionally they offer protection against foreign opportunistic pathogens such as viruses, bacteria and fungi and thus inhibiting or even killing those predators that are attacking these sessile organisms (Wink, 2001). Moreover, secondary metabolites assist in the pollination and dispersion of the seeds of some plants, where they attract pollinators (animals) through scented monoterpenes or carotenoids in some flowers and, in return, these plants provide nutrients and nectar for the pollinators (Wink, 2003). These phytochemicals are also known to adapt to any environmental stresses (biotic or abiotic) and changes (Balandrin. et al., 1985). Furthermore, some secondary metabolites are used as means of communication between plants and microorganisms that work and communicate symbiotically (Wink, 2001). *Claviceps purpurea*, a saprophytic fungus, and a perfect example of mutualistic symbiosis, provides its host plant with secondary metabolites, ergort alkaloids. These defensive compounds help the plant escape from the invasion of certain herbivores and, in return, this endophytic fungus takes up water and nutrients from this plant for its own survival (Ahimsa-Müller et al., 2007; Wink, 2008).

Secondary metabolites were, for ages, also used in cosmetics, fragrances, food, beverages, and traditional medicine (Ammon & Wahl, 1991; Balandrin & Klocke, 1988; Balandrin. et al., 1985; Bates, 1985) due to their ability to fight against bacteria, oxidants, diabetes, inflammation, and pain (Rosario & Josephine, 2015; Upadhayay et al., 2012). They were eventually used as active compounds in pharmaceutics and neutraceuticals (Koul et al., 2008; Markman & Mekhail, 2002; Qu & Chen, 2014; Zheng et al., 2014).

Therefore, secondary metabolites had a major role in the commerce and in making plants important sources in different fields of medicine, especially in curing some diseases, which is why they came to be called medicinal plants (Sofowora et al., 2013).

Researchers have found that most of the phytochemicals present in trees and plants come from five precursor pathways which are as follows: 1) acetyl coenzyme A as a precursor of saponins, steroids and polyketides (flavonoids, anthraquinones), 2) shikimic acid for tannins, cinnamic acids, indole and aromatic acids, 3) glycolysis as a precursor of sugars and gallic acid, 4) active isoprene for different terpenoids and e) citric acid cycle for alkaloids (Wink, 2001).

Noteworthy, most trees are dicotyledonous flowering plants that produce mainly triterpenoid saponins; however, the remaining plants are monocotyledonous ones (Moses et al., 2014).

2.4.1. Chemical composition and structure of saponins

As mentioned above, saponins which are secondary metabolites produced by a wide variety of plants, are amphiphilic bioactive compounds made up of a hydrocarbon skeleton which is a hydrophobic steroid, triterpenoid or alkaloid part called sapogenin or aglycone. The latter is connected via a glycosidic linkage (either ether or ester**)**, mainly at carbon C-3 (Singh & Sharma, 2019), to one or more hydrophilic sugar chains called glycones which are mainly

oligosaccharides (Dib et al., 2016; Güçlü-Üstündağ & Mazza, 2007; Hostettmann & Marston, 1995b; Vinarova et al., 2015). This physiochemical property explains, their nature of being biosurfactants, emulsifying, and forming micelles in water, hence their use in many detergents (İbanoğlu & İbanoğlu, 2000; Sarnthein-Graf & La Mesa, 2004; Wang et al., 2005). One sugar moiety connected to the aglycone part makes the saponin a monodesmosidic one, two sugar moieties connected to the sapogenin and three oligosaccharides linked to the aglycone part give bidesmosidic and tridesmosidic saponins, respectively (Natori et al., 1981). Also, the main sugars that could be found in saponins are the hexoses D-glucose (Glc), D-galactose (Gal), D-xylose (Xyl), D-glucuronic acid (GlcA), D-fucose (Fuc), L-rhamnose (Rha), L-arabinose (Ara), and the pentose D-apiose (Api) (El Aziz et al., 2019; Kregiel et al., 2017).

Depending on the nature of their hydrophobic part, aglycone, saponins are classified into three groups: triterpenoid, steroidal and alkaloidal (figures 2 and 3). Also, table 1 shows the structural differences among the three groups. In a study conducted on plants species in Central Asia, it has been found that out of 1730 plants, 627 contained triterpenoid saponins and as for the steroidal saponins, they existed in 127 species (Kareru et al., 2008).

Saponins	# C atoms	OH group	COOH group	Spiro-Carbon	# O atoms	# N atoms
Triterpenoid	30	1 at C-3 (ether bond)	1 at C-17 (ester bond)	_____	4	0
Steroidal	27	1 at C-22 (acetal linkage)	_____	1	3	0
Alkaloidal	27	1	_____	1	2	1

Table 1: A comparative table showing the composition and structures of the three different types of saponins.

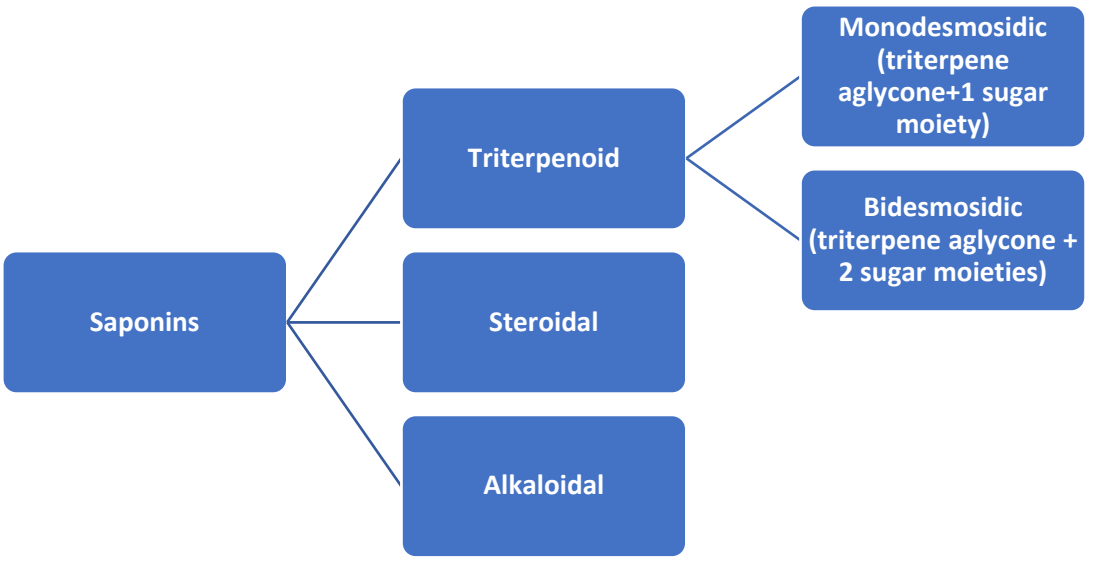


Figure 2: A schematic depicting the three types of saponins: triterpenoid, steroidal and alkaloidal. Also, triterpenoid saponins could be either monodesmosidic (aglycone+1 sugar) or bidesmosidic (aglycone + 2 sugars).

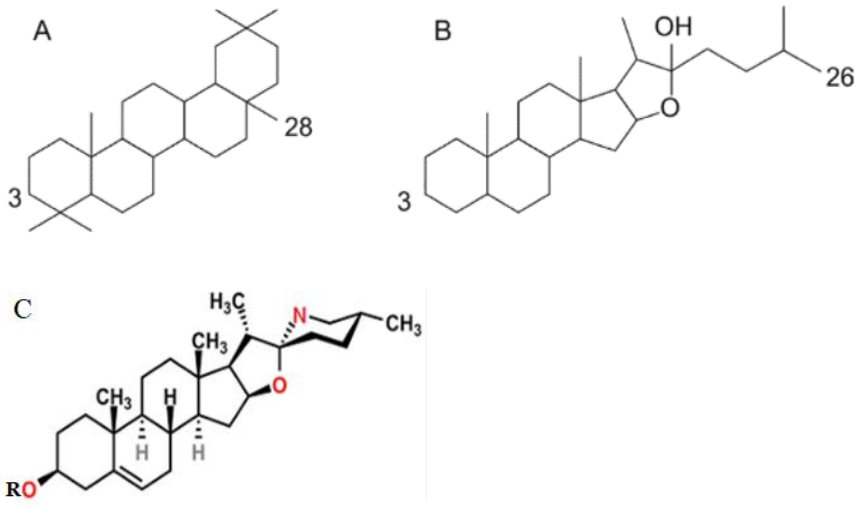


Figure 3: A representation of the chemical structure of the three types of aglycones: (A) Triterpenoid, (B) Steroidal, and (C) Alkaloidal sapogenins (Najjar, 2017). (A) a pentacyclic, 30 C atoms compound, with 1 OH, at C-3 (ether bond) and 1 COOH at C-28 (ester bond). (B) a 27 C atoms compound with two heterocyclic rings furan and pyran rings, with 1 OH at C-22 (acetal linkage) and no COOH. (C) 27 C atoms compound similar to steroidal saponin but instead of a pyran ring, it has a piperidine ring.

2.4.1.1. Triterpenoid saponins

Triterpenoid saponins, also considered glycosylated triterpenes (K. Hostettmann & A. Marston, 1995) are produced by a wide variety of plants but mainly dicotyledonous ones (Moses et al., 2014). Triterpenes come from terpenes (Dewick, 2002) which classification depends on the number of isoprene units that they are derived from. Each isoprene unit (C_5H_8) is composed of five carbon atoms (5 Cs) and, thus, the characterization of terpenes goes as follows: one isoprene unit (5 Cs) is considered a hemiterpene, two isoprenes make the monoterpenes group with 10 carbon atoms; the same classification is applied to the remaining terpenes with sesquiterpenes

comprising 15 carbon atoms, diterpenes having 20 carbons (Cs), sesterpenes containing 25 Cs, triterpenes with 30 Cs and tetraterpenes with 40 Cs (Ashour et al., 2018; Dewick, 2002; El Aziz et al., 2019; Martin et al., 2003). Consequently, triterpenoid saponins have 30 carbon atoms (figure 3a), but they lack a spiro-carbon (El Aziz et al., 2019).

In addition, the main chemical structure of triterpenoid saponins is either tetra- or penta- cyclic; the former having lanostane, cucurbitane, dammarane and tirucallane as the main classes and the latter giving rise to four main categories which are: oleanane, lupane, hopane and ursane (Furtado et al., 2017; Liu, 1995; Singh & Sharma, 2015). Oleanane triterpene being the structure that predominates among medicinal plants (Kareru et al., 2008), was initially discovered in the Chinese plant *Panax japonicum* (Liu, 1995; Szakiel et al., 2005; Vincken et al., 2007).

Triterpenoid saponins can as well have an acyclic (squalene saponin), monocyclic, bicyclic, tricyclic and hexacyclic chemical structures; but, they exist and occur in a minor quantity (Sheng & Sun, 2011).

Moreover, this class of saponins contains four oxygen atoms in their structure, along with one hydroxyl group (-OH) and one carboxyl group (-COOH) on carbons number 3 (via an ether bond) and 17 (via an ester bond), respectively (Cheng et al., 2015; El Aziz et al., 2019; Sporn et al., 2011; Wei et al., 2015). The position of the oxygen atoms goes as follows: one of them is linked to the carbon number three (C-3) via an ether bond, two oxygen atoms are attached to carbon number 17 (C-17) with an ester bond and the fourth oxygen at carbon number 24 in the form of an alcoholic group (-CH_2 -OH) that is not attached (El Aziz et al., 2019) (table 1).

Furthermore, triterpenoid glycosides can also be categorized as monodesmosidic (figure 2) with a triterpene aglycone linked to one sugar unit at C-3, or bidesmosidic (figure 2) having their aglycone part attached to two sugar moieties: one being at C-3 (ether bond) and the other being

at either C-28 (ester bond) or at C-24 (ether bond). Thus the classification of this type of glycosides depends on the number of sugar units attached to the aglycone part of them (Güçlü-Üstündağ & Mazza, 2007; Madland, 2013). Furthermore, triterpenoid saponins are mostly found in the following plants: soybeans, quillaja and alfalfa (Haralampidis et al., 2002).

2.4.1.2. Steroidal saponins

This class of saponins is made up of 27 carbon atoms (figure 3b), with rings having a tetracyclic structure as well as two heterocyclic rings which are furan and pyran rings (Nabavi et al., 2020; Podolak et al., 2010). The former being an aromatic ring that contains one oxygen and four carbon atoms, and the latter being a non-aromatic ring comprising five carbons and one oxygen, with both rings having between them a spiro-carbon atom (Chaieb, 2010; El Aziz et al., 2019). It is important to mention that steroidal saponins possess one hydroxyl group at C-22 (acetal linkage) (Hardman, 1987; Hostettmann & Marston, 1995b) but lack the carboxyl group (El Aziz et al., 2019) and that their glycone and aglycone parts are attached to each other via an ether bond at C-3 (Chaieb, 2010). In addition, two forms of steroidal saponins are known: a) spirostanol and b) furostanol and are depicted in figure 4 (Saxena et al., 2013; Sparg et al., 2004). The above-two types are composed of a ring system with spirostanol having a “hexacyclic ABCDEF-ring system” (closed F ring) and furostanol containing a “pentacyclic ABCDE-ring system” with an additional F ring that is open (figure 4) (Sautour, Mitaine-Offer, et al., 2007; Yang et al., 2006).

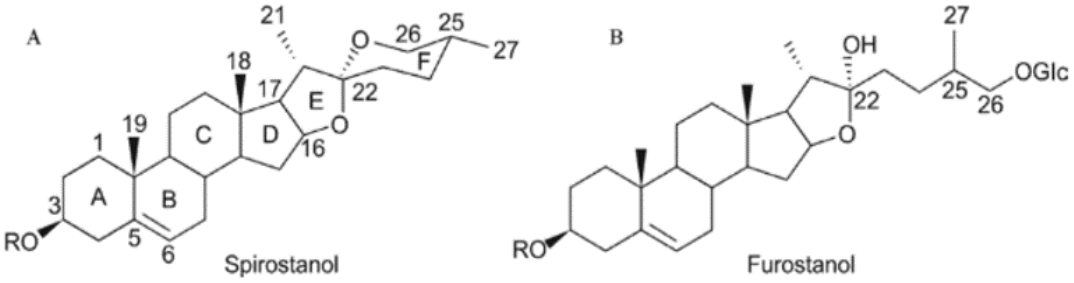


Figure 4: Structural characterization of the two types of steroidal saponins (A) Spirostanol and (B) Furostanol (Najjar, 2017). (A) ABCDEF- hexacyclic ring system with a closed F ring; (B) ABCDE-pentacyclic ring system with an extra F ring that is open.

Also, same as triterpenoid saponins, this class can happen as bidesmosidic, in the case of furostanol, with one sugar moiety at C-3 and the other at C-26 (via an ether bond) (Majinda, 2012).

Moreover, steroidal saponins predominate among the following plants: yucca, tomato and oats (Haralampidis et al., 2002).

2.4.1.3. Alkaloidal saponins

Glykoalkaloids also called alkaloid saponins share several similarities in structure with those of steroidal saponins, where they both have 27 carbon atoms (figure 3c), one hydroxyl group, two heterocyclic rings, one spiro- carbon and they both lack a carboxyl group. However, the main difference in structure between these two classes of saponins is that instead of having a pyran ring like that in steroidal saponins, alkaloid glycosides have a piperidine ring which contains five carbon atoms and one nitrogen atom instead of an oxygen (table 1) (Abed el Aziz et al., 2017; Augustin et al., 2011; Ginzberg et al., 2009; Hostettmann & Marston, 1995a, 1995b; Itkin et al., 2013).

2.5. Biosynthetic pathway of saponins

To better understand the above mentioned three classes of saponins and their chemical origin, a brief discussion about their aglycone's biosynthetic pathway (figure 5) will be discussed shortly.

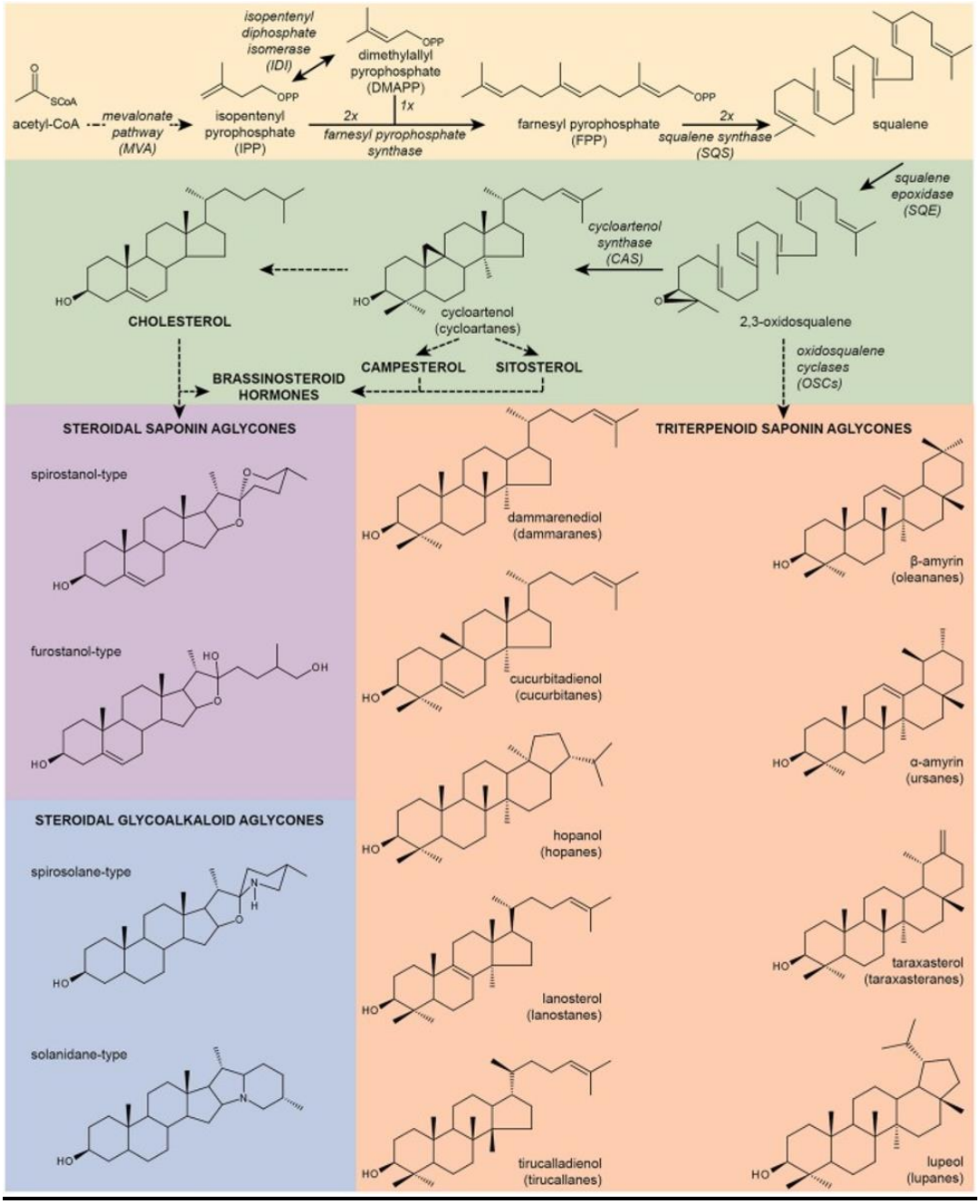


Figure 5: A schematic representation of the biosynthetic pathway of the different types of saponin's hydrophobic parts (Moses et al., 2014). The very first step of this pathway involves the conversion of Acetyl- CoA through the mevalonate pathway (MVA) and a series of additional reactions into the linear compound squalene, composed of 30 carbons. This precursor will be oxidized into 2,3- oxidosqualene and which in turn will undergo cyclization and eventually converted into either triterpenoid saponins's sapogenins (in pink), cycloartenol (for plants) (green) or cholesterol, the precursor of steroidal (purple) and alkaloidal (blue) aglycones. Dashed arrows indicate the presence of multiple steps and the enzymes' and pathways' names are written in italic.

The first step of this pathway includes the conversion of the precursor Acetyl- CoA, through the mevalonate pathway (MVA), into isopentyl pyrophosphate (IPP) (Goodwin, 1970), a terpene precursor composed of 5 Cs in its backbone (Buhaescu & Izzedine, 2007; Moses et al., 2014). This step is catalyzed by the rate-limiting enzyme 3- hydroxy- 3- methylglutaryl- CoA reductase (HMGR) (Goldstein & Brown, 1990).

Second, the isomerization of IPP, catalyzed by the enzyme isopentenyl diphosphate isomerase (IDI), results in dimethylallyl pyrophosphate (DMAPP), which is the isomeric form of IPP (Agranoff et al., 1960).

The third step of the pathway includes the formation of farnesyl pyrophosphate (FPP), an isoprene farnesyl having 15 carbon atoms in its backbone. This step is achieved when two units of IPP and one unit of DMAPP are condensed (Cornforth et al., 1966; Moses et al., 2014; Thimmappa et al., 2014). This reaction is catalyzed by the enzyme farnesyl pyrophosphate

synthase (Moses et al., 2014). Noteworthy, FPP is the immediate precursor of saponin that underwent a prenylation reaction, which means that, the farnesyl group was covalently added to a cysteine group close to the C-terminus of the protein (Poulter & Rilling, 1978; Poulter & Satterwhite, 1977).

Next, a second condensation reaction catalyzed by squalene epoxidase (SQE), but this time of two FPP units, produces the committed precursor squalene (30 Cs (linear)). The latter subsequently undergoes an epoxidation reaction catalyzed by the enzyme squalene synthase (SQS), leading to the intermediate compound 2,3- oxidosqualene (Moses et al., 2014). The latter has multiple fates, where, it could either lead to the formation of triterpenoid saponin aglycones with polycyclic structures, due to its cyclization by several oxidosqualene cyclases (OSC) (Kannenberg & Poralla, 1999; Moses et al., 2014; Thimmappa et al., 2014). These structures include oleananes, ursanes, taraxasterol, lupeol, dammaranes, cucurbitanes, hopanes, lanostanes, and tricullanes (Vincken et al., 2007). The second possibility is that 2,3- oxidosqualene could be cyclized by the enzyme cycloartenol synthase (CAS) (Ohyama et al., 2009), resulting in cycloartenol, a precursor of triterpenes and having a tetracyclic structure (Inagaki et al., 2011; Phillips et al., 2006; Racolta et al., 2012; Xue et al., 2012). Eventually, cycloartenol will give rise to different phytosterols (Ohyama et al., 2009; Shibuya et al., 2004), mainly found in angiosperms, such as cholesterol composed of 27 Cs, campesterol having 28 carbon atoms and sitosterol with 29 Cs (Moses et al., 2014).

In addition, the oxygenation and glycosylation of the 27-C cholesterol leads to the generation of the steroidal saponin aglycones spirostanol and furostanol (Thakur et al., 2011). Moreover, cholesterol is also considered the precursor of glycoalkaloid aglycones that in turn will have an

amine group and the resulting two types are: spirosolane and solanidane (Ginzberg et al., 2009; Itkin et al., 2013).

Finally, tailoring enzymes such as oxidoreductases will adjust the aglycones. The latter will undergo further glycosylation, with the appropriate number of sugars mainly at C-3 (OH group via an ether bond) or C-28 (COOH group via an ester bond) or on both positions. As a results saponins will form (Friedman, 2006; Vincken et al., 2007) and eventually their solubility in water increases (Sawai & Saito, 2011).

2.6. Bioactivity of saponins

Many studies were extensively conducted to reveal the biological properties of saponins (table 2) (Francis et al., 2002; Lacaille-Dubois & Wagner, 1996). One of the most striking characteristics of saponins is their ability to lyse erythrocytes (hemolytic activity) due to their high affinity for membrane sterols, and eventually releasing hemoglobin (Oda et al., 2000; Seeman et al., 1973). Saponins were used by as fish poisons (Francis et al., 2001), since they exhibit ichthyotoxic effects through their ability to gain access to the respiratory epithelia, where they induce damage to them and as a result killing of the fish by suffocation (Milgate & Roberts, 1995; Roy et al., 1990). In addition, it has been reported that saponins of the medicinal plant *Styrax officinalis* has the ability to kill *Cornu aspersum*, a terrestrial gastropod (Dib et al., 2016). This molluscicidal effect, which can be due to the saponins's ability to gain access to the mollusk's soft body wall and damage it (Chaieb, 2010; Lacaille-Dubois & Wagner, 1996), gave a promising advantage to control several diseases such as the freshwater snails disease schistosomiasis (Hostettmann, 1980). Moreover, different studies have shown that saponins have antibacterial properties due to their ability to damage the bacterial cell wall and hence inhibit the growth of bacteria and even cause them to lyse (Hostettmann & Marston, 1995b;

Naidu, 2000). Furthermore, saponins have an important role in lowering serum cholesterol levels (hypocholesterolemic effect) directly and indirectly (Milgate & Roberts, 1995). This is done through two mechanisms, that have a common hallmark: a) the capacity to block intestinal absorption through the establishment of insoluble complexes with cholesterol (Malinow et al., 1977) and b) the intestinal inhibition of bile salts reabsorption which induces an increase in the cholesterol formation of bile salts in the liver and eventually leading to a high reduction of cholesterol in the serum (Sidhu & Oakenfull, 1986; Vinarova et al., 2015) .

Besides these reported properties, studies on rats have proven that saponins have hypoglycemic properties, due to their direct effect on β- cells that in turn increase levels of insulin in the blood and eventually reduce blood sugar levels (Petit et al., 1993).

An interesting fact about saponins, is that they can exhibit anticarcinogenic activities (Güçlü-Üstündağ & Mazza, 2007) on several cell lines such as leukemia, prostate cancer and breast cancer (Bachran et al., 2008; Yıldırım & Kutlu, 2015). This is because, these secondary metabolites can prevent the progression of cancerous cells and can induce apoptosis through the caspase 3 pathway (Liu et al., 2000).

Additionally, these secondary metabolites can act as adjuvants by enhancing the effect of oral vaccines (Güçlü-Üstündağ & Mazza, 2007). This is done through their ability to strengthen the immune system's response by acting as adjuvants, increasing the absorption of certain molecules and stimulating the production of additional antibodies; therefore, amplifying the potency of oral vaccines (Cheeke, 2000).

Other properties of saponins, such as being anti-viral, anti-inflammatory and immune system boosters bioactive agents, are discussed extensively in other papers (Liu, 1995).

Moreover, these phytochemicals help protect plants from gastropods, herbivores plant invaders and even from other invasive plants (Chaieb, 2010; Lacaille-Dubois & Wagner, 1996; Mitchell-Olds et al., 1998). Furthermore, it has been shown that saponins isolated from the fruit pericarp of the tree *Sapindus mukorossi* exhibited strong fungicidal effects on the two opportunistic fungi *Venturia inaequalis* and *Botritis cinerea* (Porsche et al., 2018).

Biological activity of saponins:
Hemolytical activity (Oda et al., 2000)
Ichthyotoxic effect (Francis et al., 2001; Roy et al., 1990)
Molluscicidal effect (Dib et al., 2016)
Antimicrobial effect: - Antibacterial effect (Hostettmann & Marston, 1995b; Naidu, 2000) - Antiviral effect (Liu, 1995) - Fungicidal effect (Porsche et al., 2018)
Anticarcinogenic effect (Güçlü-Üstündağ & Mazza, 2007)
Hypocholesterolemic effect (Milgate & Roberts, 1995)
Hypoglycemic effect (Petit et al., 1993)
Adjuvant activity (Liu, 1995)
Anti-inflammatory effect (Liu, 1995)
Immune system boosters (Liu, 1995)

Table 2: Extensive selection of saponins' biological activities that they offer for humans, plants and for the biodiversity.

2.7. Antifungal activity of saponins against *C. albicans*

As previously stated, saponins exhibit a wide variety of biological activities (table 2) important for plants and human health. Various papers and studies regarding the antifungal activity of saponins against *C. albicans* have delineated the presence of several medicinal plants. The latter produced triterpenoid and other steroidal saponins possessing remarkable antifungal properties against *C. albicans*.

Several papers have reported the antifungal properties that triterpenoid and steroidal saponins offer against *C. albicans*. Tables 3 and 4 represent different types of medicinal plants-producing the two groups of saponins along with their levels of antifungal potency against *C. albicans*. These saponins are known for their conspicuous antifungal properties against several fungal strains especially the pathogenic antifungal-resistant strains of *C. albicans*. In addition to fighting off diseases caused by the latter fungus.

All of these studies have incorporated different antifungal susceptibility tests (*in vitro*) against *C. albicans* in order to depict the antifungal properties of these saponins, as well as their potency. Plus, they determined whether *C. albicans* was resistant or susceptible to these compounds so that the latter could be used to treat fungal infections caused by *C. albicans*. Also, some have tested the effectiveness of saponins *in vivo* against *C. albicans*.

2.7.1. Antifungal susceptibility tests

2.7.1.1. Broth microdilution technique

The broth microdilution technique is an *in vitro* method used to report the effectiveness of a saponin (or drug) against *C. albicans* and whether the latter was susceptible or resistant to this antimicrobial agent. This is done through the determination of the minimum inhibitory

concentration (MIC) (µg/mL) and the minimum fungicidal concentration (MFC) (µg/mL) (Abbaszadeh et al., 2014; Carson et al., 1995; Takahagi-Nakaira et al., 2009). With MIC being the lowest concentration of the saponin (or antifungal drug) that will inhibit *C. albicans* growth (Carson et al., 1995; Ekabo et al., 1996) and MFC being the lowest concentration of the saponin (or antifungal drug) that significantly decreased/diminished the number of colony-forming units (CFU) (David & Sudarsanam, 2013). Therefore, the lower the MIC and MFC, the more effective the saponin is.

2.7.1.2. Disk diffusion technique

Another *in vitro* method used to determine the susceptibility of *C. albicans* to the used saponin (or antifungal drug) is the disk diffusion technique (Bauer, 1966). It is characterized by the zone of inhibition (mm), on a petri dish, and which is the zone where there is no visible growth of *C. albicans* (Patel et al., 2011). Thus, the larger the diameter of the inhibition zone, the better the saponin.

2.7.2. Antifungal activity of triterpenoid saponins against *C. albicans*

Triterpenoid saponins with antifungal properties can include those that have the following aglycones: hederagenin acid (figure 6-1), phytolaccagenin acid (figure 6-2), and oleanolic acid (figure 6-3). The general structure of each one of them is depicted in figure 6. In addition, triterpenoid saponins with antifungal effects against *C. albicans* can include other sapogenin chemical structures such as: jujubogenin (figure 6-4), holostane (figure 6-5), and β-escin (figure 6-6) and others which are going to be mentioned in the next sections.

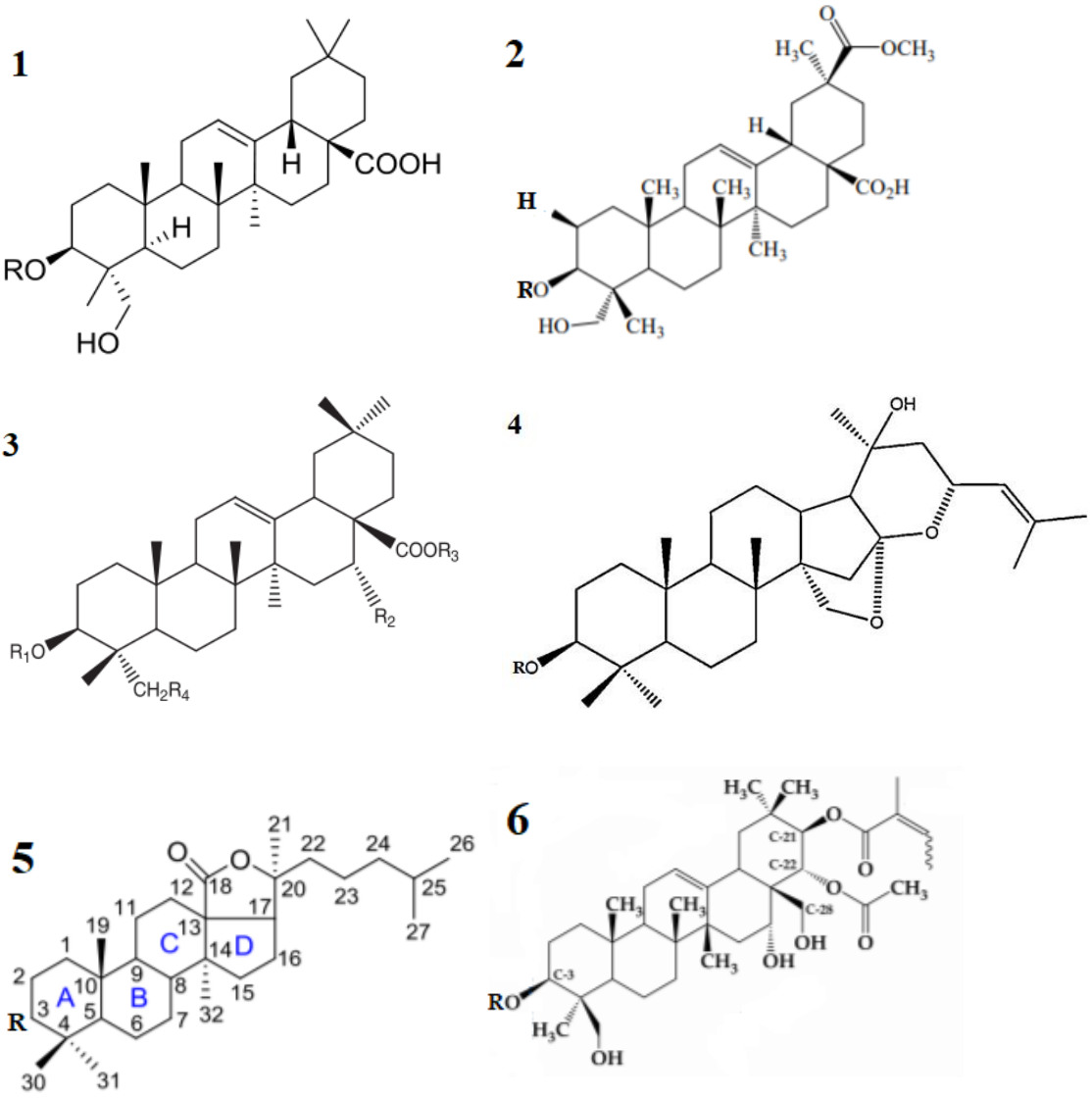


Figure 6: General structure of the aglycones of well-known antifungal triterpenoid saponins. 1: Hederagenin acid (Hu et al., 2018), 2: Phytolaccagenin acid (Liberto et al., 2010), 3: Oleanolic acid (Njateng et al., 2015; Zhong et al., 2001), 4: Jujubogenin (Higuchi et al., 1984; Ribeiro et al., 2013), 5: Holostane (Bahrami & Franco, 2016), and 6: β-escin (Dargel et al., 2019). *R in compounds 1, 4, 5, and 6 and R_1 in compound 3, represent the hydrophilic part of the saponin attached to C-3 of the aglycone of the corresponding saponin.

All of the above compounds along with the plants that produce them, and their relevant families are depicted in table 3. The last mentioned also includes, the antifungal susceptibility results of the compounds and that determine their levels of effectiveness.

Family	**Plant**	**Aglycone type**	**Compound**	**Susceptibility tests results**
Amaranthaceae	*Anabis articulate*	Oleanolic acid	Saponins **(140-142)** *	NTI
Araliaceae	*Hedera helix*	Hederagenin acid	Saponins α- hederin **(31)** and δ-hederin **(32)**	MIC (µg/mL): - **(31)**: 25 - **(32)**: NA[5]
	Kalopanax pictus	Hederagenin acid	Kalopanax saponins: A **(1)**, B **(2)** *, I **(3)**, and H **(4)** *	$D_{\text{zone of inhibition}}$ (mm): - **(1)**: 9.0 - 14.5 - **(2)** and **(4)**: 0 -**(3)**: 8.3 -17.8 MIC (µg/mL): - **(1)** and **(3)**: 25 - **(2)** and **(4)**: >200
	Polyscias fulva	Hederagenin acid	Saponins **(27)**, **(28)**, **(29)** *, and **(30)** *	MIC/MFC (µg/mL): - **(27)**: 100/ NA[8] - **(28)**: 12.5/25 - **(29)** and **(30)**: NA[8]

		Oleanolic acid	- Sapogenin **(54)** - Saponins **(55)**, **(56)**, and **(57)** *	MIC/MFC (µg/mL): - **(54)** and **(57)**: NA[8] - **(55)**: 100/NA[8] - **(56)**: 50/NA[8]
Asteraceae	*Melanthera elliptica*	Oleanolic acid	Saponins **(46)**, **(47)** *, **(48)**, and **(49)** *	MIC (µg/mL): - **(46)**: 64 - **(47)**: NA[7] - **(48)**: 128 - **(49)**: 16
Caryophyllaceae	*Acanthophyllum gypsophiloides*	Others	Saponins **(144,145)**	$D_{\text{zone of inhibition}}$ (mm) (0.55 mg/disc): - **(144)**: 10 - **(145)**: 7
	Dianthus calocephalus Boiss	Others	_____	NTI
	Silene dichotoma Ehrh. Subsp.	Others	_____	NTI

Chenopodiaceae	*Chenopodium quinoa*	Hederagenin acid	**(8)** * and **(9)** *	MIC (µg/mL): (8) and (9): NA[9']
		Phytolaccagenic acid	Saponins **(40)** *, **(41)**, and **(42)** *	MIC (µg/mL): - **(40)** and **(42)**: NA[9] - **(41)**: ≤ 100
Fabaceae	*Glycyrrhiza glabra* L.	Others	Saponins glycyrrhizic acid **(169)** and 18β-glycyrrhetinic acid **(170)**	NTI
Hippocastanaceae	*Aesculus hippocastanum* L.	β-escin	- Saponins escins: Ia **(157)**, Ib **(158)**, IIa **(159)**, IIb **(160)**, IIIa **(161)**, IIIb **(162)**, IV **(163)**, V **(164)**, and VI **(165)** - Saponins ioescins: Ia **(166)**, Ib **(167)**, and V **(168)**	NTI
Holothuriideae	*Bohadschia bivittata* Mitsukuri	Holostane	Saponin bivittoside D **(156)**	MIC_{80} (µM): 2.80
	Bohadschia marmorata	Holostane	Saponins marmoratoside **(151)**, 17α-	MIC_{80} (µM): - **(151)**: 2.81

			hydroxyimpatienside **(152)**, marmoratoside B **(153)**, 25- acetoxy bivittoside **(154)**	- **(152)**: 2.78 - **(153)**: 44.44 - **(154)**: 43.13
	Holothuria impatiens	Holostane	Saponin impatienside A **(155)**	MIC_{80} (µM): 2.81
Leguminoseae	*Lupinus angustifolius*	Oleanolic acid	Saponins **(58)** and **(59)**	MIC (µg/mL): - **(58)**: 30 - **(59)**: 25
		Others	Saponin soyapogenol A **(143)**	MIC (µg/mL): 25
Loganiaceae	*Buddleja madagascariensis*	Oleanolic acid	Mimengoside A **(150)**	MIC (µg/mL): 50
Myrsinaceae	*Maesa lanceolata* Forsskal var *golungensis* Welw.	Oleanolic acid	Saponins **(64-69)**	NTI
Phytolaccaceae	*Phytolacca tetramera*	Phytolaccagenic acid	Saponins phytolaccosides B **(37)**, E **(38)**, and F **(39)**	MIC (µg/mL): - **(37)**: 125 - **(38)** and **(39)**: NA[6]
	Anagallis arvensis	Oleanolic acid	Saponins AnC (Desglucoanagalloside B) **(60)**, AnA	MIC (µg/mL): - **(60)**: 1 - **(61)**: 2

Primulaceae			(Anagallosaponin I) **(61)**, AnB **(62)**, and Desglucoanagalloside A (Anagallosaponin IV) **(63)**	- **(62)** and **(63)**: 4
	Cyclamen cilicium Boiss et Heldr var. intarninaturn	Oleanolic acid	Saponins desglucocyclamin I **(70)**, cyclamen **(71)**, and isocyclamin **(72)**	NTI
	Cyclamen coum tuber	Oleanolic acid	Saponins cyclaminorin **(76)**, desglucocyclamin **(77)**, cyclacoumin **(78)**, and mirabilin lactone **(79)**	NTI
Primulaceae	Cyclamen mirabile	Oleanolic acid	Saponins cyclaminorin **(73)**, desglucocyclamin **(74)**, cyclamin **(75)**	MIC (µg/mL): **(73-75)**: 160
Quillajaceae	*Quillaja saponaria*	Oleanolic acid	Saponins **(80-139)**	NTI
	Colubrina retusa	Jujubogenin	Jujubogenin saponin **(149)**	MIC (µg/mL): 50

Table 3: Plant-producing triterpenoid compounds (sapogenins/saponins) with different aglycones. Hederagenin acid (yellow), phytollacagenic acid (dark green), oleanolic acid (orange), jujubogenin (light grey), holostane (blue), β-escin (light red), and others (vivid red). The

Rhamnaceae	*Zizyphus joazeiro*	Jujubogenin	Jujubogenin saponins 1 **(146)**, 2 **(147)**, and 3 **(148)**	NTI
Ranunculaceae	*Clematis tangutica*	Hederagenin acid	Saponins **(35)** * and **(36)** *	$D_{zone\ of\ inhibition}$ (mm): **(35)** and **(36)**: NA^0
Sapindaceae	*Lecaniodiscus cupanioides*	Hederagenin acid	Saponins **(33)** and **(34)**	IC_{50} (µg/mL): - **(33)**: 10 - **(34)**: 8.5
	Paullinia pinnata	Oleanolic acid	- Sapogenin friedelin **(50)** - Saponins aridanin **(51)**, **(52)**, and lotoidoside **(53)**	MIC (µg/mL): - **(50)** and **(51)**: NA^2 - **(52)** and **(53)**: 3.125
	Sapindus mukorossi	Hederagenin acid	Sapindosides A **(12)**, B **(13)**, C **(14)**, and D **(15)**	MIC (µg/mL): **(12)** and **(13)**: 25 **(14)**: 50 **(15)**: 100
	Sapindus mukorossi (pulp)	Hederagenin acid	- Saponins **(16)**, **(17)**, **(19)**, α- hederin **(20)**, **(21)**, **(22)**, **(23)**, **(24)**, **(25)**, and **(26)** - Sapogenin **(18)**	MIC (µg/mL): - **(20)**: 16 - **(19)**, **(21)**, **(24)**, and **(26)**: 32 - **(16)**, **(22)**, and **(25)**: 64 - **(23)**: 128

				- **(17)** and **(18)**: NA[7]
		Oleanolic acid	Saponins **(43)**, **(44)**, and **(45)**	MIC (µg/mL): - **(43)**: NA[7] - **(44)**: 16 - **(45)**: 8
	Sapindus saponaria	Hederagenin acid	S1 **(10)** and S2 **(11)**	NT
	Serjania salzmanniana	Hederagenin acid	**(5)**, salzmannianosides A **(6)**, and B **(7)**	MIC (µg/mL): **(5)** and **(6)**: ≤ 16 **(7)**: NA[3]

table also includes the families of the plants and the susceptibility tests results of the compounds determining their levels of potency.

*: bidesmosidic saponins

NT→ not tested

NTI → not tested individually
NA→ not active at the highest test concentration:
- NA[0]: not active (zone of inhibition), NA[1]: >20 µg/mL, NA[2]: ≥ 100 µg/mL, NA[3]: ≥ 125 µg/mL, NA[4]: >128 µg/mL, NA[5]: >200 µg/mL, NA[6]: >250 µg/mL, NA[7]: >256 µg/mL, NA[8]: >400 µg/mL, NA[9]: ≤ 500 µg/mL, NA[9']: >500 µg/mL, NA[10]: >800 µg/mL.

2.7.2.1. Triterpenoid saponins with hederagenin acid aglycone

(Kim et al., 1998) studied the antifungal effect of the triterpenoid saponins, kalopanaxsaponins A **(1)**, B **(2)**, I **(3)**, and H **(4)**, possessing a hederagenin acid aglycone and that were previously isolated from the plant *Kalopanax pictus* (Araliaceae) (Park et al., 1998), against *C. albicans*-causing candidiasis.

The disk diffusion results have shown that only A **(1)** and I **(3)** exhibited good antifungal activities against *C. albicans*. They produced inhibition zones between 9 and 14.5 mm and between 8.3 and 17.8 mm, respectively, for concentrations between 50 and 200 µg/mL. As for saponins B **(2)** and H **(4)**, they haven't produced any inhibition zone at any of the reported concentrations (inactive).

For further accuracy, they tested the MICs of the four saponins: A **(1)** and I **(3)** had both the same MIC values (25 µg/mL) and were considered active against *C. albicans*. Whereas B **(2)** and H **(4)** were inactive against *C. albicans*, exhibiting both MICs > 200 µg/mL.

The potent kalopanaxsaponin A **(1)**, which exhibited an MIC value of 25 µg/mL and good diameter of inhibition zone, had in its aglycone a free carboxyl group at C-17 and two monosaccharides at C-3. However, kalopanaxsaponin B **(2)** which had the same sugar chain as in the potent saponin A **(1)**, exhibited no antifungal activity with an MIC value of > 200 µg/mL and 0 mm of inhibition zone diameter. Maybe, the presence of an additional sugar chain at C-28 instead of the free COOH at C-17, might have impaired the activity of the saponin. As for saponin I **(4)**, it was as potent as saponin A **(1)**, with an MIC value of 25 µg/mL, and having the same aglycone but the only difference is that saponin I **(4)** had three monosaccharides at C-3. This additional sugar in the C-3 glycosidic chain, might be the reason why saponin I **(4)** produced slightly better inhibition zones results than saponin A **(1)**. In spite of having the same sugar chain as saponin I **(4)**, saponin H **(3)** had an additional sugar chain at C-28 instead of a free

carboxyl group at C-17, which might have impaired its activity (diameter of inhibition zone= 0 mm and MIC > 200 µg/mL).

Another study performed by (Ekabo et al., 1996), where they isolated, from the plant *Serjania salzmanniana* (Sapindaceae), three saponins 1 **(5)**, 3 (salzmannianoside A) **(6)** and 4 (salzmannianoside B) **(7)** having hederagenin aglycones. Only saponins 1 **(5)** and 3 **(6)** exhibited potent antifungal properties against *C. albicans*, with MIC values of ≤ 16 µg/mL. They had in their aglycone, a free carboxyl group at C-17, and had three monosaccharides at C-3. As for saponin 4 **(7)**, even though it had the same aglycone as the potent saponins 1 **(5)** and 3 **(6)**, it exhibited no antifungal activity (MIC ≥ 125 µg/mL) against *C. albicans*. This might be due to the additional monosaccharide in the sugar chain at C-3, having a total of four monosaccharides.

In addition, two saponins, 2 **(8)** and 3 **(9)**, from *Chenipodium quinoa* (Chenopodiaceae) (Woldemichael & Wink, 2001) were considered inactive, neither of them exhibited antifungal effects on *C. albicans* (MICs of both > 500 µg/mL). This is because both saponins, regardless of the number of sugars in their sugar chains at C-3, had an additional sugar moiety at C-28 , instead (bidesmosides) of a free COOH at C-17, that rendered them inactive.

Moreover, (Tsuzuki et al., 2007) reported the strong to moderate activity of the buthanolic (n-BuOH) extract of *Sapindus saponaria* (Sapindaceae) against *C. albicans* (MIC and MFC values between 300 and 600 µg/mL). The classification of a compound's strength is determined after (Aligiannis et al., 2001) and (Duarte et al., 2005) who suggested that those having an MIC up to 500 µg/mL are said to be strong. Also, when the MIC is between 600 µg/mL and 1500 µg/mL the compounds are said to be moderate and when the MIC is larger than 1600 µg/mL, then they are considered weak. Accordingly, fraction F was isolated from n-BuOH extract, via column chromatography. The obtained fraction presented strong fungistatic and fungicidal activities

(both MIC and MFC between 75 and 150 µg/mL). Then, two triterpenoid saponins 1 **(10)** and 2 **(11)**, with hederagenin aglycones, were isolated from fraction F via activity-guided fractionation. They both had a free COOH at C-17 and three monosaccharides at C-3, with an additional acetoxy group (OAc) in the sugar chain of saponin 2 **(11)**. However, each of the compounds 1 **(10)** and 2 **(11)** alone was not tested for its antifungal activity against *C. albicans*. Hence, it is important to do so in the future since the extracts of *Sapindus saponaria* presented great results.

Furthermore, (Kimata et al., 1983) extracted, from *Sapindus mukorossi* (Sapindaceae), four triterpenoid saponins, sapindosides A **(12)**, B **(13)**, C **(14)**, and D **(15)** and (Favel et al., 1994) tested them against *C. albicans* (MIC> 200 µg/mL ➔ inactive). The potent sapindosides A **(12)** and B **(13)** exhibited the same MIC results of 25 µg/mL. They both had three monosaccharides at C-3 (A: Ara, Rha, Ara; B: Ara, Rha, Xyl). In addition, compound C **(14)** produced good results (MIC= 50 µg/mL), but were inferior to A **(12)** and B **(13)**, maybe this was because sapindoside C **(14)** had the terminal Ara sugar in the form of a furanosyl (Ara_f) instead of a pyranosyl (Ara_p) (in the case of A **(12)**). A **(12)** and C **(14)** had almost the same hydrophilic part but the terminal Ara in the form of furanosyl reduced the activity. Also, saponin D **(15)** was less effective (MIC= 100 µg/mL) than all other compounds, due to the excessive increase in sugar units at C-3 (six monosaccharides). In another study, (Hu et al., 2018) extracted ten saponins and one hederagenin sapogenin from the pulp of *Sapindus mukorossi* (Sapindaceae) and reported their MICs (MIC> 256 µg/mL➔ inactive):

- Saponin 2 **(16)**: COOH at C-17, OH at C-23, and three monosaccharides at C-3 (with terminal Ara_f+ acetoxy group (OAc) ➔ MIC= 64 µg/mL.

- Saponin 3 **(17)**: COOH at C-17, CH_2OAc at C-23, and three monosaccharides at C-3➔ MIC> 256 µg/mL

- Sapogenin 9 **(18)**: same aglycone as saponin 2 but lacks a sugar chain at C-3 ➔ MIC> 256 µg/mL.

- Saponin 10 **(19)**: same aglycone as saponin 2, and one monosaccharide at C-3 ➔MIC= 32 µg/mL.

- Saponin 11 **(20)**: same aglycone as saponin 2, and two monosaccharides at C-3 ➔ MIC= 16 µg/mL.

- Saponin 12 **(21)**: same aglycone as saponin 2, and three monosaccharides at C-3 (with terminal Ara_p) ➔ MIC= 32 µg/mL.

- Saponin 13 **(22)**: same aglycone as saponin 2, and three monosaccharides at C-3 (with terminal Ara_p + OAc group) ➔ MIC= 64 µg/mL.

- Saponin 14 **(23)**: same aglycone as saponin 2, and three monosaccharides at C-3 (with terminal Ara_p + two OAc groups) ➔ MIC= 128 µg/mL.

- Saponin 15 **(24)**: same aglycone as saponin 2, and three monosaccharides at C-3 (with terminal Ara_p) ➔ MIC= 32 µg/mL.

- Saponin 16 **(25)**: same aglycone as saponin 2, and three monosaccharides at C-3 (with terminal Ara_f + OAc group) ➔ MIC= 64 µg/mL.

- Saponin 17 **(26)**: same aglycone as saponin 2, and three monosaccharides at C-3 (with terminal Ara_f two OAc groups) ➔ MIC= 32 µg/mL.

The reason behind these results is the following: the most potent saponin among these compounds, saponin 11 (α- hederin) **(20)** had two monosaccharides at C-3. Then, saponin 3 **(17)** which had an acetoxy group at C-23 instead of an OH was lacking any activity (MIC> 256

µg/mL). Also, the sapogenin 9 **(18)** lacking the hydrophilic part, did not exhibit any antifungal effect against *C. albicans*. Plus, the potent saponins 2 **(16)** and 16 **(25)** had the same MIC (64 µg/mL) and oligosaccharide chains, with Ara as the terminal sugar in furanosyl form with an acetoxy group attached to it. When a second OAc group was added to the same sugar chain, such as in the potent saponin 17 **(26)**, the activity of the latter was enhanced with an MIC of 32 µg/mL. Second, saponins 10 **(19)** and 12 **(21)** had respectively one and three monosaccharides (terminal Ara_p), that presented the same potency (MIC= 32 µg/mL). However, when an acetoxy group was added to the hydrophilic part of the potent saponin 13 **(22)** (the same sugar chain as saponin 12 **(21)**), its activity was reduced (MIC= 64 µg/mL). Third, when a second OAc group was added to the same sugar chain, in the case of saponin 14 **(23)**, the antifungal activity kept on decreasing to reach a moderate level (MIC= 128 µg/mL). Fourth, when the potent saponin 15 **(24)** was devoided of any acetoxy group in its sugar chain (same hydrophilic part as potent saponin 12 **(21)**), its antifungal effect increased (MIC= 32 µg/mL). This could be further explained that when the terminal sugar is Ara_p (saponins 12 **(21)**, 13 **(22)**, 14 **(23)**, 15 **(24)**), the presence and increase in the number of OAc groups attached to it will eventually lead to a decrease in the antifungal properties of the saponin against *C. albicans*. Nevertheless, in the case of a terminal Ara_f (saponins 2 **(16)**, 16 **(25)**, 17 **(26)**), the presence and increase in the number of OAc in the oligosaccharide chain will increase the effect of the saponin against the tested fungus. Adding to the above interpretation, the presence of an acetoxy group on C-23 of the aglycone, instead of an OH group, will directly deplete the effect of the according saponin (saponin 3 **(17)**).

Additionally, (Njateng et al., 2015) extracted four hederagenin triterpenoid saponins from *Polyscias fulva* (Araliaceae): three of which have their structures already been discovered: saponins 7 **(27)** (Joshi et al., 1992), 8 **(28)** (Zhong et al., 2001), and 10 **(29)** (Grishkovets et al.,

1996)), along with a new one: saponin 11 **(30)**. They studied the antifungal activity of the four compounds against *C. albicans* (MIC> 400 µg/mL ➔ inactive; MFC > 400 µg/mL ➔ no fungicidal effect). Saponin 7 **(27)** was found to have a moderate fungistatic effect against *C. albicans* (MIC= 100 µg/mL) and lacked any fungicidal activity (MFC> 400 µg/mL). It had one monosaccharide at C-3 and an OH at C-23. As for its analogue, saponin 8 **(28)**, it shared the same aglycone and hydrophilic part at C-3 but with an additional monosaccharide (Rha) in the sugar chain. This extra sugar increased greatly its fungistatic and fungicidal effects (MIC/MFC= 12.5/12.5 µg/mL). Concerning saponins 10 **(29)** and 11 **(30)**, which had the same sugar chain as the potent saponin 8 **(28)**; however, the presence of an extra sugar moiety at C-28 (bidesmosides), deprived them from any activity (both MIC/MFC > 400 µg/mL).

Another plant-producing triterpenoid saponins with hederagenin aglycones, is *Hedera helix* (Araliaceae). (Vidal-Ollivier et al., 1989) extracted from this plant two triterpenoid saponins: α-hederin **(31)** and δ- hederin **(32)**. Only the former was effective against *C. albicans*, producing an MIC of 25 µg/mL, and possessing two monosaccharides at C-3 (Ara and Rha). As for δ-hederin **(32)** which had only one sugar at C-3, was considered inactive with an MIC >200 µg/mL.

As well, (Adesegun et al., 2008) isolated from *Lecaniodiscus cupanioides* (Sapindaceae), two already known hederagenin saponins (Encarnación et al., 1981) with a hydroxyl property at C-23 and a free COOH at C-17. The potent compound 1 **(33)**, which had three sugars at C-3 (Ara, Rha, and Ara_f as terminal sugar) and a half maximum inhibitory concentration 50 (IC_{50}) of 10 µg/mL. Regarding compound 2 **(34)**, which also had the same sugar chain at C-3, exhibited almost similar results as those of compound 2, with an IC_{50} of 8.5 µg/mL.

Lastly, (Du et al., 2003) studied the antifungal activity of two bidesmosidic triterpenoid saponins from *Clematis tangutica* (Ranunculaceae). Saponin 1 **(35)** was completely inactive producing <6 mm diameter zone with >80 µg/disc; and saponin 2 **(36)** showed a very slight activity but it was too weak (25 µg/disc of saponin 2 **(36)** produced <6 mm zone of inhibition). Therefore, this further confirmed the little to no activity of bidesmosidic triterpenoid saponins.

Thus, triterpenoid saponins with a hederagenin aglycone, must possess a free COOH at C-17 and be monodesmosidic i.e. having one sugar chain, composed of either two or three monosaccharides, at C-3 (longer chain: 3 monosaccharides give better results). However, any additional sugar (above three) will weaken the activity and even deplete it. In addition, the shape of the terminal sugar (if Ara), whether it was a pyranose or furanose will affect greatly the MIC, with pyranose giving better results than furanose. Additionally, the presence of an acetoxy group in the aglycone will inhibit the antifungal effect of the saponins. Nonetheless, a saponin with a trisaccharidic chain having a terminal Ara_p with an OAc attached to it will reduce its antifungal properties. This is the complete opposite in the case of a saponin of trisaccharidic chain with a terminal Ara_f. In this case, the increased number of OAc attached to the terminal Ara_f will produce better MIC results. Also, a hydroxyl moiety in the aglycone (either at C-16 or C-23) will increase the activity. All of these properties will allow them to exert great antifungal effect against *C. albicans*.

2.7.2.2. Triterpenoid saponins with phytolaccagenin acid aglycones

Three phytolaccosides B **(37)**, E **(38)**, and F **(39)**, were isolated from *Phytolacca tetramera* (Phytolaccaceae) (Escalante et al., 2002). Only saponin B **(37)** exhibited moderate antifungal effect on *C. albicans*, with an MIC value of 125 µg/mL (MIC ≥ 250 µg/mL ➔ saponin inactive). As for the remaining two saponins E **(38)** and F **(39)**, they were both deprived of antifungal activity, having MICs >250 µg/mL. The reasons behind these results are the following: saponin B **(37)** had in its aglycone a free COOH at C-17, a hydroxyl group at C-2 (also called 2β- OH) and one monosaccharide at C-3. However, even though saponin E **(38)** had the same aglycone as the moderate saponin B **(37)** (OH at C-2 and free COOH at C-17), the additional sugar in the hydrophilic part (total of two monosaccharides), rendered it inactive. As for the last saponin, phytolaccoside F **(39)**, in spite of having a free COOH at C-17, the lack of 2β- OH and also the increase in the number of sugars in the glycone (total of three monosaccharides) at C-3, completely impaired its activity. For further clarification, other studies have reported that whenever a saponin with an oleanolic acid aglycone lacked a hydroxyl group at C-2 of the aglycone, was considered too weak or devoided of activity (A. Favel et al., 1994).

(Woldemichael & Wink, 2001) also isolated three saponins (4 **(40)**, 5 **(41)**, and 6 **(42)**) with a phytolaccagenin aglycone, from *Chenipodium quinoa*. Only saponin 5 **(41)** possessed antifungal properties against *C. albicans*, producing a MIC value of ≤ 100 µg/mL. However, saponins 4 **(40)** and 6 **(42)** were deprived from antifungal activities. In spite of having one monosaccharide at C-3, saponin 4 was deficient of 2β- OH, and had an additional sugar moiety at C-28 (bidesmosidic saponin) which depleted its activity (MIC ≤ 500 µg/mL). Also, the increase in the number of sugar chain at C-3, the lack of 2β- OH, and the extra sugars at C-28, have contributed greatly to the inoperativity of saponin 6 **(42)**. Concerning saponin 5 **(41)**, it had two monosaccharides at C-3 (instead of one) and lacked an OH at C-2. However, the presence of an

aldehyde group in its aglycone (at C-17), amplified its antifungal activity and eventually saponin 4 **(40)** was active against *C. albicans*, exhibiting thereby a lower MIC (≤ 100 µg/mL) than the other saponins.

Hence, a triterpenoid saponin with a phytolaccagenin aglycone possessing antifungal properties against *C. albicans* should have the following: a sugar chain at C-3 but with shorter carbohydrate chains (one monosaccharide), and a free COOH at C-17 instead of a sugar moiety at C-28 (which will make the saponin bidesmosidic). Also, the presence of an aldehyde group in the saponin's aglycone is a plus, which will improve the antifungal activity.

2.7.2.3. Triterpenoid saponins with oleanolic acid aglycone

(Hu et al., 2018) isolated three oleanolic acid saponins (1 **(43)**, 7 **(44)** and 8 **(45)**) from the pulp of *Sapindus mukorossi* and tested them against *C. albicans* (MIC> 256 µg/mL ➔ inactive). Saponin 8 **(45)**, exhibited the most potent antifungal activities among the three compounds (MIC= 8 µg/mL). It had three sugars at C-3 (Ara, Rha, Ara_p), with the terminal Ara sugar in its pyranosyl form. However, saponin 1 **(43)**, the analogue of 8 **(45)**, was deficient in antifungal activity (MIC> 256 µg/mL), having three monosaccharides at C-3 (Ara, Rha, Ara_f) with the terminal sugar Ara being in the furanosyl form. The latter being the reason behind the lack of activity, which was seen in sapindoside C that had its activity reduced after having its terminal Ara sugar in its furanosyl form (Kimata et al., 1983). As for saponin 7 **(44)**, having three monosaccharides at C-3 (Xyl, Rha, Ara) produced potent results (MIC= 16 µg/mL) but slightly inferior to saponin 8 **(45)**. This is due to the nature of the terminal sugar (Xyl instead of Ara).

(Tagousop et al., 2018) studied the antifungal activities of four oleanolic acid saponins from *Melanthera elliptica* (Asteraceae), against *C. albicans* (MIC> 256 µg/mL ➔ inactive). The

monodesmosidic saponins 1 **(46)** (Vidal-Ollivier et al., 1989), 3 **(48)** (Paphassarang et al., 1989) and the bidesmosides 2 **(47)** (Vidal-Ollivier et al., 1989) and 4 **(49)** (Tan et al., 1999), have their structures already known. Saponins 1 **(46)** (1 monosaccharide at C-3) and 3 (2 monosaccharides at C-3) showed good antifungal properties with MIC= 64 µg/mL and moderate effect with MIC= 128 µg/mL, respectively. Even though saponin 3 **(48)** had an extra sugar as compared to 1 **(46)**, it yet, was less effective, maybe this is due to the nature of the sugar attached to glucose at C-3. They also had both COOH at C-17. However, these values were lower than those produced by *S. mukorossi* saponins (**(45)** 8 and **(44)** 16 µg/mL) (Kimata et al., 1983), maybe due to the decreased number of sugars in the hydrophilic part of the compounds of *M. elliptica*. As for the remaining bidesmosides, 2 **(47)** and 4 **(49)**, the former was inactive (MIC > 256 µg/mL) and the latter, surprisingly, exhibited great antifungal activity (MIC= 16 µg/mL).

In other studies, four compounds were extracted from *Paullinia pinnata* (Sapindaceae) (Lunga et al., 2014): the oleanolic acid sapogenin 3 **(50)** (friedelin) (Klass et al., 1992; S. B. Mahato & Kundu, 1994), saponins 5 **(51)** (aridanin) (Abdel-Kader et al., 2001; Ngassapa et al., 1993), 6 **(52)** (Ngassapa et al., 1993), and 7 **(53)** (lotoidoside E) (Hamed et al., 2005); and were tested against *C. albicans* (MIC ≥ 100 µg/mL ➔ inactive). Sapogenin 3 **(50)** and saponin 5 **(51)** had no effect on the tested fungus (MIC of both ≥ 100 µg/mL). However, saponin 6 **(52)**, the analogue of saponin 5 **(51)**, exhibited potent activities (MIC= 3.125 µg/mL). Even though 5 **(51)** and 6 **(52)** shared the same sugar chain and almost a similar aglycone; but the presence of an additional hydroxyl group at C-16 greatly enhanced its activity. As for saponin 7 **(53)**, which was also the analogue of saponin 5 **(51)** but with a longer sugar chain (additional Gal at C-3), and an extra OH at C-16, exhibited better antifungal results with MIC= 3.125 µg/mL.

Moreover, (Njateng et al., 2015) thoroughly reviewed the fungistatic and fungicidal activities (MIC and MFC > 400 µg/mL ➔ inactive) of four compounds from *Polyscias fulva*. The following were: compound 4 oleanolic acid sapogenin **(54)** (Zhong et al., 2001), and saponins 5 **(55)** (Zhong et al., 2001), 6 **(56)** (Grishkovets et al., 1996), and 9 **(57)** (bidesmoside) (Ahmed et al., 2009). Compounds 4 **(54)** (sapogenin) and 9 **(57)** (bidesmoside) were inactive at the highest concentration tested (both MIC/MFC > 400 µg/mL). Whereas saponin 5 **(55)**, having two monosaccharides at C-3, only moderately blocked the growth of *C. albicans* (MIC= 100 µg/mL) and did not show any fungicidal activity (MFC > 400 µg/mL). Also, saponin 6 **(56)** (analogue of saponin 5 **(55)**) which had an additional OH at C-16 , showed better fungistatic results than 5 **(55)** (MIC= 50 µg/mL); but also lacked fungicidal effect (MFC > 400 µg/mL).

Furthermore, (Woldemichael & Wink, 2002) isolated from *Lupinus angustifolius* (Leguminoseae) the triterpenoid saponins 4 **(59)** and 2 **(58)** (Bialy et al., 1999) that produced MIC values of 25 and 30 µg/mL, respectively. Saponin 2 **(58)** had three monosaccharides at C-3 (GlcA, Gal, Rha), one OH at C-22, one sugar at C-21. Also, saponin 4 **(59)** had four monosaccharides at C-3 (GlcA, Gal, Rha, Rha), one OH at C-22 and one sugar at C-21. In spite of having an additional sugar moiety (at C-21) other than at C-3, they both presented good fungistatic results. The presence of a hydroxyl group at C-22 and the increased sugar length at C-3 might have contributed to the enhanced activity.

Additionally, four triterpenoid saponins were isolated from the plant *Anagallis arvensis* L., which belongs to the Primulaceae family (Soberón et al., 2017). The latter along with the Myrsinaceae family are the only two families that produce oleanolic acid saponins that are very infrequent in nature. These saponins usually have an epoxy bridge between carbons number 13

and 28 (13β, 28- epoxy bridge) (Foubert et al., 2008). The four compounds under study were the following:

- Saponin 1 **(60)**: Anagallisin C (AnC) (also named Desglucoanagalloside B)

- Saponin 2 **(61)**: Anagallisin A (AnA) (also named Anagallosaponin I)

- Saponin 3 **(62)**: Anagallisin B (AnB)

- Saponin 4 **(63)**: Desglucoanagalloside A (also called Anagallosaponin VIII).

(Soberón et al., 2017) performed the microdilution technique to determine the MIC of the four compounds against two strains of *C. albicans* (ATCC10231 and 12-99). First, all four compounds (with 13β, 28- epoxy bridge) were potent against the strain ATCC10231, with MIC values of 1, 2, 4, and 4 µg/mL for saponins 1 **(60)**, 2 **(61)**, 3 **(62)**, and 4 **(63)**, respectively. AnC (saponin 1) **(60)** produced the best results among all tested saponins. Also, when they were tested against 12-99 *C. albicans* strain, all four compounds gave the same results as when they were tested against ATCC10231. The reason behind these findings is that even though AnA **(61)** and AnB **(62)** had the same sugar chain at C-3, the presence of an OH group (AnA **(61)**) instead of a ketone group (AnB **(62)**), at C-16 gave better results. As for AnC **(60)** and Desgluconagalloside A **(63)** that had the same sugar chain at C-3 (four monosaccharides), exhibited different MIC results (MIC_{AnC}= 1 µg/mL, $MIC_{Desgluconagalloside\ A}$= 4 µg/mL). This is because AnC **(60)** had an OH at C-16, and an OH at C-23, which was absent in Desgluconagalloside A **(63)**, and eventually enhanced its activity. In addition, the presence of a carboxylic group at C-22 in compound 4 **(63)** might have slightly reduced its activity.

Another plant producing oleanolic acid saponins with a 13β, 28- epoxy bridge is *Maesa lanceolata* Forsskal var *golungensis* Welw. from the family Myrsinaceae (Foubert et al., 2008).

(Sindambiwe et al., 1998) isolated a mixture of six triterpenoid saponins **(64→69)** called maesasaponin mixture B, from this plant. The latter was found to impede the growth of *C. albicans* at an MIC of 100 µg/mL (moderate activity). However, individual saponins were not tested against the assigned fungus.

Other plants belonging to the Primulaceae family (possess 13β, 28- epoxy bridge) are *Cyclamen cilicium* Boiss et Heldr var. intarninaturn, *Cyclamen mirabile,* and *Cyclamen coum tuber*.

First, (Abbasołu & Türköz, 1995) found that the MIC of the total saponins extract of *Cyclamen cilicium* Boiss et Heldr var. intarninaturn Meikle was 0.15%. However, they did not determine the activity of individual saponin of this plant, so, it is important in the future to do so. *C. cilicium* produces three major triterpenoid saponins: desglucocylamin I **(70)** (four sugar at C-3), cyclamen **(71)** (five sugars at C-3), and isocyclamin **(72)** (five sugars at C-3). They all share the same aglycone properties, which are the presence of the 13β, 28-epoxy bridge and a hydroxyl group at C-16, increasing thereby their antifungal effects (Yayli et al., 1998)

Second, (Çaliş, Şatana, et al., 1997) isolated from *Cyclamen mirabile*, three triterpenoid saponins: 1 **(73)** (cyclaminorin with three sugars at C-3) and 2 **(74)** (desglucocyclamin with four monosaccharides at C-3) that were previously extracted from *Cyclamen coum* Miller (Yayli et al., 1998) and 4 **(75)** (cyclamin with six sugars at C-3) (Reznicek et al., 1989) and studied their effects against *C. albicans* (MIC> 320 µg/mL ➔ inactive). They all exhibited the same moderate effects (MIC= 160 µg/mL). They all had 13β,28- epoxy bridge and an OH at C-16.

Third, (Sajjadi et al., 2016) tested the fungistatic and fungicidal effects of saponin extracts of *Cyclamen coum tuber*. They have found that these extracts were able to block *C. albicans* growth and even kill it, for MICs and MFCs between 5 and 32 µg/mL. However, in this study,

individual saponins were not studied. Additionally, some of this plant's saponins were already isolated and their structures were known, with oleanolic acid being the aglycone. They also had the epoxy bridge between C-13 and C-28. These compounds were the following: saponin 1 **(76)** (cylaminorin with three monosaccharides at C-3), saponin 2 **(77)** (desglucocyclamin with four sugars), saponin 3 **(78)** (cyclacoumin with four monosaccharides), and saponin 4 **(79)** (mirabilin lactone with five sugars and a lactone function between C-28 and C-30). It is important to study the effect of each of these saponins alone (Çaliş, Yürüker, et al., 1997)

Quillaja saponaria (Quillajaceae) had its total saponin extract (QTS) studied against *C. albicans* (Moghimipour et al., 2014). QTS inhibited the growth of the latter at an MIC of 10^4 µg/mL, which is considered a quite weak activity. Also 2 x 10^4 µg/mL of QTS produced a zone of inhibition of 16.5 mm in diameter. In this study, the higher the concentration, the better were the results, so QTS was considered a good antifungal but only in large test concentrations. This might be due to the nature of the sixty triterpenoid bidesmoside saponins **(80 → 139)** which were isolated from *Q. saponaria* and having an aglycone (quillaic acid) of β- amyrin series (oleanane) (Fleck et al., 2019). These saponins mainly have a branched trisaccharide unit at C-3 and two monosaccharides at C-28 (Kite et al., 2004). Nevertheless, saponins of *Q. saponaria* can also be monodesmosides but in very minor quantities (Fleck et al., 2019).

Besides these listed plants, (Maatalah et al., 2012) studied the antifungal activity of the saponin extracts of *Anabis articulate* (Amaranthaceae) against *C. albicans*, by performing the disk diffusion method. They have found that 500 µg/mL of this extract produced a diameter of zone of inhibition of 8.8 mm on the plate. Nevertheless, they did not perform this technique on each saponin alone. This plant is known to produce triterpenoid saponins, three of which have their structures already known (bidesmosidic saponins with oleanolic acid aglycone) (Salah et al.,

2019). The latter were: saponins 2 **(140)**, 3 **(141)**, and 4 **(142)**, and they all shared the same aglycone (OH at C-23) and sugar chains (1 sugar at C-3 and C-28).

Triterpenoid saponins possessing an oleanolic acid aglycone have different properties that make them good antifungal agents. The latter mean having one sugar chain at C-3 (three monosaccharides), hydroxyl groups at C-16, and/or C-22 and C-23. Also, the presence of a hydroxyl group at C-2 (2β-OH) enhances the antifungal properties of the compound. In addition, the 13β, 28- epoxy bridge, such as in the Primulaceae and Myrsinaceae families, adds great strength to the saponins. Moreover, the nature of the terminal sugar is very important (Ara_p better than Xyl better than Ara_f). Furthermore, monodesmosidic saponins are better antifungals than bidesmosidic ones. However, the presence of a carboxylic group in the aglycone could decrease the antifungal activity of the compound against *C. albicans*.

2.7.2.4. Triterpenoid saponins with other aglycone types

Many plant producing triterpenoid saponins with different and numerous aglycone types were reported over the years. These medicinal plants that were found to inhibit the growth, and in some cases kill *C. albicans*, are the following; *Lupinus angustifolius* (Leguminoseae), *Acanthophyllum gypsophiloides* (Caryophyllaceae), *Ziziphus joazeiro* (Rhamnaceae), *Colubrina retusa* (Rhamnaceae), *Buddleja officinalis* (Loganiaceae), *Bohadschia marmorata* (Holothuriideae), *Aesculus hippocastanum* L. (Hippocastanaceae), *Dianthus calocephalus* Boiss, *Silene dichotoma Ehrh subsp Dichotoma Davis* (Caryophyllaceae), and *Glycyrrhiza glabra* L. (Fabaceae).

First, other than oleanolic acid saponins, *Lupinus angustifolius* also produces a potent soyapogenol A saponin **(143)** with an MIC of 25 µg/mL (Arao et al., 1997; Bialy et al., 1999;

Woldemichael & Wink, 2002). The latter is a dicarboxylic saponin with two hydroxyl groups (one at C-21 and the other at C-22). It is also the analogue of the potent oleanolic acid saponin 2 **(58)** MIC= 25 µg/mL), but with an additional sugar at C-3 (branched tetrasaccharide: GlcA, Gal, Rha, Rha).

Second, *Acanthophyllum gypsophiloides* Regel produced two biodesmosidic saponins 1 **(144)** and 2 **(145)**, having both the same branched trisaccharide chain at C-3 (Ara, Gal, Glc) and sugar chain at C-28 (Xyl, Xyl, Rha, Quinovone, Fuc). The only difference was in their aglycones, where saponin 1 **(144)** possessed an OH at C-16, whereas saponin 2 **(145)** did not (Khatuntseva et al., 2012). According to the literature, bidesmosides are basically deprived of antifungal activity due to the additional sugar moiety at C-28 (Du et al., 2003; Njateng et al., 2015; Woldemichael & Wink, 2001; Zhang et al., 2006). But it was not the case in this study, since both bidesmosidic saponins showed good inhibitory results against *C. albicans*, in the disk diffusion technique. 0.55 mg/disc of saponins 1 **(144)** and 2 **(145)** produced zones of inhibition of 10 and 7 mm in diameter, respectively. It is important to mention that these compounds were active against the tested fungus, only at an acidic pH (pH=4) (at neutral pH ➔ both inactive), that might explain the obtained results. This was evident with other studies that reported the effective antifungal properties of saponins against fungi at an acidic pH (Kulakovskaya et al., 2005). Since little is known about the positive effect of bidesmosidic triterpenoid saponins against *C. albicans*, further studies need to be done in order to understand the basis of this activity.

Third, (Ribeiro et al., 2013) studied the fungistatic effect of three *Zizyphus joazeiro* (juà) saponins (MIC> 12500 µg/mL ➔inactive), also called juà saponins **(146➔148)**, and that were previously isolated from this plant (Higuchi et al., 1984). All three presented a new sapogenin

called jujubogenin, and they all were active against *C. albicans* (MIC=156 µg/mL). Nevertheless, these saponins should be tested each one alone in the future.

Fourth, (Li et al., 1999) isolated a jujubogenin triterpenoid saponin (compound 1 **(149)**), produced by *Colubrina retusa*, which is very similar to those produced by *Zizyphus joazeiro* (Higuchi et al., 1984). It had a hydroxyl group at C-20 and a branched trisaccharidic chain at C-3 (Ara_f as terminal). This saponin **(149)** was found to inhibit *C. albicans* growth with an MIC value of 50 µg/mL, making it a good antifungal.

Fifth, Mimengoside A (16- dehydroxysaikogenin G) **(150)**, a triterpenoid saponin previously extracted from *Buddleja officinalis* (Ding et al., 1992), had four monosaccharides at C-3. This saponin that is also produced by *Buddleja madagascariensis* (Loganiaceae) was tested against *C. albicans* and inhibited the latter at an MIC of 50 µg/mL (Emam et al., 1996),

Sixth, (Yuan et al., 2009) extracted from *Bohadschia marmorata* four new triterpenoid saponins of holostane aglycones (1: Marmoratoside **(151)**, 2: 17α- hydroxyimpatienside **(152)**, 3: marmoratoside B **(153)** and 4: 25- acetoxy bivittoside D **(154)**), along with two already known saponins (5: impatienside A **(155)** from *Holothuria impatiens* (Holothuriideae) (Sun et al., 2007) and 6: bivittoside D **(156)** from *Bohadschia bivittata* Mitsukuri (Holothuriideae) (Kitagawa et al., 1981, 1989). All of the compounds, except for saponins 3 **(153)** and 4 **(154)**, exhibited potent antifungal results against *C. albicans*. Saponins 1 **(151)** and 5 **(155)** had the same MIC_{80} values of 2.81 µM, and saponin 6 **(156)** gave MIC_{80} of 2.80 µM. As for the potent saponin 2 **(152)** (analogue of 5 **(155)**), it gave the best results (MIC_{80}= 2.78 µM). The slightly better results it produced as compared to saponins 1 **(151)** and 5 **(155)**, is due to the presence of an extra hydroxyl group at C-17 of its aglycone. Concerning the compounds 3 **(153)** and 4 **(154)**, they had a hydroxyl and acetoxy group, respectively, at C-25 of their sapogenin and which might

have negatively impacted their activity. As a result, a drastic increase in MIC_{80} of saponins 3 **(153)** (MIC_{80}= 44.44 µM) and 4 **(154)** (MIC_{80}= 43.13 µM) was seen. This could be further explained by the lack of the acetoxy group at C-25 in saponin 6 **(156)** (same aglycone as saponin 4 **(154)**) and which eventually enabled it to exhibit good MIC_{80} results (2.80 µM). Also, 12 α-OH, that is present in all six saponins, has been mentioned to be crucial for the antifungal activity of a compound (KITAGAWA et al., 1989).

In addition, (Abbasołu & Türköz, 1995) have reviewed other medicinal plant producing triterpenoid saponins. They studied the fungistatic activity of the total saponin extract of each plant against *C. albicans*. The following were, *Aesculus hippocastanum* L. (MIC= 0.31%), *Dianthus calocephalus* Boiss (MIC= 0.63%), *Silene dichotoma* Ehrh. Subsp. *dichotoma* Davis (MIC= 0.63%) and *Glycyrrhiza glabra* L. (MIC= 0.63%), producing good antifungal results against the tested fungus. Therefore, each saponin of these plants should be alone thoroughly investigated in the future. This is done through the determination of its chemical structure, i.e. aglycone group along with the hydrophilic part and other functional groups. All of these structures are worth discovering later on in order to perceive their contribution in managing *C. albicans* infections. Among these five listed plants, *A. hippocastanum* has been already examined for its saponin content, and (Zhang et al., 2010) have reported the presence of twelve escin saponins (also named β- escin) comprising mainly three sugars at C-3 (Zhang et al., 2010). The latter were: escins Ia **(157)**, Ib **(158)**, IIa **(159)**, IIb **(160)**, IIIa **(161)**, IIIb **(162)**, IV **(163)**, V **(164)**, and VI **(165)** and the ioescins Ia **(166)**, Ib **(167)**, and V **(168)** (YOSHIKAWA et al., 1994; Yoshikawa et al., 1996; Yoshikawa et al., 1998).

In addition, *G. glabra* L. was studied and had its total saponin extract (GTS) tested against *C. albicans* (MIC= 10^4 µg/mL, and 20 µg/mL of GTS → $d_{\text{zone of inhibition}}$= 15.33 mm) (Moghimipour

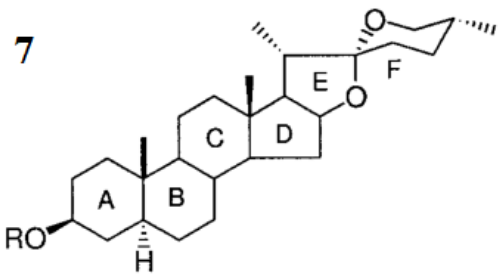
et al., 2014). The higher the concentration of GTS, the better the results were. However, GTS was considered very weak when looking at its antifungal susceptibility results (high MIC and diameter of zone of inhibition). Additionally, this plant is known to have an active biocompound, glycyrrhizic acid **(169)**, a triterpenoid saponin, that however, was found to be inactive against *C. albicans* (MIC and MFC> 200 µg/mL) (Messier & Grenier, 2011). Nevertheless, another triterpenoid saponin 18- β glycyrrhetinic acid **(170)**, isolated from *G. glabra*, exhibited inhibitory effect on the growth of the tested fungus, at an acidic pH (Fiore et al., 2005; Pellati et al., 2009; Tsukiyama et al., 2002).

It is important to conduct further studies in order to better understand the reason behind the antifungal effect of these saponins. The latter must be compared to other researches so that a clearer conclusion about the role of their aglycones and hydrophilic part have in their antifungal activities. In addition, OH groups in the aglycone have a positive impact in increasing the potency of a saponin. Also, bidesmosidic saponins might be only effective under acidic conditions (pH <7).

2.7.3. Antifungal activity of steroidal saponins against *C. albicans*

Steroidal saponins exerting antifungal effects against *C. albicans* include those that have the following aglycones (figure 7) : tigogenin (figure 7-7), sarsapogenin (figure 7-8), smilagenin (figure 7-9), hecogenin (figure 7-10), diosgenin (figure 7-11), tigogenyl (7-12), chlorogenin (figure 7-13), β-chlorogenin (figure 7-14), pennogenin (figure 7-15), and dioscin (figure 7-16) and some others (table 4).

7



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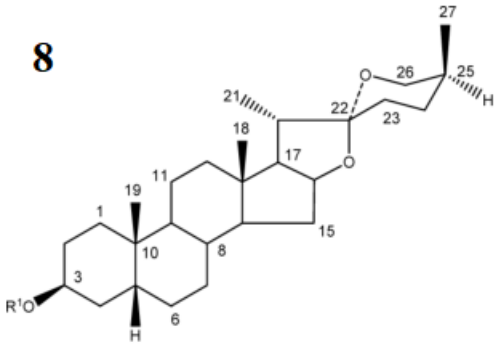

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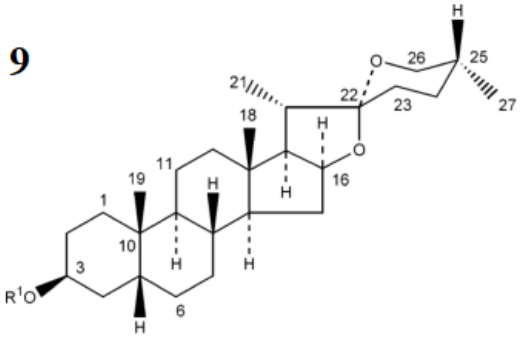

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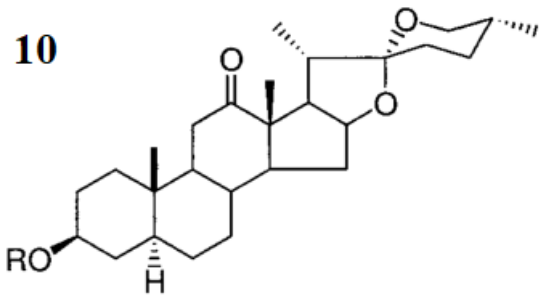

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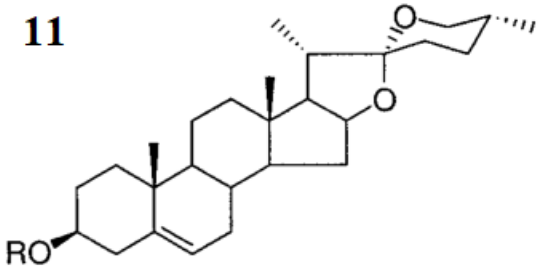

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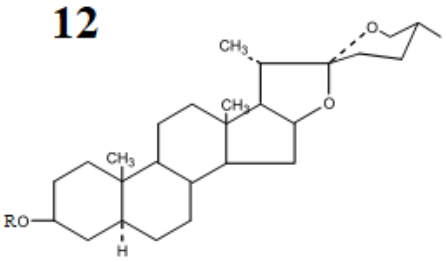

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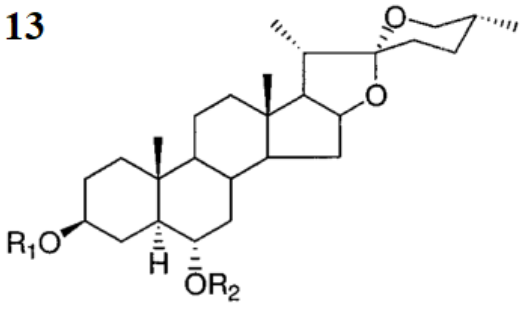

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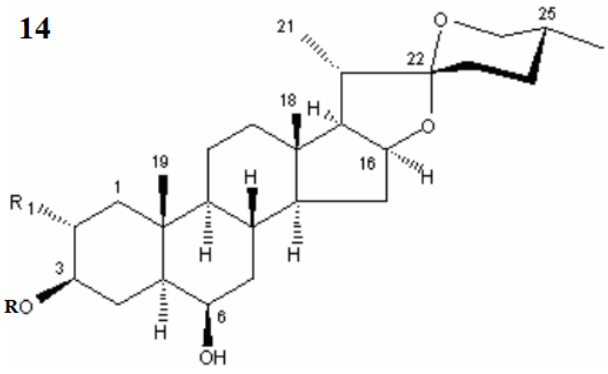

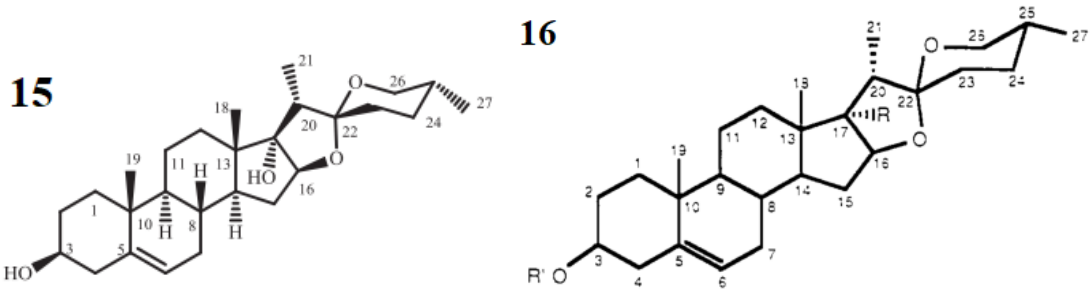

Figure 7: General structure of the aglycones of common antifungal steroidal saponins. 7: Tigogenin (Yang et al., 2006), 8: Sarsapogenin (Belhouchet et al., 2008), 9: Smilagenin (Sautour et al., 2005), 10: Hecogenin (Yang et al., 2006), 11: Diosgenin (Yang et al., 2006), 12: Tigogenyl (Favel et al., 2005), 13: Chlorogenin (Yang et al., 2006), 14: β- chlorogenin (Chincharadze & Kel, 1979), 15: Pennogenin (Shafiq ur et al., 2015), and 16: Dioscin (Hufford et al., 1988). *R in compounds 7, 10, 11, 12, and 14 and R_1 in 8, 9, and 13 and R' in 16 represent the hydrophilic part attached to C-3 of the aglycone of the corresponding saponin.

Family	**Plant**	**Aglycone type**	**Compound**	**Susceptibility tests results**
Alliaceae	*Allium leucanthum*	β-chlorogenin	Saponins **(226)**, eruboside **(227)** and yayoisaponin C **(228)**	MFC (µg/mL): - **(226)**: 12.5 - **(227)**: 25 - **(228)**: 50
		Chlorogenin	Saponin **(223)** and sapogenin **(224)**	MIC/MFC (µg/mL):

Asparagaceae	*Agave americana*			**(223)** and **(224)**: NA[1]
		Hecogenin	- Saponins **(201-206)** - Sapogenin **(207)**	MIC/MFC (µg/mL): - **(201-203)** and **(205-207)**: NA[1] - **(204)**: 20/20
		Tigogenin	- Sapogenin **(171)** - Saponins **(172,173)**	MIC/MFC (µg/mL): - **(171)**: NA[1] - **(172)**: 5/5 - **(173)**: 10/10
	Polianthes tuberosa	Chlorogenin	Saponin **(225)**	MIC/MFC (µg/mL): 10/10
		Hecogenin	Saponins **(208,209)**	MIC/MFC (µg/mL): - **(208)**: 20/NA[1] - **(209)**: NA[1]
		Tigogenin	Saponins **(174,175)**	MIC/MFC (µg/mL): - **(174)**: 5/5 - **(175)**: 20/NA[1]
	Ruscus aculeatus L.	Others	Convallamarogenin saponins **(243-247)**	NTI
	Yucca gloriosa L.	Others	Tigogenyl saponins **(262,263)**	MIC (µg/mL): - **(262)** and **(263)**: 3.12

Dioscoreaceae	*Dioscorea cayenensis*	Diosgenin	Saponins **(218)** ** and **(219,220)**	MIC (µg/mL): - **(218)**: NA[5] - **(219)**: 12.5 - **(220)**: 100
	Dioscorea panthaica prain et Burk	Others	____	NTI
	Dioscorea parviflora	Diosgenin	Saponins **(214-217)**	MIC/MFC (µg/mL): - **(214)** and **(217)**: NA[1] - **(215)**: 10/NA[1] - **(216)**: 2.5/2.5
Fabaceae	*Trigonella foenum graceum* L.	Others	Saponins **(232-235)**, neotigogenin saponin **(236)**, and yamogenin saponin **(237)**	NTI
	Allium rotundum L.	Others	Agigenin saponins **(248,249)**, β-chlorogenin **(250,251)**, and diosgenin saponins **(252)**	NTI
	Allium sativum	β-chlorogenin	Saponins **(229)** ** and **(230)**	MIC (µg/mL): - **(229)**: NA[10] - **(230)**: 25

Liliaceae	*Asparagus acutifolius* L.	Sarsapogenin	Saponins **(187-189)** **, and **(190-193)**	MIC (µg/mL): - **(187-189)**: NA[5] - **(190)** and **(191)**: 12.5 - **(192)** and **(193)**: 50
	Asparagus officinalis L.	Sarsapogenin	Saponin AS-I **(186)**	MIC (µg/mL): 20-30
	Lilium candidum L.	Others	Diosgenin saponin **(253)**, isonarthogenin **(254)** and saponins **(255-257)** and **(258)** **	NTI
	Smilax aspera subsp. *mauritanica*	Sarsapogenin	Saponins **(180,181)** and curillin G **(182)** **, asparagoside E **(183)** **, asparoside A **(184)** **, and asparoside B **(185)** **	MIC (µg/mL): - **(180)** and **(182-185)**: NA[5] - **(181)**: 25
	Smilax medica	Smilagenin	Saponins **(194)**, **(195)**, **(196)** **, disporoside **(197)**, and **(198-200)**	MIC (µg/mL): - **(194)**, **(195)** and **(197)**: 25 - **(196)** and **(200)**: NA[5]

Table 4: Plant-producing steroidal compounds (sapogenins/saponins) with different aglcyones. Tigogenin (light orange), sarsapogenin (maroon), smilagenin (pink), hecogenin (dark

				- **(198)** and **(199)**: 12.5
	Yucca aloifolia	Tigogenin	Saponins **(260,261)**	NTI
	Yucca schidigera	Others	Saponins **(264-277)**	MIC (µg/mL): - **(264)**: 6.25 - **(265, 266)** and **(274)**: 12.5 - **(268)**: 100 - **(270)**: 50 - **(273)**: 25 - **(267)**, **(269)**, **(271,272)** and **(275-277)**: NA[2]
Melanthiaceae	*Trillium govanianum*	Diosgenin	Saponins **(221,222)**	MIC (µg/mL): - **(221)**: 2.5 - **(222)**: NA[1]
		Others	- Saponins govanoside A **(278)**, borassoside E **(279)** - Sapogenin pennogenin **(280)**	MIC (µg/mL): - **(278)**: 5 - **(279)**: 2.5 - **(280)**: NA[1]

	Trillium grandiflorum	Dioscin	Saponin TG-I **(281)**	MIC (µg/mL): 1.56
Poaceae	*Avena sterilis*	Others	____	NTI
Scrophulariaceae	*Digitalis cariensis* Boiss.	Others	Timosaponin H1 **(259)**	NTI
	Digitalis lanata	Others	Tigogenin saponins **(238-240)**, gitogenin saponin **(241)**, and digitogenin saponin **(242)**	NTI
Solanaceae	*Capsicum frutenscens*	Others	Saponins CAY-I **(231)**	IC_{90} (µg/mL): 7.7
	Trigonella foenum aculeatus L.	Others	____	NTI
Zygophyllaceae	*Tribulus terrestris* L.	Tigogenin	Saponins TTS-9 **(176)**, TTS-12 **(177)**, TTS-13 **(178) ****, and TTS-15 **(179)**	MIC_{80} (µg/mL): - **(176)** and **(178)**: NA[4] - **(177)**: 1 - **(179)**: 2.3
		Hecogenin	Saponins TTS-8 **(210)**, TTS-10 **(211)**,	MIC_{80} (µg/mL): - **(210-212)**: NA[4] - **(213)**: 41.7

			TTS-11 **(212)**, and TTS-14 **(213)**	

khaki), diosgenin (light blue), chlorogenin (navy blue), others (dark purple), and dioscin (light purple). The table also includes the families of the plants and the susceptibility tests results of the compounds determining their levels of potency.

**: furostanol steroidal saponins
NT→ not tested
NTI→ not tested individually
NA→ not active at the highest test concentration:
- NA^0: not active (zone of inhibition), NA^1: >20 µg/mL, NA^2: ≥ 100 µg/mL, NA^3: ≥ 125 µg/mL, NA^4: >128 µg/mL, NA^5: >200 µg/mL, NA^6: >250 µg/mL, NA^7: >256 µg/mL, NA^8: >400 µg/mL, NA^9: ≤ 500 µg/mL, $NA^{9'}$: >500 µg/mL, NA^{10}: >800 µg/mL.

2.7.3.1. Steroidal saponins with tigogenin aglycones

(Yang et al., 2006) tested the MICs and MFCs of the previously isolated sapogenin tigogenin (compound 23) **(171)** and tigogenin saponins 1 **(172)** and 2 **(173)** from *Agave Americana* (Asparagaceae) (Jianming et al., 2002), against *C. albicans*. In addition to compounds 3 **(174)** and 4 **(175)**, tigogenin saponins, from *Polianthes tuberosa* (Asparagaceae) (Jin, Zhang, & Yang, 2004). 20 µg/mL was assigned to be the highest concentration for testing: any value that was above it, automatically made the saponin inactive. Accordingly, the saponins were marked as inactive (NA) (MIC and MFC > 20 µg/mL, marginal (MIC and MFC= 20 µg/mL), moderate (MIC and MFC= 10 µg/mL), or potent (MIC and MFC < 10 µg/mL) (Yang et al., 2006). Following the aforementioned standards, saponin 3 **(174)** was considered potent, exhibiting both MIC and MFC values of 5 µg/mL. It had four sugars in its glycosidic chain at C-3. Then, saponin 1 **(172)** which was the analogue of the potent saponin **(174)** but with a longer sugar chain (same chain but with an additional rhamnose unit), presented the same MIC and MFC values of that of compound 3 **(174)**. This means that an extra rhamnose won't reduce the activity of saponin 1 **(172)** against *C. albicans*. However, saponin 4 **(175)** was also the analogue of

saponin 3 **(174)**, having the same sugar chain but with an additional xylose unit. The presence of the latter at this position instead of rhamnose, made the saponin's fungistatic activity marginal with an MIC value of 20 µg/mL. Also, saponin 4 **(175)** had no fungicidal effect on *C. albicans* as it produced an MFC value > 20 µg/mL. Sapogenin 23 **(171)** which was clearly lacking a sugar chain at C-3, exhibited no antifungal effect against *C. albicans* (MIC and MFC >20 µg/mL).

As for saponin 2 **(173)**, which almost had the same sugar chain as the potent saponin 1 **(172)**, but the replacement of Xyl (2-1) by Glc (2-1) decreased its antifungal activity leaving it as moderate (both MIC and MFC= 10 µg/mL).

Additionally, four tigogenin spirostanol saponins TTS-9 **(176)**, TTS-12 **(177)**, TTS-15 **(179)** and one furostanol saponin TTS-13 **(178)**, produced by *Tribulus terrestris* L. (Zygophyllaceae), were tested for their activity against *C. albicans.* This was done through the determination of their MIC_{80} (the lowest concentration of the saponin, that will inhibit 80% of the growth of *C. albicans*). Also, any value of MIC_{80}> 128 µg/mL, marked immediately the saponin as inactive and lacking an antifungal activity. So, TTS-9 **(176)** which had only two monosaccharides at C-3 (< 4 sugars), presented no antifungal activity (MIC> 128 µg/mL). As for TTS-12 **(177)**, it presented the best antifungal properties with an MIC_{80}= 1 µg/mL (< MIC_{80} of the positive control fluconazole= 1.4 µg/mL), where it had five sugars in its hydrophilic part. However, the furostanol TTS-13 **(178)**, the analogue of TTS-12, was deprived of activity (MIC_{80} > 128 µg/mL). Even though it had the same sugar chain at C-3 as that of TTS-12 **(177)**, but the presence of an additional sugar moiety at C-26 (open F ring) (bidesmosidic saponin) might be the reason behind the inoperativity of TTS-13 (Zhang et al., 2006). This might explain why other studies have stated that the presence of a closed F ring is crucial for the proper antifungal

functioning of a saponin (Tian et al., 2017a). Maybe this will prevent having a second point of attachment for another sugar chain on the aglycone (since a furostanol has an additional sugar moiety at C-26 of its open F ring) (Majinda, 2012). Moreover, TTS-15 **(179)** was also potent (MIC_{80}= 2.3 µg/mL), having four monosaccharides at C-3 but instead of Xyl it was replaced with a Glc which slightly decreased its activity. For further certainty, and since TTS-12 **(177)** gave better results, it was tested for its *in vivo* effects against *C. albicans*-infected rats vaginally. The results have shown that 30 and 60 mg/Kg of TTS-12 **(177)** was able to significantly decrease the number of infected rats, and 60 mg/Kg almost cleared the infection from all rats fourteen days post infection (Zhang et al., 2006). Furthermore, *C. albicans* is known for its ability to form biofilms on solid surfaces. So, in the same study TTS-12 **(177)** was also effective against *C. albicans* biofilm hyphal formation and was able to block the development of hyphal cells (Zhang et al., 2006).

Thus, antifungal saponins with tigogenin algycones must have at least four monosaccharides at C-3. The presence of five sugars at the aforementioned position will increase the antifungal properties of the saponin. This depends on the type of the sugar attached at C-3, since the potency of the saponin will increase according to the following classification: Rha > Xyl > Glc. Noteworthy, the presence of glucose at the position (2-1) (Glc (2-1)) will reduce the activity of the compound.

2.7.3.2. Steroidal saponins with sarsapogenin aglcyones

(Belhouchet et al., 2008) studied the antifungal activities of five saponins previously obtained from the roots of *Smilax aspera* subsp. *mauritanica* (Liliaceae) (MIC> 200 µg/mL➔ saponin inactive). The six studied compounds were two sarsapogenin saponins 1 **(180)** (Agrawal, 2003; Agrawal et al., 1998) and 3 (curillin G) **(181)** (Sharma & Sharma, 1993) and four furostanols: 2

(182), 4 (asparagoside E) **(183)** (Goryanu, 1984), 5 (asparoside A) **(184)** and 6 (asparoside B) **(185)** (Sharma et al., 1982). Only compound 3 **(181)** (two monosaccharides at C-3) was active against *C. albicans* with an MIC of 25 µg/mL; however, saponins 1 **(180)**, 2 **(182)**, and 4 **(183)**, 5 **(184)**, and 6 **(186)** showed no signs of antifungal activity (MIC > 200 µg/mL). The lack of activity in furostanols 2 **(182)**, 4 **(183)**, 5 **(184)**, and 6 **(186)** also supports latter findings, because of the opened F ring, allowing thereby an additional point of attachment of another sugar chain on the aglycone (at C-26). As for saponin 1, although it had three monosaccharides at C-3; however, the sugar chain was a linear one instead of being branched which could be the reason behind its decreased antifungal effect. This was further validated by (Shimoyamada et al., 1990) who isolated a sarsapogenin saponin, AS-I **(186)** (Eggert & Djerassi, 1975), from *Asparagus officinalis* L. (Liliaceae), and which was tested for its activity against *C. albicans* (MIC> 200 µg/mL➔ saponin inactive). AS-I **(186)** had a branched sugar chain at C-3, composed of three monosaccharides and which eventually exhibited good antifungal properties against the tested fungus, producing an MIC value ranging between 20 and 30 µg/mL.

For further confirmation about the effect of a branched trisaccharidic chain (at C-3) on *C. albicans* growth, (Sautour, Miyamoto, et al., 2007) worked on seven steroidal saponin produced by *Asparagus acutifolius* (Liliaceae). Three of which were furostanols (1 **(187)**, 2 **(188)**, and 3 **(189)**) and the remaining four (4 **(190)**, 5 **(191)**, 6 **(192)**, and 7 **(193)**) were sarsapogenin spirostanol saponins. All seven compounds were studied for their activities against *C. albicans* (MIC> 200 µg/mL ➔ saponin inactive). Only spirostanol saponins influenced the tested fungus (12.5 < MIC < 50 µg/mL), whereas the furostanol saponins were inactive at the highest test concentration (MIC> 200 µg/mL). The latter results further confirmed the reduced and diminished antifungal activity of saponins, with an open F ring, against *C. albicans*. Regarding

the spirostanol saponins **(190→193)**, all four compounds exhibited good antifungal results, having a branched trisaccharidic chain at C-3. But saponins 4 **(190)** and 5 **(191)** were clearly better antifungals (MICs of both= 12.5 µg/mL) than compounds 6 **(192)** and 7 **(193)** (MICs of both= 50 µg/mL). The reason behind this outcome and enhanced activity, was due to the replacement of Glc by Xyl at C-2 of the sugar (Glc) attached to C-3 of the aglycone. Accordingly, this pinpoints the relevance between the types of sugars in the hydrophilic chain of the saponin and its antifungal impact.

Regarding steroidal saponins with sarsapogenin aglcyones, the presence of a sugar chain of at least two monosaccharides at C-3 represents good MIC results. In addition, if the hydrophilic part was composed of three monosaccharides, the chain must be branched (instead of linear) in order for the saponin to be active. Otherwise, the compound will have its activity depleted. Additionally, the nature of the monosaccharide attached to the sugar at C-3 is very important for the antifungal effect against *C. albicans*.

2.7.3.3. Steroidal saponins with smilagenin aglycones

(Sautour et al., 2005) isolated from *Smilax medica* (Liliaceae) three smilagenin spirostanol saponins: two of which were new (compounds 1 **(194)** and 2 **(195)**) and an already known one (compound 4: disporoside A **(197)**) (J. Li et al., 2017), and one furostanol (compound 3) **(196)**. All four saponins underwent susceptibility tests against *C. albicans* (MIC > 200 µg/mL ➔ saponin inactive). Only saponins 1 **(194)**, 2 **(195)**, and 4 **(197)** were active against *C. albicans*, all producing the same MIC value (25 µg/mL). Noteworthy, saponins 2 **(195)** and 4 **(197)** had a branched sugar chain composed of three monosaccharides and exhibited good antifungal effects,

which also confirms the importance of a branched trisaccharidic chain at C-3 (Belhouchet et al., 2008; Sautour, Miyamoto, et al., 2007; Shimoyamada et al., 1990). As for the furostanol saponin 3 **(196)**, it was marked as inactive with an MIC> 200 µg/mL having an additional sugar moiety at C-26.

Later on, (Sautour et al., 2006) extracted from *Smilax medica* two smilagenin saponins, 1 **(198)** and 2 **(199)**, along with a known one (compound 3) **(200)** (Agrawal et al., 1985). Only saponins 1 **(198)** and 2 **(199)** presented antifungal properties against *C. albicans* producing the same MIC value (12.5 µg/mL). Compounds 1 **(198)** and 2 **(199)** had two monosaccharides at C-3 and four monosaccharides at C-3. However, saponin 3 **(200)**, which had only one sugar at C-3, did not have any effect on *C. albicans*. These two results could explain that spirostanol saponins with a smilagenin aglycone possess an antifungal activity, only if they had sugar chain longer than one monosaccharide at C-3. This is also in agreement with the findings of other works (Takechi et al., 1991).

Hence, steroidal saponins with smilagenin aglycones exert their antifungal effects better when they have two or four monosaccharides or a branched trisaccharidic chain at C-3.

2.7.3.4. Steroidal saponins with hecogenin aglycones

(Yang et al., 2006) studied the antifungal activities of eight hecogenin compounds against *C. albicans* (MIC and MFC > 20 µg/mL ➔ compound inactive). These compounds were the following: 14 **(208)** and 15 **(209)** were hecogenin saponins isolated from *Polianthes tuberosa* (Jin, Zhang, & Yang, 2004; Jin et al., 2003), and 8 **(201)**, 9 **(202)**, 10 **(203)**, 11 **(204)**, 12 **(205)**, and 13 **(206)** were hecogenin saponins along with the hecogenin sapogenin (compound 26) **(207)** from *Agave Americana* (Jin et al., 2003). Among all these compounds, only saponins 11 **(204)**

and 14 **(208)** showed some activity against *C. albicans*; although they were weak/marginal, exhibiting MIC/MFC of 20/20 and 20/ >20, respectively. So, saponin 11 **(204)** showed marginal inhibitory and fungicidal effects, as for saponin 14 **(208)**, it was only able to weakly inhibit fungal growth. In addition, compounds 8 **(201)**, 9 **(202)**, and 10 **(203**) had one, two, and three monosaccharides, respectively at C-3, which made them all inactive (MIC and MFC values > 20 µg/mL). Saponin 10 **(203)** had also Glc (2-1) which also contributed greatly to its inoperativity. Moreover, all of these compounds (8→13 **(201→206)**, 14 **(208)**,15 **(209)**, and 26 **(207)**) had a ketone group at C-12 of their aglycone.

So, even though saponin 11 **(204)** had the same sugar chain as the potent tigogenin 3 **(174)** (MIC/MFC= 5/5 µg/mL) (Jin, Zhang, & Yang, 2004); however, the presence of Glc (2-1) in its sugar chain and the ketone at C-12 of the sapogenin, left the saponin inactive. Regarding phytochemical 13 **(206)**, which had the same hydrophilic part as the potent tigogenin 1 **(172)** (5 monosaccharides and MIC/MFC= 5/5 µg/mL) (Jianming et al., 2002), had its antifungal activity impaired due to the presence of a ketone group at C-12 of the aglycone. Additionally, compound 14 **(208)** had the same sugar moiety at C-3 as that of the marginal tigogenin 4 **(175)** (Jin, Zhang, & Yang, 2004) and exhibited the same results. As for saponin 15 **(209)**, which almost shared the same glycone as that of 14 **(208)**, but the replacement of Xyl (2-1) with Glc (2-1) and the presence of the ketone at C-12, directly depleted its activity. Concerning the hecogenin sapogenin (compound 26) **(207)**, it exhibited no antifungal activity due to the absence of the oligosaccharidic chain at C-3.

Moreover, other hecogenin saponins TTS-8 **(210)**, TTS-10 **(211)**, TTS-11 **(212)**, and TTS-14 **(213)** were isolated from *Tribulus terrestris* L. (Zhang et al., 2006). They all had their MIC_{80} tested against *C. albicans* (MIC_{80}> 128 µg/mL ➔ saponin inactive). Only TTS-14 **(213)** showed

moderate antifungal properties against *C. albicans* (MIC_{80}= 41.7 µg/mL), although it had the same sugar chain as that of the potent tigogenin TTS-15 **(179)** (MIC_{80}= 2.3 µg/mL). However, the presence of the ketone at C-12 of its sapogenin, had reduced its effect on *C. albicans*. As for the remaining compounds that had a ketone at C-12 and were devoided of activity (MIC_{80}> 128 µg/mL): TTS-8 **(210)** had two monosaccharides, TTS-10 **(211)**, and TTS-11 **(212)** both had a linear trisaccharidic chain at C-3 which explains their lack of antifungal activity (Belhouchet et al., 2008).

Hecogenin steroidal saponins are not considered good antifungals as they present very weak to no activity against *C. albicans*. The reason behind this lies in the fact that this type of saponins possesses a ketone group at C-12. The latter either reduces dramatically the antifungal properties of the saponin or renders it completely inactive. Also, the type (absence of Glc (2-1)) and the number of sugars adds to the role of these compounds (between branched trisaccharides and five monosaccharides → increase in activity).

2.7.3.5. Steroidal saponins with diosgenin aglycones

Four diosgenin saponins 19 **(215)**, 20 **(216)** (Ming et al., 2002), 18 **(214)** , and 21 **(217)** (Jin, Zhang, Li, et al., 2004) were isolated from *Dioscorea parviflora* (Dioscoreaceae). (Yang et al., 2006) studied their growth inhibitory and fungicidal effects on *C. albicans* (MIC and MFC > 20 µg/mL ➔ saponin is inactive). Saponin 20 **(216)** presented excellent antifungal susceptibility results (MIC and MFC= 2.5 µg/mL), due to the presence of a branched trisaccharidic chain at C-3 of its sapogenin. As for saponin 19 **(215)**, it had the same number and almost same sugar part

as that of compound 20 **(216)**. However, the presence of Glc (2-1) replacing Rha (2-1) decreased the activity of saponin 19 **(215)** rendering it moderate (MIC= 10 µg/mL) and depriving it from its fungicidal effect (MFC > 20 µg/mL). Regarding saponins 18 **(214)** and 21 **(217)**, they only had one and two monosaccharides, respectively; also, saponin 18 **(214)** had Glc (2-1). They both produced no effect (both MIC and MFC > 20 µg/mL).

Another plant, *Dioscorea cayenensis* (Dioscoreaceae) (Sautour et al., 2004), produced two diosgenin saponins 2 **(219)** and 3 **(220)**. The latter was the analogue of the former, but with a longer sugar chain. Their activities were tested against *C. albicans* through the determination of their MIC (MIC> 200 µg/mL ➔ inactive saponin). Compound 2 **(219)** had three monosaccharides at C-3 (branched chain) with an MIC value of 12.5 µg/mL and hence viewed as potent. However, saponin 3 **(220)** had a reduced MIC of 100 µg/mL (moderate activity), and this was due to the extended sugar unit at C-3 (total of four monosaccharides). In addition, this plant also produced a furostanol saponin (compound 1) **(218)** and that had the same sugar chain at C-3 as that of saponin 3 **(220)**; yet was devoided of antifungal activity (MIC> 200 µg/mL ➔ inactive) due to the additional sugar moiety at C-26.

Moreover, one diosgenin saponin 2 **(221)** and one diosgenin sapogenin 4 **(222)**, from *Trillium govanianum* (Melanthiaceae), were tested against *C. albicans* (MIC> 20 µg/mL ➔ inactive saponin) (Ismail et al., 2015). Saponin 2 **(221)** exhibited excellent MIC values of 2.5 µg/mL, hence marked as potent. The reason behind its potency might be due to the branched trisaccharidic chain at C-3. As for the sapogenin (compound 4) **(222)**, the lack of a sugar chain at C-3 of its aglycone made it inactive (MIC > 20 µg/mL).

Diosgenin saponins are excellent antifungals only if they have three monosaccharides, but in a branched way. Less than this number of sugars will diminish the activity of the saponin, and four monosaccharides will lessen it.

2.7.3.6. Steroidal saponins with chlorogenin aglycones

Two chlorogenin saponins, 16 **(223)** and 17 **(225)**, were isolated from *Agave Americana* (J.-M. Jin et al., 2004) and *Polianthes tuberosa* (Jin, Zhang, & Yang, 2004), respectively, along with a chlorogenin sapogenin (compound 27) **(224)** from *Agave Americana* (J.-M. Jin et al., 2004). So, (Yang et al., 2006) studied their impact on *C. albicans* (MIC > 20 µg/mL➔ compound inactive). Only saponin 17 **(225)** showed moderate activity (MIC/MFC= 10/10 µg/mL), having the same sugar chain as the previously discussed marginal hecogenin saponin 14 **(208)** (MIC/MFC= 20/ >20 µg/mL) but without a ketone group. This further assure that the presence of a ketone at C-12 (as in the hecogenin saponin 14 **(208)**) weakens and almost impairs the antifungal activity of the compound. As for saponin 16 **(223)** and sapogenin 27 **(224)**, they were deficient of activity due to the lack of a sugar chain at C-3.

Another β- chlorogenin saponin, compound 3 **(226)** (Sobolewska et al., 2016), was extracted from *Allium leucanthum* (Alliaceae) and tested for its influence on three *C. albicans* strains (Mskhiladze et al., 2008). Having four monosaccharides in its aglycone, it was able to greatly exhibit fungicidal effects on all three types, producing the same MFC values (12.5 µg/mL). However, eruboside **(227)**, a second β-chlorogenin saponin produced by the same plant *A. leucanthum*, displayed higher MFC results (25 µg/mL) than saponin 3 **(226)** against *C. albicans* strains (Mskhiladze et al., 2008). The latter results owned to the extended xylosyl unit in compound 3 **(226)** which contributed to the enhanced MFC results, but eruboside **(227)** had a glucosyl unit replacing the Xyl. This further confirms the importance of an extended Xyl sugar

unit instead of a Glc which was seen in other studies (Sautour, Miyamoto, et al., 2007; Yang et al., 2006; Zhang et al., 2006). Same thing goes for yayoisaponin C **(228)**, a third β- chlorogenin saponin from the same plant *Allium leucanthum* (Mskhiladze et al., 2008), which had an extra Glc instead of Xyl, exhibited an MFC value of 50 µg/mL for all strains.

In addition, (MATSUURA et al., 1988) worked on two steroidal saponins (1 **(229)** and 3 **(230)**), from *Allium sativum* (Liliaceae), one furostanol (compound 1) **(229)** and one β-chlorogenin spirostanol (compound 3) **(230)** (Sobolewska et al., 2016). The furostanol **(229)** having an additional sugar moiety at C-26 was inactive (MIC > 800 µg/mL); whereas the spirostanol **(230)** inhibited *C. albicans* growth at a concentration of 25 µg/mL. Saponin 3 **(230)** presented results similar to those of eruboside **(227)** (β-chlorogenin saponin) (same as the MFC value), previously isolated from *Allium leucanthum* (Mskhiladze et al., 2008). They both had the same number of sugars at C-3 with the same additional monosaccharide Glc instead of Xyl.

Thusly, steroidal saponins with (β-) chlorogenin aglycones represent better antifungal results when they possess four monosaccharides at C-3. Also, depending on the type of the extended sugar in the chain, the saponin will exhibit the appropriate potency (Xyl > Glc).

2.7.3.7. Other steroidal saponins

Other steroidal saponins such as CAY-1 **(231)**, produced by *Capsicum frutenscens* (Solanaceae) (Lucca et al., 2002), was considered as a potent saponin producing an inhibitory concentration (IC_{90}) of 7.7 µg/mL. IC_{90} was considered the concentration that impeded a minimum of 90% of *C. albicans* growth.

In addition, more plants-producing steroidal saponins and endemic to Turkey, were reported by (Abbasołu & Türköz, 1995). The following were: *Lilium candidum* L. (Liliaceae), *Allium rotundum* L. (Liliaceae), *Avena sterilis* L. (Paceae), *Trigonella foenum graecum* L. (Fabaceae), *Digitalis cariensis* Boiss. (Scrophulariaceae), *Digitalis lanata* (Scrophulariaceae), *Yucca aloifolia* L. (Liliaceae), and *Ruscus aculeatus* L. (Asparagaceae). Their saponin extracts were tested for their antifungal activities against *C. albicans* and they all presented good results with low MIC values ranging between 0.15% (*A. rotundum* L.) and 1.25 % (*Trigonella foenum aculeatus* L. (Fabaceae)). Thus, these medicinal plants' saponins are worth farther investigation regarding their chemical structures: aglycone types and sugar chains (length and types of sugar units) and their contribution to treating fungal infections. Scientist have already studied and reported some of these saponins' structures; however, they have not been tested yet for their antifungal activities against *C. albicans*. First, *T. foenum graecum* L. contains four known diosgenin saponins **(232→235)** (Gangrade & Kaushal, 1979; Mahato et al., 1982; Varshney & Jain, 1979; Varshney et al., 1984), one neotigogenin saponin **(236)** (Mahato et al., 1982), and one yamogenin saponin **(237)** (Bogacheva et al., 1976; Bogacheva et al., 1977). Second, *D. lanata* produces five saponins, three of which have tigogenin aglycones **(238→240)** (Tschesche & Balle, 1963; Tschesche et al., 1972; Tschesche & Wulff, 1963), one gitogenin **(241)** (Tschesche et al., 1972) and one digitogenin **(242)** (Tschesche & Wulff, 1963). Third, *R. aculeatus* L. produces five convallamarogenin saponins **(243→247)** (Bombardelli et al., 1972; Mahato et al., 1982). Fourth, (Maisashvili et al., 2008) have identified five known saponins from *A. rotundum* L.. Two of them had agigenin aglycones **(248-249)** (Kravets et al., 1990; Sata et al., 1998), two β- chlorogenin saponins **(250-251)** (Chincharadze & Kel, 1979), and one diosgenin saponin **(252)** (Espejo et al., 1982). Fifth, (Mimaki et al., 1999) isolated five spirostanol **(253 →257)** and

one furostanol **(258)** saponins from *L. candidum* L., having a diosgenin aglycone (saponin 1) **(253)** (Agrawal et al., 1985) and isonarthogenin aglycone (saponin 2) **(254)** (Blunden et al., 1986; Minato & Shimaoka, 1963). Sixth, (Zengin et al., 2020) have identified one timosaponin H1 **(259)** from *D. cariensis* Boiss. Seventh, two tigogenin saponins **(260-261)** were identified from *Y. aloifolia* L. (Bahuguna & Sati, 1990).

In addition, (Favel et al., 2005) have reported the presence of two tigogenyl saponin: 1 (yuccaloeside B) **(262)** and 2 (yuccaloeside C) **(263)**, from *Yucca gloriosa* L. (Asparagaceae), and that were potent antifungal against *C albicans* (MIC of both was 3.12 µg/mL). Saponins 1 and 2 had at C-3 five and six sugar units, respectively.

Moreover, (Miyakoshi et al., 2000) studied the effect of fourteen saponins, produced by *Yucca schidigera* (Liliaceae) against *C. albicans* (MIC > 100 µg/mL ➔ saponin inactive). Seven of them are already known (1 **(264)**, 3 **(266)**, 5 **(268)**, 7 **(270)**, 9 **(272)**, 13 **(276)** (Tanaka et al., 1996) and 6 **(269)** (Niwa et al., 1988)) and the other seven were new (2 **(265)**, 4 **(267)**, 8 **(271)**, 10 **(273)**, 11 **(274)**, 12 **(275)**, and 14 **(277)** (Miyakoshi et al., 2000). To begin with saponin 1**(264)** (mixture of sarsapogenin and sarsapogenin) which was the most active against *C. albicans* (MIC = 6.25 µg/mL), had a branched chain of three monosaccharides. Saponin 2 **(265)**, had the same aglycone as saponin 1 **(264)**, and almost the same branched trisaccharidic chain as the hydrophilic part. But instead of a Glc attached to C-3, it was replaced with a Gal, which slightly decreased its activity (MIC= 12.5 µg/mL). Concerning compound 3 **(266)**, had the same aglycone and almost the same sugar chain as saponin; however, Xyl was replaced with a Glc attached to Glc (at C-3). The nature of the sugar attached to Glc at C-3 might explain the reduced activity of saponin 3 (MIC= 12.5 µg/mL). Compounds 4 **(267)** and 12 **(275)** also shared the same sugar chain as the potent compound 1 **(264)**; nevertheless, the presence of the ketone

group at C-12 had depleted their activities (MIC > 100 µg/mL ➔ inactive). Additionally, saponin 5 **(268)** had a marginal effect (MIC= 100 µg/mL), although it shared the same sugar chain as that of the potent compound 2 **(265)**, but the hydroxyl group at C-2 had dramatically decreased its activity (as opposed to some triterpenoid saponins). Also, saponin 6 **(269)** the analogue of compound 5 **(268)** but with a shorter sugar chain, had also a 2β- OH which impaired its activity (MIC> 100 µg/mL). Same results were seen in saponin 14 **(277)** (MIC > 100 µg/mL) which had the same hydrophilic part as saponin 6 **(269)** and a hydroxyl group at C-2 of the aglycone. As well, saponins 13 **(276)** and 14 **(277)** having a 2β- OH were inactive against *C. albicans* (MIC > 100 µg/mL). As for the remaining saponins (including 1 **(264)**, 2 **(265)**, and 3 **(266)**) 7 **(270)**, 10 **(273)**, and 11 **(274)** which lacked either a 2β-OH or a ketone at C-12, were able to inhibit the growth of *C. albicans* at concentrations ranging between 6.25 and 50 µg/mL (Miyakoshi et al., 2000). However, those having a 2β- OH, such saponins 5 **(268)**, 6 **(269)**,13 **(276)** and 14 **(277)**, and a ketone at C-12, such as saponins 4 **(267)** and 12 **(275)**, presented very weak to no activity MIC≥ 100.

The study performed by (Miyakoshi et al., 2000) further explains the importance of a branched trisaccharidic chain at C-3 which contributes to the increase in the antifungal properties. Also, the presence of a ketone group at C-12 decreases and might diminish the effect of the saponin. Surprisingly, and as opposed to triterpenoid saponins, the presence of a 2β- OH in the aglycone of a saponin depletes its activity against *C. albicans*.

Another study performed by (Yang et al., 2018) who extracted the saponin extract, Huang Shanayo extract (HSE), from the plant *Dioscorea panthaica* prain et Burk. (Dioscoreaceae). HSE is known to comprise more than thirty steroidal saponins, some of which were already determined (Wang et al., 2015). However, in this study (Yang et al., 2018), they only studied the

antifungal and fungicidal effect of HSE against *C. albicans* (not each saponin alone). 64 µg/ mL of HSE was able to completely kill *C. albicans* (MFC→ 0 $\log_{10}$ CFU), and 32 µg/ mL of HSE significantly decreased the percent viability of *C. albicans* cells by 80%. To further study the effects of HSE, they tested its ability to block HSE adhesion to polysterene surfaces (one of the first steps of biofilm infection). HSE was able to suppress 80% of *C. albicans* adhesion at a concentration of 64 µg/ mL. Additionally, this saponins extract concentration (64 µg/ mL) was also able to significantly repress 90% of biofilm formation and development of hyphal cells. So, HSE accomplished two important tasks: a) inhibiting *C. albicans* to solid surfaces, and b) antibiofilm activity. Hitherto, no other studies were reported on the antifungal effect of individual saponins of *Dioscorea panthaica* prain et Burk. against *C. albicans*. Therefore, based on the excellent results of HSE in this study, it is crucially worth to study the impact of each saponin alone on the tested fungus and eventually could be used as an alternative to prevent and treat *C. albicans* biofilm formations.

Lastly, (Shafiq ur et al., 2015) isolated from *Trillium govanianum* (Melanthiaceae) three steroidal compounds: the saponins 1 (govanoside A) **(278)** and 2 (borassoside E) **(279)**, and the sapogenin 3 (pennogenin) **(280)**, and studied their effect against *C. albicans* (MIC> 20 µg/mL ➔ inactive). The latter was deprived of antifungal activity (MIC> 20 µg/mL), due to the lack of the hydrophilic part. Surprisingly, and even though saponin 1 **(278)** (linear trisaccharidic chain at C-3) had a second point of sugar attachment (C-24) other than at C-3, it presented excellent MIC results (MIC= 5 µg/mL). Maybe this is because the F ring was closed, and which have contributed to the antifungal activity. Also, saponin 2 **(279)**, which had a branched trisaccharidic chain at C-3, presented the best result among the three compounds, exhibited an MIC value of 2.5 µg/mL.

Plus, TG-I **(281)**, a dioscin saponin isolated from the plant *Trillium grandiflorum* (Melanthiaceae) (Hufford et al., 1988), and having a branched trisaccharidic chain at C-3, produced the best antifungal results against *C. albicans* (MIC= 1.56 µg/mL).

Just as triterpenoid saponins, steroidal saponins with different sapogenins than the ones listed above, need further investigation. This means that more exploration on the different types of genins and sapogenins should be done, i.e. common functional groups, types and number of monosaccharides. Eventually, these findings will allow scientists to tackle more easily the appropriate saponins for the treatment of infections caused by *C. albicans*.

It has been speculated that whenever a steroidal saponin with a spirostanol form is active, its analogue furostanol will automatically be devoided of activity (Sautour et al., 2004; Yang & Li, 1996). This explains the results reported by different papers such as those presenting the steroidal saponins of *A. acutifolius* (Sautour, Miyamoto, et al., 2007), *T. terrestris* (Zhang et al., 2006), *S. medica* (Sautour et al., 2005), and *A. sativum*. (MATSUURA et al., 1988). Other studies have highlighted the importance of a closed F ring (spirostanol saponin) which plays a major role in the contribution to the antifungal effect of a saponin (Tian et al., 2017b). Somehow, these two properties seem to be interrelated.

2.7.4. Structural basis of the antifungal potency of saponins

Saponins have different aspects and traits that make them good antifungal agents. They could range from functional groups, to number and types of sugars. However, their position is what determines their levels of potency. All of these characteristics combine and exert their effects on the fungal cell wall of *C. albicans* in order to inhibit its growth or even terminate it.

2.7.4.1. Antifungal mode of action of saponins against *C. albicans*

The exact mode of action of saponins against *C. albicans* is poorly understood and reported. However, several studies have reported that the main determinant of the antifungal activity of saponins lies in their aglycone part. The latter binds directly to the ergosterol in the fungal cell membrane, to form extramembranous aggregates that will lead to the death of the fungus. Thus, once the ergosterol is extracted from the membrane, pores will form, and the fungal membrane will lose its vital contents (Augustin et al., 2011; Damke et al., 2011; Gyawali & Ibrahim, 2014; Osbourn, 2003). In our study, we found that the hydrophilic part of a saponin has a major contribution to its antifungal effect against *C. albicans*. In fact, the presence of a sugar chain at C-3 is very crucial for saponins to have a good antifungal effect. Also, depending on the number and the type of the monosaccharides in the chain, the saponin exhibits different antifungal potencies. The nature and number of the hydrophilic sugar residues in saponins alter the hydrophilic-lipophilic balance (HLB), and hence, change their detergent-like effect. Obviously, the emulsifying properties of saponins have a direct bearing on their capability of lysing thereby *C. albicans*. This was observed in almost all studied saponins (both triterpenoid or steroidal); nonetheless, the number of monosaccharides residues exceeds a threshold value, the antifungal effect is decreased or lost (Ekabo et al., 1996; Favel et al., 1994; Kimata et al., 1983; Sautour et al., 2004; Yang & Li, 1996; Yang et al., 2006).

2.7.4.2. Different properties that affect the antifungal effect of saponins

Various characteristics that saponins hold, contribute greatly to their potency against *C. albicans*. However, they are not applicable to all of them as each category of saponins (depending on the aglycone) has its own features. Nevertheless, one common hallmark is shared among both types of saponins, which is the presence of a hydrophilic part at C-3, that is very crucial for their proper functioning. Some triterpenoid saponins require shorter sugar chains, other longer.

However, the latter does not mean that the activity of a compound is enhanced with the increase of the number of sugars added at C-3. Thus, the antifungal effect of saponins is limited to the number of monosaccharides at C-3. Any value above or below the limits, will deteriorate/diminish the effect of the compound.

Almost all triterpenoid saponins have a free COOH at C-17 (monodesmosidic) instead of an additional sugar moiety at C-28 (bidesmosidic). The only effective type of saponins was found to be monodesmosides, since any extra point of sugar attachment on the aglycone (at C-28) will impair the activity of the compound (Du et al., 2003; Njateng et al., 2015; Woldemichael & Wink, 2001; Zhang et al., 2006). Nevertheless, very rare cases of bidesmosidic saponins being active against *C. albicans* were reported; but only under acidic conditions (pH<7) (Fiore et al., 2005; Khatuntseva et al., 2012; Kulakovskaya et al., 2005; Pellati et al., 2009; Tsukiyama et al., 2002).

In addition, the presence and position of functional groups such as OH, and OAc in triterpenoid saponins are very important. In other words, the existence of an OAc group in the aglycone will impair the saponin's activity (Hu et al., 2018; KITAGAWA et al., 1989; Yuan et al., 2009). Nonetheless, OAc attached to the terminal sugar (in the case of Ara) in a triterpenoid saponin, will increase the activity of the compound only if Ara was in its furanosyl form (OAc+ pyranosyl ➔loss of function) (Kimata et al., 1983). Moreover, OH groups at any of the following positions: C-2 (2β-OH), C-16, C-22, C-23, and C-24, will contribute to an enhanced antifungal effect (Adesegun et al., 2008; Lunga et al., 2014; Njateng et al., 2015; Soberón et al., 2017; Woldemichael & Wink, 2002). Furthermore, the potency of a triterpenoid saponin will be affected positively by the presence of an aldehyde group (Woldemichael & Wink, 2001). Noteworthy, the 13β, 28- epoxy bridge that dominates among saponins produced by certain plant

families (Primulaceae and Myrsinaceae) is reported to be powerful in enhancing the antifungal efficacy against *C. albicans* (Foubert et al., 2008; Soberón et al., 2017; Yayli et al., 1998). Also**,** carboxylic groups in the aglycone of triterpenoid saponins are known to decrease their antifungal properties (Soberón et al., 2017).

As for steroidal saponins, the types of sugars in the hydrophilic part are very important (in the order of decreasing potency Rha> Xyl> Glc). Also, the presence of Glc (2-1) will dramatically weaken the activity and render the saponin inactive most of the times (Jin, Zhang, & Yang, 2004; Miyakoshi et al., 2000; Mskhiladze et al., 2008; Yang et al., 2006; Zhang et al., 2006).

Additionally, a trisaccharide chain at C-3, in steroidal saponins, must be branched rather than linear (Belhouchet et al., 2008; Li et al., 1999; Sautour, Miyamoto, et al., 2007; Shimoyamada et al., 1990). This will lead to great antifungal effects against *C. albicans*. Also, steroidal saponins have their activity weakened whenever a hydroxyl group is present at C-2 (2β-OH) of the aglycone (contrary to some triterpenoid saponins) (Miyakoshi et al., 2000). Moreover, a ketone group at C-12 of the aglycone will cause a drastic decrease in the effect of the phytochemical under study or even impair it. Thus, hecogenin saponins are the least effective against *C. albicans* (presence of ketone at C-12) (Yang et al., 2006; Zhang et al., 2006). Furthermore, steroidal saponins are not operative whenever the number of monosaccharides at C-3 is less than two (Sautour et al., 2006; Takechi et al., 1991).

Plus, it was proposed that the closed F ring contributes to the antifungal effect of a steroidal saponin (Tian et al., 2017a). This proposition was further validated when it was stated that spirostanol saponins are effective, whereas their analogue furostanols are not (Sautour et al., 2004; Yang & Li, 1996). This means that whenever an F ring is open (furostanol), it will allow the attachment of an extra sugar moiety. The latter will result in a bidesmosidic steroidal

saponin. So, in both triterpenoid and steroidal saponins, monodesmosidic saponins are better and great antifungal agents rather than bidesmosidic ones.

3. Conclusion

Resistance of fungal strains to the widely used azoles and polyenes causes a massive threat to humans and to the biodiversity. The basic measures that people usually apply are not as powerful and efficacious against pathogenic resistant strains of *C. albicans*. This is because the latter have adopted new mechanisms of evasion in order to escape the drugs used against them. Therefore, as an alternative solution, medicinal plants have proven to have a positive impact on human health and biodiversity over the years. This is because of the phytochemicals they produce, especially saponins. After several conducted studies, these secondary metabolites have demonstrated to be effective and potent against different resistant fungal strains where they reduced, even totally inhibited their growth and in some cases killed them. They exert their effects by acting on the fungal cell membrane and destructing its wholeness and eventually inhibiting the growth of the fungus or killing it. Different susceptibility techniques, such as broth microdilution and disk diffusion techniques, are employed in order to detect the ability of a saponin to act as an antifungal agent. The latter could either exert fungistatic or fungicidal or both effects on *C. albicans*.

In addition, saponins possess several properties that help them become better antifungal agents. The functional groups such as hydroxyl, carboxyl, aldehyde, and acetoxy groups are some of those major properties. Their position on the aglycone or hydrophilic part determine the saponins' levels of potency. Sometimes, the presence of one of them increases the antifungal effect of a compound; but might decrease it in a different one. Therefore, each saponin has its

own characteristics that affect its function. However, the ketone functionality has proven to deteriorate the effect of the phytochemical, such as in the hecogenin steroidal saponins. Moreover, additional and very major features, are the nature (type) and number of the monosaccharides in the hydrophilic part. Also, the structure of the terminal sugar is very crucial (in the case of Ara), and influences the potency of the compound.

Furthermore, monodesmosides exert the greatest antifungal effects against *C. albicans*; although, a minority of bidesmosides might be effective but only under acidic conditions.

Finally, the saponin with the best antifungal activity was the oleanolic acid triterpenoid saponin Anagallisin C (AnC) **(60)** from *A. arvensis* scoring an MIC of 1 µg/mL (Soberón et al., 2017). The second best antifungal saponin was the dioscin steroidal saponin TG-I **(281)** from *T. grandiflorum* with an MIC of 1.56 µg/mL (Hufford et al., 1988). Also, the steroidal saponin TTS-12 **(177)**, from *T. terrestris* presented the best MIC_{80} results (MIC_{80}= 1 µg/mL) and which were less than the MIC_{80} of the positive control fluconazole (1.4 µg/mL) (Zhang et al., 2006). Thus, these three compounds (along with other saponins mentioned earlier) could be further used as alternatives of the antifungal drugs FLC and AMB that *C. albicans* have acquired resistance to them. Hence, avoiding the rapid rise of antifungal resistance. Accordingly, it is important to consider adopting new ways of treating fungal infections such as with triterpenoid and steroidal saponins found in the medicinal plants, mentioned in the above sections.

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