Evaluation of *Corchorus* Saponins for their Therapeutic Potential

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Declaration

It is hereby delared that this research study has been done for partial fullfillment of requirement for the degree of Master of Sciences in Biomedical Engineering. This work has not been taken from any publication. I hereby also declare that no portion of work referred to in this thesis has been submitted in support of an application for another degree or qualification in this university or other institute of learning.

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Dedicated to :

My Parents & Siblings along with

my beloved Uncle M. Imran Abid (late) and his family

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"Praise [is due] to Allah, Who has favored us over many of His believing servants."

[Al-Quran 27:15]

First of all, I would like to pay my best regards and gratitude to Allah Almighty for the blessings He bestow upon me, no doubt I would not have been able to achieve this purpose without His blessings. My efforts are of no worth without His mercy and help. O my Lord! Accept this; verily you are all Hearing and all knowing. . I dedicate my work to my mother, a strong and gentle soul who dedicated her own life to grow me up and taught me to have faith in Allah. I would like to thank my father for earning an honest living for us and supporting me throughout my research work. I would like to express my deepest appreciation to my supervisor Dr. Nosheen Fatima Rana for her help and guidance during my research work. I would like to thank G.E.C committee members of my MS thesis Dr. Nabeel Anwer, Dr. Adeeb Shehzad (NUST) and Dr. Abida Raza (NORI Islamabad) for their support throughout my research work.

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List of Acronyms and Abbreviations

Abbreviation	Meaning
ADR	Adverse drug reaction
AA%	Percentage antioxidant activity
BHA	Butylated hydroxyanisol
CD8+	Cluster of Differentiation 8
DMSO	Dimethyl sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FTIR	Fourier Transform Infra-Red spectroscopy
ICD	Inhibitory Concentrations in Diffusion
LB	Lysogeny broth
MIC	Minimum inhibitory concentrations
MRSA	Methicillin-resistant *Staphylococcus aureus*
Pet. ether	Petroleum ether
ROS	Reactive oxygen species
SSSS	Staphylococcal scalded skin syndrome
TLC	Thin layer chromatography
WHO	World Health Organization
% Yield	Percentage yield
Amox	Amoxicillin

Abstract

Saponins are extensively investigated due to their pharmacological importance in treatment of cancer, diabetes, inflammation and cardiovascular diseases. The role of saponins in the treatment of infectious diseases has also been explored. Their wide medicinal applications are due to variety in their structure. Novel saponins are continuously being searched for their broad spectrum activities. *Corchorus depressus* (family *Tiliacae*) have been used as folk medicine for the treatment of various ailments such as urinary disorders, urinary associated inflammation, injuries and fever. However the therapeutic potential of *C. depresssus* have been very less explored. In this study antimicrobial activity of various extracts of *C. depresssus* branches was determined using Disc Diffusion method. Phytochemical screening of the extracts was done using chemical methods. The branches of *C. depresssus* were found to be rich in saponins and identified as triterpenoid saponins through Fourier Transform Infra-Red spectroscopy (FTIR) analysis. *Corchorus* saponins were isolated and found to be effective against various gram positive and gram negative pathogens. They also showed substantial free radicals scavenging activity. Their mechanism of action is found to be bactericidal.

1. Introduction

1.1 Antibiotic resistance

The discovery of antibiotics was a revolutionary development in the field of medicines in the era of 20th century. These were the cure for many fatal infections and diseases. Millions of people have been saved by using these drugs (Drews 2000). Now a days antibiotics are also important not only for treating infections but also for prophylactic coverage of patients having high risk e.g. patients going through surgeries, organ transplantation, chemotherapy and other intensive care situations (Ciulla, Starr et al. 2002). However, this increased usage of antibiotics has encounter an emerging serious problem of this era that is antibiotic resistance (Walsh and Wright 2005).

In the middle of 20th century, Penicillin was produced in huge amounts and it had reduced the number of deaths caused by microbial infections. But within four years, microbes began to develop resistance against penicillin (Sneader 2005). It was supposed that almost all bacterial infections worldwide have become resistant and un-curable. So more discoveries have been made and pharmaceutical industries have produced some new drugs against resistant microbes (Yim, Huimi Wang et al. 2006). Generally microorganism have an ability to transfer drug-resistance, by genetic means, to their next generations against those therapeutic agents which are being used to cure infections (Salyers, Gupta et al. 2004).

1.1.1. Multi-drug resistance

In past 40 years, relatively few new antibiotics have been discovered. Excessive use of antibiotics has resulted in emergence of many multidrug resistant strains of bacterial pathogens such as multi drug resistant *S. aureus* and erythromycin resistant *E. coli* (Finland 1979). There

is a serious requirement of research to find out new drugs that are more effective to fight against the bacterial infections (Adegoke, Iberi et al. 2011).

Due to multi-drug resistance in bacteria, the need for screening new compounds from plants that have antimicrobial activity is increased. There are several reasons to choose bioactive compounds from plant sources such as, extraction from plants is cheaper and they have negligible rate of adverse drug reaction (ADR) which otherwise is very high in synthetic pharmaceutical drugs (Chariandy, Seaforth et al. 1999).

1.2. Reactive oxygen species (ROS)

Our immune system face new challenges every day in the form of hazardous chemicals and pathogenic substances. There are different kinds of reactive species such as reactive oxygen species and reactive nitrogen species. They are also known as antimicrobial molecules that contain hydrogen peroxide, hydroxyl, superoxide anions and nitrite radicals. ROS generation plays an important role in cell signaling and are considered essential for various biological processes (Halliwell 1994). They also cause oxidative-stress cell damage by reacting with biomolecules such as proteins, lipids, enzymes and DNA/RNA. But to balance lethal oxidative stress, biological system have developed a defense mechanism known as antioxidative system. Recently, naturally plant-isolated phytochemicals have gained huge attention as modulators of ROS generations as well as a treatment strategy for inflammatory and aging diseases (Beatty, Koh et al. 2000).

1.3. *Corchorus depressus* and its role in therapeutics

Corchorus depressus (Linn.) Stocks (Tiliacae) is a deep rooted, branched perennial herb with elliptical shaped leaves and yellow flowers, mostly found in the sandy South-middle areas of

Pakistan (Kataria, Rao et al. 2013). Various parts of this plant have been used as a folk medicines for the treatment of various ailments. Whole plant is used to cure urinary disorders, urinary-associated inflammation, injuries and fever (Qureshi and Raza Bhatti 2008).

Corchorus contains bioactive compounds such as saponins, terpenoids, phenolics, sterols, carbohydrates, glycosides and fatty acids (Khan, Bano et al. 2006). These bioactive compounds mediate different pharmacological activities such as anti-convulsive, anti-pyretic, anti-estrogenic and anti-cancer (Del Villar-Martínez, García-Saucedo et al. 2005). Experimental work shows that the therapeutic effect attributed to this specie of *Corchorus* is scavenging of reactive oxygen species (ROS) generation, thus exerting antioxidant effect (Akinpelu, Aiyegoro et al. 2010).

1.4. Aims and Objectives

For centuries, plant-derived phytochemicals have been used for the treatment and prevention of various disorders. Pathogens infection is alarming risk to human health and a notable increase is seen globally in the bacterial diseases. These bacterial pathogens utilize different mechanism in order to survive in a variety of host lesions. Meanwhile some bacteria have modified genetic characteristics that cause survival as well as resistance to currently available drugs in the market. Drug resistance and poor public health systems are major feature of failure for existing drugs to control bacterial diseases in underdeveloped and developing countries (Theuretzbacher 2011) (Miyakis, Pefanis et al. 2011). Therefore, urgent attention is needed to overcome challenge of drug resistance among the gram *positive* and gram negative bacteria.

The objectives of the study were:

1- To determine the phytochemical constituents, antibacterial and antioxidant activity of *Corchorus depressus* branches extracts, isolated with different solvents such as ethanol, methanol, chloroform, n-butanol and H_2O.
2- To determine phytochemical composition and antibacterial activity by chemical and disc diffusion method.
3- To determine antimicrobial effect of these extracts against gram positive and negative bacterial strains including *Enterococcus faecalis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi and Escherichia coli.*
4- To determine Minimum inhibitory concentrations (MIC) of these extracts
5- To determine the antioxidant effect for assessing the scavenging activity of extracts in regards to ascorbic acid by employing DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay.
6- Extraction of Saponins and testing for their therapeutic efficacy and determining mode of action.

2. Literature review

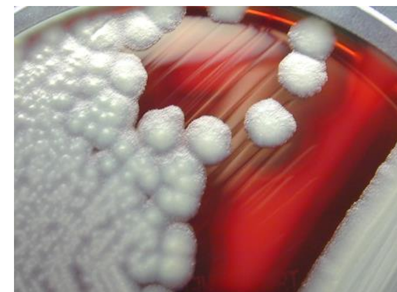
2.1. Pathogenic bacteria

Pathogen is a broad term which means anything that can induce illness and infection. Normally the term pathogen is used to designate an infectious agent such as microbes including virus, bacteria, and fungi. They cause disease in their hosts including plants, animals and human being. There are many pathogenic bacterial strains known for causing infections and diseases such as typhoid, pneumonia, and tetanus. Bacterial strains are generally divided in to two groups, gram positive and gram negative.

2.1.1. Gram positive strains

Gram-positive bacteria take up the crystal violet stain and appear to be purple-colored under a microscope due to the presence of thick peptidoglycan layer in the its cell wall . This type of bacteria include *Bacillus cereus, Staphylococcus aureus* and *Enterococcus faecalis.*

2.1.1.1. Bacillus cereus

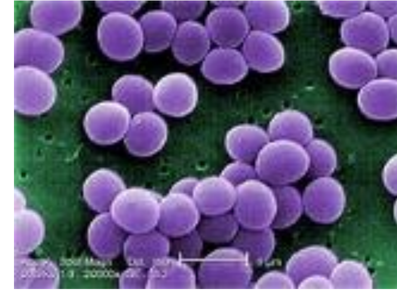


Bacillus cereus is a rod-shaped, gram-positive, facultative anaerobic bacterium. Some strains are beneficial for human beings and some are harmful causing foodborne illness (Ryan, Ray et al. 2004). It is responsible for causing “Fried rice” syndrome by infecting fried rice dishes placing at room temperature for a long time (Asaeda, Caicedow et al. 2005). This strain can produce endospores that are well protective and cannot be easily destructed.

It is also responsible for causing nausea, vomiting and diarrhea (Kotiranta, Lounatmaa et al. 2000). It can contaminate improperly cooked food by producing endospores (Baron, Chang et

al. 1994). They can survive in food cooked at 100°C or less temperature (Roberts, Baird-Parker et al. 1996). The other reason of contamination is improper refrigeration of food that allows spores to germinate (McKillip 2000). Bacterial growth leads to production of enteroxins that are highly resistant to heat and acid treatment and cause diarrheal and emetic syndrome (Ehling-Schulz, Fricker et al. 2004).

2.1.1.2. *Staphylococcus aureus*



Staphylococcus aureus is gram-positive bacterium found in human respiratory tract and on skin (Kluytmans, Van Belkum et al. 1997). It can cause skin infections such as boils, respiratory tract disease such as sinusitis and food poisoning, though it is not always pathogenic. It also produce toxic proteins that bind with cell surface and inactivate the antibodies (Cole, Tahk et al. 2001)

It does not always shows symptoms of infections. S. aureus can survive weeks to months without showing any symptoms (Cimolai 2008).

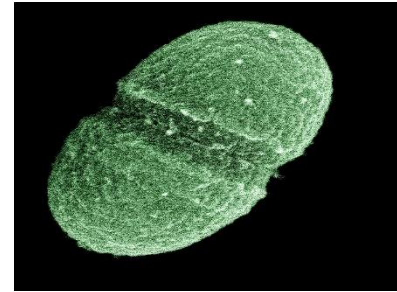
S. aureus is prevalent in patients having atopic dermatitis. It is found in active areas of body such as armpits and scalp and form large pimples. This can further leads to staphylococcal scalded skin syndrome (SSSS). Ritter's disease is the sever form of that disease that can be observed in neonates (Curran and Al-Salihi 1980).

Exotoxin produced by S. aureus can be classified into three groups that are all associated with different types of diseases (Dinges, Orwin et al. 2000).

Infections can be treated by using antibiotics such as penicillin but in some countries penicillin resistance is present among S. aureus. Therefore oxacillin or flucloxacillin is used instead of

penicillin. For some serious infections such as endocarditis combination therapy with gentamicin is used that is controversial due to the damage to kidneys (Phuong, Kneen et al. 1999).

2.1.1.3. *Enterococcus faecalis*



Enterococcus faecalis is a facultative anaerobic (Arias, Contreras et al. 2010) gram-positive bacterium. It is found in gut of human and other mammals (Ryan, Ray et al. 2004).

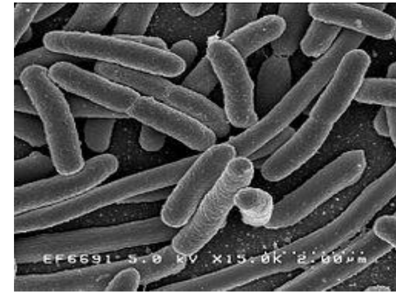
E. faecalis can cause life threatening infections in hospitals where high levels of antibiotic resistance is present in this specie (Ryan, Ray et al. 2004). In 30% to 90% cases, *E. faecalis* is found in root canal-treated teeth (Molander, Reit et al. 1998). Root canal-treated teeth has nine times greater presence of *E. faecalis* in primary infections (Rôças, Siqueira Jr et al. 2004).

E. faecalis shows resistance to several antibiotics such as aminoglycosides, aztreonam and clindamycin. It is also resistant to penicillin, oxacillin and commonly to vancomycin (Courvalin 2006, Amyes 2007). Vancomycin resistant *E. faecalis* can be treated by nitrofurantoin (Zhanel, Hoban et al. 2001). Ampicillin is also preferable when bacteria are susceptible (Arias, Contreras et al. 2010).

2.1.2. Gram negative strains

Gram-negative bacteria do not preserve the crystal violet stain due to the presence of thin layer of peptidoglycan in cell wall . This class of bacteria include *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa*.

2.1.2.1. *Escherichia coli*



Escherichia coli (E. coli) is gram negative, rod shaped, facultative anaerobic bacterium. It is commonly found in gut endothermic organisms. Some serotypes of E. coli can cause food poisoning and food contamination but most of strains cause no harm to their hosts (Daniels, Mackinnon et al. 2002). These harmless strains are known as normal flora of intestine and can play role in the production of Vitamin K_2 and can resist the proliferation of harmful bacteria in the gut (Bentley and Meganathan 1982, Hudault, Guignot et al. 2001).

Intestinal flora is consisted of about 0.1% of E. coli and other facultative anaerobic bacteria (Eckburg, Bik et al. 2005). Pathogenic strains of this bacterium can transfer through oral and fecal routs and can cause disease. They are capable of surviving outside the host for a limited amount of time that make them indicator for testing samples of fecal contamination(Feng, Weagant et al. 2002).

E. coli can also cause infections of urinary tract and neonatal meningitis. Some of its strains can cause hemolytic-uremic syndrome, mastitis and gram-negative pneumonia(Todar 2006).

2.1.2.2. *Salmonella typhi*

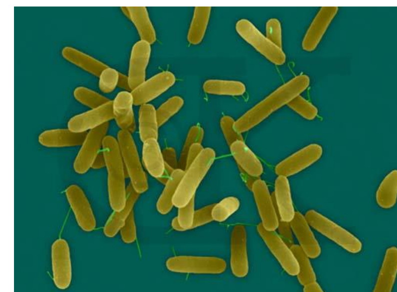
The actual name of *Salmonella typhi* is *Salmonella enterica* subsp. *Enterica* is one of the subspecies of *Salmonella enterica* that is an aerobic, flagellated, rod-shaped, gram-positive bacterium (Giannella and Salmonella 1996). There are many members of this species that are pathogenic.

The worldwide known disease caused by the food or fecal contamination of this bacterium is typhoid fever (Carrasco, Morales-Rueda et al. 2012, Ifeanyi 2014). It is also known as enteric fever, infantile remittent fever, slow fever, nervous fever, pathogenic fever and abdominal typhus. Typhus resembles to typhoid in symptoms but is caused by different bacterial specie (Cunha and Cunha 2008).

In the treatment of typhoid, resistance is not as such a serious problem so far. Normally, treatment is started with ciprofloxacin (Thaver, Zaidi et al. 2005, Fraser, Goldberg et al. 2007, Parry and Beeching 2009) or third generation of cephalosporin that is cefotaxime (Soe and Overturf 1987, Wallace, Yousif et al. 1993, Dutta, Mitra et al. 2001). For oral alternative cefixime is a suitable choice (Bhutta, Khan et al. 1994, Phuong, Kneen et al. 1999).

2.1.2.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an aerobic gram-positive bacterium (Ryan, Ray et al. 2004). It is an opportunistic pathogen of human as well as of plants (Giannella and Salmonella 1996). (Anzai, Kim et al. 2000).



P. aeruginosa is a common bacterium that can cause several diseases in humans inhabiting in soil, skin flora and water resources. It can survive in hypoxic conditions. It can produce infection in organisms having reduced immunity. It can cause infections in lungs, kidneys and urinary tract (Baltch and Smith 1994). As it can survive in moisture containing surfaces, it can contaminate surgical equipment such as catheters. It has been used in oil spills because of its property to degrade hydrocarbons and tar balls (Itah and Essien 2005, Geller, Iversen et al. 2010). It can also cause blood infections along with infections of burns and wounds in

immunocompromised persons (Todar 2006). It can be treated with several antibiotics including colistin and polymyxin B (Hachem, Chemaly et al. 2007).

2.2. Drug resistant bacteria

Some species of bacteria are known to be resistant to different antibiotics that are well known in the market. These bacteria include Methicillin-resistant *Staphylococcus aureus* (MRSA) that is very common for causing nosocomial infections. Infections caused by MRSA are very difficult to treat because MRSA has developed the resistance against several known antibiotics available in the market. Vancomycin is a glycopeptide type of drug known as an effective antibiotic for curing MRSA infections. However, late studies reported the presence of such *Staphylococcus aureus* that are vancomycin-resistant (Adwan and Mhanna 2008).

The other example of such bacteria is *Pseudomonas aeruginosa* that is also known for causing nosocomial infection due to its ubiquitous nature (Morrison and Wenzel 1984). It inhabit in moist places and has resistance to different known antibiotics (Trautmann, Lepper et al. 2005). Studies reported that antimicrobial efflux system could be the reason of its multi-drug resistance against multiple antimicrobial agents (Adwan, Abu-Shanab et al. 2009).

2.3. Antioxidant activity

ROS are formed during metabolic activity or through the action of ionizing radiations. Their interaction with biomolecules lead to ionizing disorders including cancer (Wiseman and Halliwell 1996). Antioxidants present in nature can be used to prevent formation of ROS related disorders (Shinwari 2010). These antioxidants are abundantly present in the medicinal plants and have a considerable role in the development of modern therapeutics (Verpoorte 2000, Sarwat and Ahmad 2012). And so, medicinal plants have arisen as substitutive health

care system to deal with the recent worldwide health issues (Shinwari and Qaisar 2011). Plants are being used as folk medicines since 4000-5000 B.C. In Pakistan, 400 out of 2000 known plants species are widely used in local medicines (Shinwari 2010). Plant derived antioxidants are also exploited commercially either as antioxidative additives or as nutritional supplements (Gülçin, Oktay et al. 2002). Synthetic antioxidants such as butylated hydroxyanisol (BHA) have also limited use in nutrition because of their carcinogenic upshot (Botterweck, Verhagen et al. 2000). And so, many plant species have been searched for new antioxidants, but generally there is still a need to find and collect additional information regarding the antioxidant potential of certain plant species as they are harmless and biologically active. Thus, in contemporary years, significant work has been conducted, focused on identification of plant materials having noteworthy antioxidant activity.

2.4. Role of plants in therapeutics

Plants are present on this planet for billions of years ago and are playing significant role in human lives. Many of them are being explored and used for the production of drugs on both domestic and industrial scale (Halliwell 2005). Plant derived compound have been used for many years in the field of conventional medicines.

2.4.1. Ethnobotanical studies

The information derived from ethnobotanical studies is very important in the favor of production, conservation and depletion of biological resources (Abo, Fred-Jaiyesimi et al. 2008). According to the statistics of World Health Organization (WHO), 80% of the world population is dependent on the drugs derived from natural biological resources to fulfill their healthcare requirements (Luyckx and Naicker 2008). A large number of people living in rural

areas are still using traditional medicines for curing common diseases due to the unavailability and deficiency of modern medicines in these poorly developed areas (Sheng-Ji 2001).

2.4.2. Phytochemicals

Now a days, studies on plant derived compounds which are used as medicines, are in progress. Several compounds such as morphine, epinephrine and atropine derived and processed from the plants are used in the medicines. Studies under the fields of ethnobotany and ethno medicines have described the importance of natural resources such as plants as pharmacological agents (Wiley and Liu 2011).

These compounds become more effective when used in combination with other compounds extracted from other plant sources (Nascimento, Locatelli et al. 2000). Numerous studies have shown that plant derived medicines are safer, cheaper and more effective than synthetic medicines to cure bacterial diseases (Vermani and Garg 2002).

Natural products have become a major focus in drug discovery. Assaying for natural compounds is a much more efficient way for discovering new antimicrobial compounds over synthetic compounds. Making synthetic compounds is very labor intensive with a low probability of producing something that has bioactivity. Whereas plants have evolved natural defense mechanisms to fend off microbial invaders and hence these compounds can be easily extracted and tested for bioactivity.

2.5. Corchorus depressus

Botanical Name: *Corchorus depressus* (Linn.)

Synonyms: Antichorus *depressus*

Family: Tiliaceae

Genus: *Corchorus*

Native name: Boh phalli, Bahu phalli

Corchorus depressus (Linn.) belonging to family Tiliaceae is a mat forming, deep rooted, extensively branched perennial herb. The branches radiating from a woody crown and growing horizontal to the ground. This herb is well known as sand binder in the desert. Fruits are encapsulated, beak and cylindrical shaped, 8-15 mm long, usually coiled upwards from the branches. Its seeds are tiny and chocolate colored.

Corchorus depressus is distributed in almost all tropical and sub-tropical areas of the world. More than 100 species of *Corchorus* are distributed in the tropical and subtropical regions of South-East Asia (Sinha, Kar et al. 2011).

2.5.1. Medicinal uses of *Corchorus depressus*

Various parts of this plant have been used as folk medicines for the treatment of various ailments. Whole plant is used to cure urinary disorders, urinary-associated inflammation, injuries and fever (Qureshi and Raza Bhatti 2008). Its leaves are used to prevent sexual disorders and improve hepatic abnormalities (Kataria, Kaur et al. 2012, Kataria, Kaur et al. 2013). Its fruits and seeds are used as demulcent and also acts as mucilage for treating gonorrhea (Kataria, Rao et al. 2013).

2.5.2. Phytochemical constituents of *Corchorus depressus*

Corchorus depressus and other species of *Corchorus* contains bioactive compounds such as saponins, terpenoids, phenolics, sterols, carbohydrates, glycosides and fatty acids (Khan, Bano et al. 2006). These bioactive compounds mediate different pharmacological activities such as anti-convulsive, anti-pyretic, anti-estrogenic and anti-cancer (Del Villar-Martínez, García-Saucedo et al. 2005).

2.6. Saponins

Saponins are mostly known as surface active, non-volatile compounds widely distributed in the wide range of plants of kingdom Plantae (Lásztity, Hidvégi et al. 1998, Oleszek 2002, Hostettmann and Marston 2005). The word "Saponins" derived from the Latin word "*sapo*" meaning 'soap' due to the forth formation when shaken well with water. These are the molecules of diverse nature and chemically characterized as triterpene and steroid glycosides. They contain nonpolar aglycones combined with one or more than one monosaccharide components (Oleszek 2002). This polar and non-polar elements arrangement is the reason of their soap-like appearance in water solution. They have various properties including bitter and sweet taste (Grenby 1991, Kitagawa 2002, Heng, Vincken et al. 2006), property to emulsify and foam formation (Price, Johnson et al. 1987), medicinal and pharmacological properties (Attele, Wu et al. 1999), hemolysis (Oda, Matsuda et al. 2000, Sparg, Light et al. 2004), along with antimicrobial, molluscicidal and insecticidal activities (Sparg, Light et al. 2004). Saponins have various uses in brewery and bakery products in addition to cosmetics (Price, Johnson et al. 1987, Petit, Sauvaire et al. 1995, Uematsu, Hirata et al. 2000) and medicines (Sparg, Light et al. 2004).

2.6.1. Classification of saponins

Although compounds have been classified previously according to their biological or phytochemical characteristics. But nowadays they can be classified on the basis of advanced knowledge of chemistry. Thus when term “saponins” come across the mind it reproduces the idea about a property of natural product classification that should be described more accurately. This becomes achievable because of the understanding of their chemical structures and their biosynthetic pathways has increased exceptionally in these days. This knowledge has encouraged the classification of natural products (e.g. saponins) on the basis of carbon skeleton biosynthesis (Connolly and Hill 1991, Xu, Fazio et al. 2004, Devon 2012).

2.6.1.1. Major classes

Primarily, saponins were classified into two major classes, the steroids and triterpenoids (Abe, Rohmer et al. 1993), both are the derivatives of a 30 carbon precursor oxidosqualene (Haralampidis, Trojanowska et al. 2002). The difference is that, steroid class of saponins are 27-C atoms containing molecules but the triterpenoid class saponins have all thirty carbon atoms in their molecule. From the biosynthetic approach, this difference is not so important because triterpenoid saponins exhibit many other carbon skeletons. However, this differentiation in these two classes is rejected in a current review (Sparg, Light et al. 2004).

2.6.1.2. Subclasses

Saponins were divided into three sub classes known as 1) triterpenoid saponins, 2) spirostanol saponins and 3) furostanol saponins. Yet this classification was not based on the difference of biosynthetic pathways, but highlighted the incidental structure elements due to secondary biotransformation. There are few more compounds such as the glycosteroid alkaloids that have been recognized as saponins (Haralampidis, Trojanowska et al. 2002). Though these

compounds have same biosynthetic origin as saponins and have a skeleton resemblance to steroids linked glycosidically to the monosaccharide components, but are not categorized as saponins. Glycosteroid alkaloids have a single nitrogen atom as a natural part of glycine structure that already classify them in a separate group.

Subclasses of the major classes are arranged on additional changes of the carbon skeletons by minor rearrangements, cleavage, degradation and homologation. Oxidation causes changes in carbon skeleton and in result, the functional groups such as hydroxyl and carbonyl groups present in different positions in carbon skeleton are oxidized. These modifications lead to cause further changes, such as formation of ether bridges, spiroketals or lactons because these functional groups are usually played role in chemical reactions. Sub-classification based on these characteristics is not discussed in this literature review as these are the type of secondary biotransformation. The basic idea behind this expectation was that similar plant orders have same types of enzymes, and they catalyze same kind of biotransformation that eventually result in similar chemical structures (Vierhuis, York et al. 2001, Umezawa 2003).

Three subclasses of saponins (i.e. triterpenoid, steroid and steroidal glycoalkaloid) have different types of plant origin. Triterpenoids are usually found in dicotyledonous plants and a few monocots. Steroid saponins are present in monocotyledonous plant (e.g. Droscoracaeae and Lilliaceae) and in few dicots as well, such as Foxglove (Hostettman, Marston et al. 1996). Oats exceptionally contain both triterpenoid and steroid saponins (Price, Johnson et al. 1987). Third member of this group that is steroidal glycol alkaloids are primarily present in the plants of family Solanaceae. Saponins extracted and purified from family Solanaceae plants and oat have been studied in detail in the reference to their potential role in plant defense mechanism against fungi (Osbourn 1996).

2.6.2. Role of saponins in therapeutics

In last two decades, a number of review articles have been published in which biological role, isolation, identification and biosynthetic activity of saponins are discussed (Kulshreshtha, Kulshreshtha et al. 1972, Mahato and Nandy 1991, Mahato and Sen 1997, Tan, Zhou et al. 1999, Connolly and Hill 2000, Sparg, Light et al. 2004).

2.6.2.1. Role in vaccines

Saponins are surface active compounds and they can trigger hemolysis of erythrocytes *in vitro,* though studies showed that this property is not due to adjuvant activity apparently (Kensil 1996). Saponins have been used as adjuvants for a long time and have been incorporated in veterinary vaccines, though they had not declared prominent results when tested on some non-mammalians (Cossarini-Dunier 1985, Grayson, Williams et al. 1987).

2.6.2.2. Role in immune system

Saponins also have the ability to induce some specific immune responses, such as inflammation (Cabral de Oliveira, Perez et al. 2001) and proliferation of monocytes (Delmas, Pierre et al. 2001). Studies have been carried out that described that saponins can provoke a strong adjuvant effect to T-dependent and T-independent antigens. It is also reported that they can induce a robust cytotoxic CD8+ lymphocyte responses and give the potential to the response when encounters mucosal antigens (Kensil 1996). Adjuvants based on saponins can do the modulation of the whole cell mediated immune system. They can also increase the antibody synthesis and above all, it require very low amount of dose adjuvant activity initiation (Oda, Matsuda et al. 2000).

Although mechanism of inducing immune responses by saponins is not understood but it is described in studies that they can induce the cytokines production such as interferons and interleukins that might facilitate their immune stimulant effects (Kensil 1996).

2.6.2.3. *Saponins as antioxidants*

Two decades ago, Zilversmit (1979) had proposed a hypothesis that atherogenesis might result from the event take place instantaneously after eating, and that it might be affected by chylomicron offcuts (Zilversmit 1979). Numerous scientists (Staprans, Rapp et al. 1994, Ursini, Zamburlini et al. 1998) extended this hypothesis and advised that nutritive lipid hydro peroxides, that may be partially produced during absorption in the alkaline pH of the intestine, are the source of chylomicron offcuts of lipid hydro peroxides, that are raised in the postprandial state. Generally, carotenoids and poly-phenols, being the chief alimentary antioxidants in diet, draw the attention of most researchers of this field. Some saponins have also been discovered to have antioxidative activity such as a group of saponins produced in legumes that is group B soya-saponins, comprehend an antioxidant moiety attached at Carbon 23 (Yoshiki, Kudou et al. 1998).

Different studies have been conducted to determine the biological properties of saponins. Triterpenoid saponin glycosides have been documented to have some considerable biological properties such as the antidiabetic effect, cytotoxicity and antitumor activity (Kambouche, Merah et al. 2009). Saponins are mostly present in the branches of plants. In a study, branches extracts of *Anabasis articulata* plant showed high values of free scavenging activity in DPPH assay, which confirmed the antioxidant activity of saponins (Benhammou, Ghambaza et al. 2013)

2.6.2.4. Saponins as Antimicrobial agents

The antimicrobial activity of saponins is determined by extracting many plants such as, *Medicago sativa*, *M. arborea*, *M. Arabica*, *Sorghum bicolor, Camellia sinensis var. sinensis* and *Acacia auriculiformis* against a selection of therapeutically important yeasts, Gram-positive and negative bacteria.

The saponins extracted from *Sorghum bicolor* by n-butanol inhibited the growth of the *Staphylococcus aureus* which showed that the saponins have inhibition against gram-positive bacterial strains but not against gram negative strains and the fungi (Oyekunle, Aiyelaagbe et al. 2006). However, in another study, saponins extracted from *M. Arabica* recorded high antimicrobial activity against Gram-positive bacteria *(Bacillus cereus, B. subtilis, Staphylococcus aureus* and *Enterococcus faecalis)* and distinct antifungal activity was also observed, especially against *Saccharomyces cerevisiae* (Avato, Bucci et al. 2006). Saponins are also examined for anti-inflammatory actions with the tea-leaf saponins extracted from the leaves of *Camellia sinensis var. sinensis.* Tea-leaf saponin exhibited relatively high antimicrobial activity against pathogenic dermal fungi with the MIC value of 10 µg/ml and also showed inhibition in the rat paw edema induced by carrageenan (Sagesaka, Uemura et al. 1996). Acaciaside A and B, two acylated bisglycoside saponins were originally isolated from *Acacia auriculiformis* which are known for their anthelminthic action. Antibacterial and antifungal tests exhibited the complete inhibition of conidial germination of *Aspergillus ochraceous* and *Curvularia lunata,* whereas relatively less inhibition against *Bacillus megaterium*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* was observed (Mandal, Babu et al. 2005). From these studies, it is concluded that the saponins have comparable antimicrobial activity both against gram positive and gram negative bacteria.

3. Materials and methods

3.1. Materials:

Following six pathogens were selected for antimicrobial test.

Gram positive bacteria

1. *Bacillus cereus* (Soil isolated)
2. *Staphylococcus aureus* (ATCC 6538)
3. *Enterococcus faecalis* (Clinically isolated)

Gram negative bacteria

1. *Salmonella typhi* (ATCC 6539)
2. *Pseudomonas Aeruginosa* (ATCC 9027)
3. *Escherichia coli* (ATCC 8739)

Nutrient Agar and Luria Bertani medium (L.B) were used as growth media for all strains except E. faecalis for which M17 medium with 3-(N-morpholino)propanesulfonic acid (MOPS) is used.

3.2. Plant selection and storage

Dry plant branches were collected and rinsed thoroughly with tap water to remove extraneous contaminants and dust and dried at room temperature. Dried samples were ground with an automated electric grinder to fine powder and stored in a sterile sealed container at room temperature.

3.3. Preparation of extracts and fractions

Extracts were prepared in methanol, ethanol, chloroform, water and n-butanol by macerating the powder sample at the concentration of 50g/1000ml in each solvent at 25°C in shaking water bath (WiseBath Model. *WSB 30*) on 160 rpm for 48 hours. The extraction solvents were separated by centrifugation with HERMLE desktop centrifuge (Model: Z326K) at 3000 rpm for 15 minutes and filtered using filter paper (Whatman no.1). Filtrates were evaporated under reduced pressure by rotary evaporation using rotary evaporator (Heidolph Laborota 4000 efficient) and stored at -20°C.

Compounds were dissolved in DMSO (Dimethyl sulfoxide) at the concentration of 100mg/ml, 50mg/ml, 25mg/ml, 10mg/ml, 5mg/ml and 1mg/ml and stored at 4°C.

3.4. Phytochemical screening

Phytochemical screening of all extracts was done by performing following tests with crude extracts of plant samples.

3.4.1. Tannins detection

0.5 g of extract was taken in a test tube and boiled in 5 ml of distilled water. Few drops of 1M ferric chloride ($FeCl_3$) were added. Solution color turned to blue-black that indicated the presence of tannins in the sample (Inalegwu and Sodipo 2013). This test was performed with all samples and results were recoded.

3.4.2. Phenolic compounds detection

Aqueous extract was taken in a test tube. 1% solution of Ferric chloride ($FeCl_3$) was prepared. 1-2 drops of $FeCl_3$ was added in the aqueous extract. Change in color of aqueous extract to blue-green confirmed the presence of phenolic compounds in the extract (Hussein 2013).

3.4.3. *Glycosides detection*

0.3 g extract was dissolved in 3 ml of distilled water and mixed with 1 ml solution of glacial acetic acid containing 2 drops of 0.1% of $FeCl_3$. This mixture was carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H2SO4 is underneath the mixture. A brown ring indicated the presence of the glycoside constituent (Mir, Sawhney et al. 2013).

3.4.4. Flavonoids detection

3 mL of extract was taken. Its color turned to intense yellow when few drops of 1 N NaOH was added. When dil. H_2S0_4 was added, it became colorless that indicated the presence of flavonoids in the sample (Arya, Thakur et al. 2001).

3.4.5. Terpenoids detection

3ml of aqueous solution was prepared of each extract and mixed with 2ml of chloroform in a test tube. 2ml of conc. H_2SO_4 was added carefully by the side of the test tube. A reddish brown colored layer was appeared at the interface of H_2SO_4 and sample mixture that indicated the presence of terpenoids (Mir, Sawhney et al. 2013).

3.4.6. Saponins detection

Froth test: 2 g of plant sample in powdered from was taken in a conical flask. 50 ml of distilled water was added and boiled in water bath (WiseBath *WSB-30*) and filtered with Whatman filter

paper no.1. 5ml of this filtrate was further mixed with 5 ml of distilled water and shaken vigorously. About 1cm thick, persistent layer of froth was observed (Gandhi, Cherian et al. 1995) on the surface of solution that indicted the presence of saponins in the sample.

3.5. Antimicrobial activity

3.5.1. Disc Diffusion Method

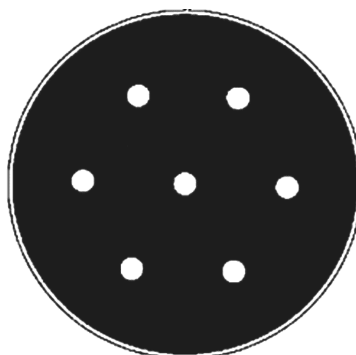
Disc diffusion method was used to determine the antibacterial activity. Tests were run according to the strategy optimized by (Bauer, Kirby et al. 1966) to evaluate the antimicrobial activity of plant extracts with few modifications.

3.5.1.1.Preparation of discs

Sterile Discs with 6 mm diameter were impregnated with amount of 10µl/disc of each plant extract with the concentration of 100mg/ml. As all extracts were made in DMSO, therefore, DMSO was also used as a negative control. One disc was also prepared with a known concentration (5mg/ml) of a standard chloramphenicol that served as a positive control. All discs were incubated in incubator (WiseCube WSG-105) at 37°C for 30 minutes.

3.5.1.2.Experiment

An overnight grown lysogeny broth (LB) culture of bacteria was spread on nutrient agar glass plates of 9cm in size with sterile glass spreader. The agar plates were left for 15 minutes in Laminar Flow cabinet (ISOCIDE Model: SCV 4A1). After that, 5 disc of extracts (Methanol, Ethanol, Aqueous, $CHCl_3$ and n-Butanol) along with a positive control (chloramphenicol). were applied on the agar plates. The negative control was placed in the center of agar plate. So total 7 discs were applied on each bacterial strain.



Plates were then placed in incubator (WiseCube WSG-105) for 24 hours at 37°C.

After 24 hours, plates were examined and inhibition zones were measured through Vernier calipers and noted. This test was repeated three times so that the reliability of results can be ensured (Zaidan, Noor Rain et al. 2005).

3.5.2. Minimum Inhibitory Concentrations

(MIC's) was determined by using method proposed by (Guerin-Faublee, Delignette-Muller et al. 1996) known as inhibitory Concentrations in Diffusion (ICD). In this test, 12 different concentration of all extracts that gave antimicrobial results were made. The concentrations were100mg/ml, 75mg/ml 50 mg/ml, 25mg/ml, 10mg/ml, 5mg/ml, 1mg/ml, 0.5mg/ml, 0.025 mg/ml, 0.01mg/ml, 0.005mg/ml and 0.001mg/ml. The disc diffusion test was again run with these concentrations. The lowest concentration that inhibit the bacterial growth on agar plate was recorded and considered as the minimum inhibition concentrations (MIC) for that particular bacterial strain (Zaidan, Noor Rain et al. 2005).

3.6. Free radicals scavenging activity test

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to determine the percentage antioxidant activity (AA%) of the extracts according to the technique proposed by Brand-Williams (Brand-Williams, Cuvelier et al. 1995). Fractions of samples were dissolved in

ethanol and made different serial dilutions (1000µg-1µg/ml). DPPH stock of 10mM concentration was prepared. Working solution of 0.1mM concentration was prepared by doing further dilutions. A single reaction consisted of 200 µl sample (dissolved in ethanol), 100 µl of DPPH solution (01.mM) and 1.2 ml of solvent (100% ethanol). DPPH is light sensitive therefore tubes containing reaction mixture were placed in a box wrapped with aluminum foil after adding it. Blank was prepared by adding 200µl sample and 1.3 ml of solvent with no DPPH. 100 µl DPPH and 1.4ml of solvent served as control. DPPH has a tendency to reduce, so when it reacts with a compound having antioxidant activity, it is reduced by accepting proton. This reaction was completed in 30 to 40 minutes. Activity was measured by observing the change in color of DPPH from deep violet to light yellow under UV-VIS spectrophotometer (BMS UV-2800) at 517nm. AA% was calculated by using equation proposed by Mensor *et al* (Mensor, Menezes et al. 2001).

$$AA\% = 100 - \left[\frac{(Abs\ of\ sample) - (Abs\ of\ blank)}{Abs\ of\ control}\right] \times 100$$

To compare the AA% values of compounds, a standard test was also performed with Ascorbic acid of 100mg-1µg/ml concentrations.

3.7. Saponins extraction

Saponins were extracted from the powdered samples in a procedure described in schematic diagram (fig. 1). 40g of powdered sample was taken in two separate thimbles (20g each). Thimbles were placed in two separate soxhlet apparatus. 200ml of 100% pure methanol was taken in each round bottom flasks. 200 ml of methanol was available for the extraction of 20g of dried powdered sample in each soxhlet. Both soxhlet were run for 3 hours. 4 cycles were completed, each cycle was of 45 minutes. After that, flasks were removed and both solvents

were mixed. Solvent was evaporated by using rotary evaporator (Heidolph Laborota 4000 efficient) at 40°C for 50 minutes. 3.4g of dried extract was obtained. This 3.4 g of extract was shaken with 1-butanol and H_2O of equal ratio (1:1) in a separating funnel. 1-butanol layer was taken and the layer of H_2O was discarded (Singh, Farswan et al. 2014).1-buanol fraction was then mixed with 35ml of pure petroleum ether (Analytical grade, 40-60°C) in separating funnel. Pet. ether dissolved the fatty acid components present in the extract. Remaining 1-butanol fraction was evaporated by using rotary evaporator (Heidolph Laborota 4000 efficient) at 80°C for 40 minutes. Crude saponins (0.72g by weight) were obtained and stored at 4°C in a sterile tube.

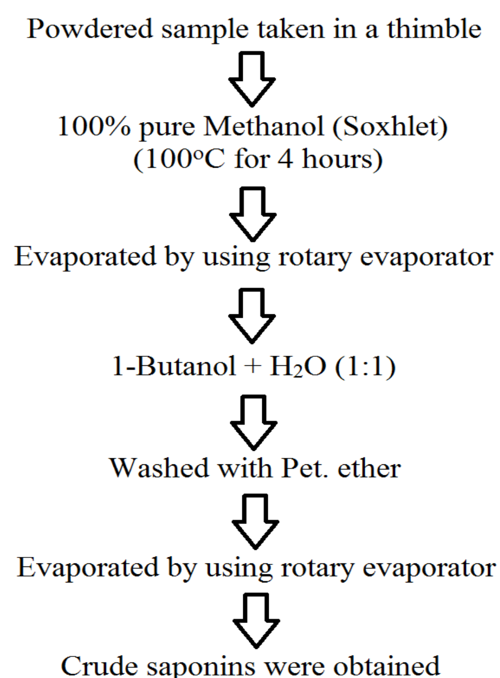


Figure 1 extraction of saponins

3.8. Detection of saponins through TLC

10µl of crude saponins (dissolved in methanol at conc. of 100mg/ml) was applied on silica gel (SiO_2) coated TLC plates (Merk KGaA, 64271, Germany) and placed in the TLC chamber containing chloroform: methanol: water (7:3:1, v/v) as mobile phase. After 2 hours, plates were removed from TLC tank and dried at 37°C. After drying, plates were sprayed with 10 % H_2SO_4 and dried at 100°C for 15minutes.

3.9. Therapeutic potential of crude saponins

Antibacterial, MICs and free radical scavenging activities of isolated saponins were determined by methods discussed previously.

3.10. Fourier Transform Infra-Red (FTIR) spectroscopy analysis of crude saponins

FTIR analysis was performed using an Agilent FTIR. 2mg crude saponins and 300mg of spectral grade potassium bromide (KBr) were compressed together in a dye to form a transparent disc. The FTIR spectrum was set at 4000-500 cm^{-1} using a Perkin Elmer FT-IR spectrometer. The internal element was a ZnSe ATR plate (50 × 20 × 2 mm) with an angle of aperture set at 45°. The sample was scanned 32 times and a spectral graph was generated using ASCII FTIR software.

3.11. Mode of action of saponins

Fresh cultures of bacteria were incubated for 4 hours. Saponins (dissolved in DMSO) were added. The controls were prepared with DMSO only. Cultures were again incubated for 6 hours

of treatment. Serial dilutions were made after 6 hours and plated on the respective agar medium. Colonies were counted after 24 hours.

4. Results

4.1. Percentage yield (%)

Percentage yield of extracts was calculated by given formula. Aqueous extract showed yield of 4.4 ± 0.4 % (Table. 1). Ethanolic and methanolic extracts exhibited the values of 4.3 ± 0.3 and 4.3 ± 0.4 respectively, whereas n-butanol and chloroform exhibited 2.9 ± 0.1 and 2.3 ± 0.1 respectively.

$$\% \, Yield = \left[\frac{weight \; of \; crude \; extract}{weight \; of \; crude \; sapmle}\right] \times 100$$

Table 1. Percentage yield of compounds.

Extracts	***% yield***
Methanol	***4.3 ± 0.4***
Ethanol	***4.3 ± 0.3***
Aqueous	***4.4 ± 0.4***
Chloroform	***2.3 ± 0.1***
n-Butanol	***2.9 ± 0.1***

4.2. Phytochemical screening

Phytochemical compositions of extracts were determined using different chemical techniques. Comparatively high amount of saponins and glycosides, whereas relatively low amounts of terpenoids, tannins and phenolic compounds were detected in methanolic extract (Table. 2). Relatively low amounts of saponins, glycosides, tannins and phenolic compounds were noticed in ethanolic extracts. In aqueous extract, only saponins, glycosides and tannins were identified whereas terpenoids, flavonoids and glycosides were spotted in chloroform extract. Butanolic extract contained just trace amount of these compounds.

Table 2. Phytochemical screening of extracts.

Extracts	***Saponins***	***Terpenoids***	***Glycosides***	***Flavonoids***	***Phenolics***	***Tannins***
Methanol	+++	++	+++	-	++	++
Ethanol	++	-	+	-	+	+
Aqueous	+	-	+	-	-	+
Chloroform	-	+	++	+	-	-
n-Butanol	-	+	+	+	+	+

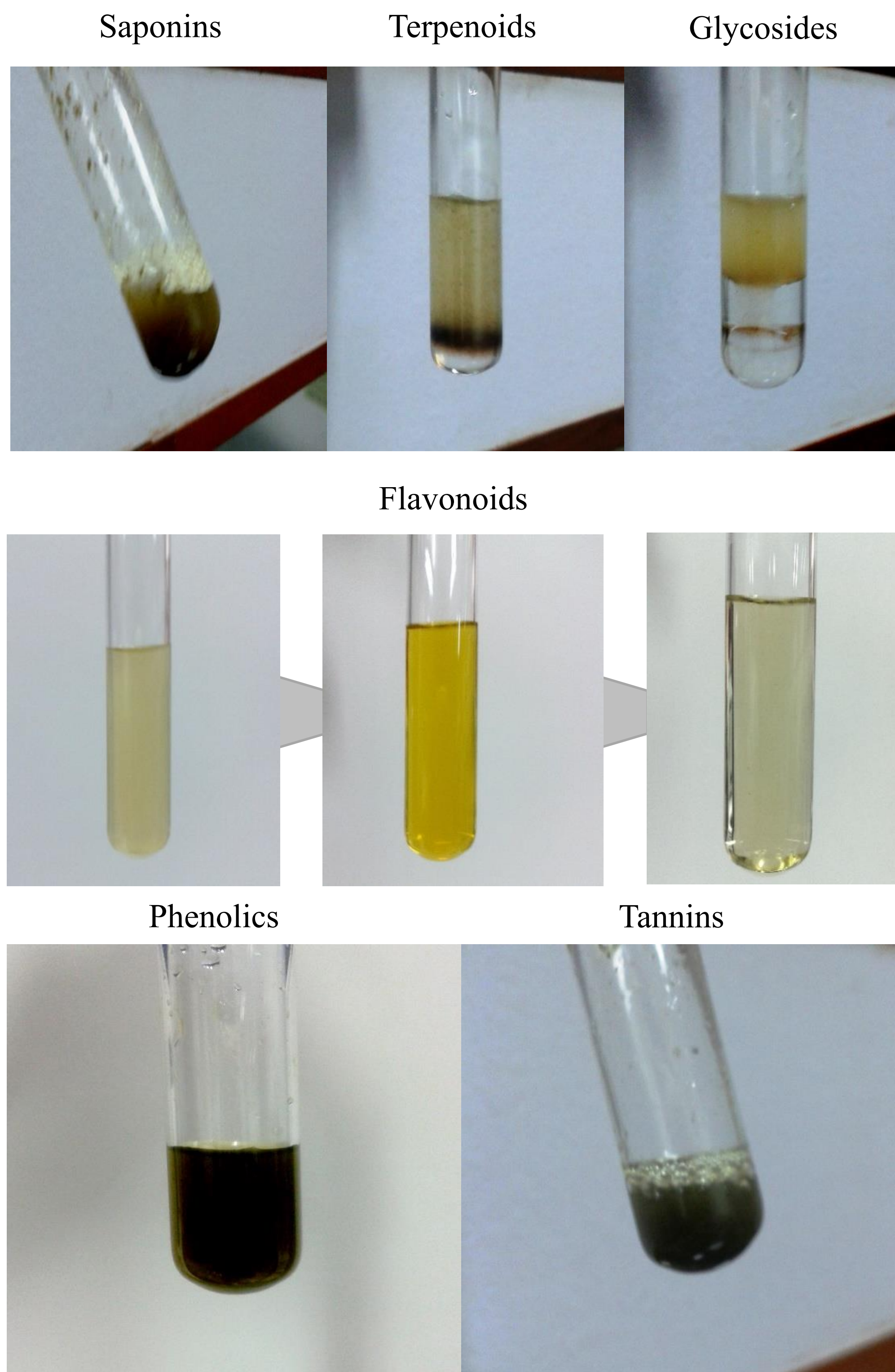


Figure 2. Phytochemical screening results of different compounds.

4.3. Antibacterial activity of extracts against gram positive strains

To assess the antibacterial effect, different strains of bacteria were exposed to various extracts of *Corchorus depresssus* branches. As shown in the Figure 3a, methanolic extracts showed significant antibacterial effect in gram positive strains i.e. *B. cereus*, *E. faecalis* and *S. aureus*. Methanolic extract inhibited microbial zone up to 11.0 - 12.0 mm. Ethanolic extract exhibited considerable inhibition zone in *S. aureus* (13.0 mm). Extract with chloroform also showed significant effect (10.0 mm) against *S. aureus*, however, ineffective against *E. faecalis* (7.3 mm). Similarly, n-butanolic extract inhibited zone was 7.0 - 8.3 mm for all gram positive strains. Aqueous extracts also showed less inhibition, ranging between 8.0 - 9.0 mm.

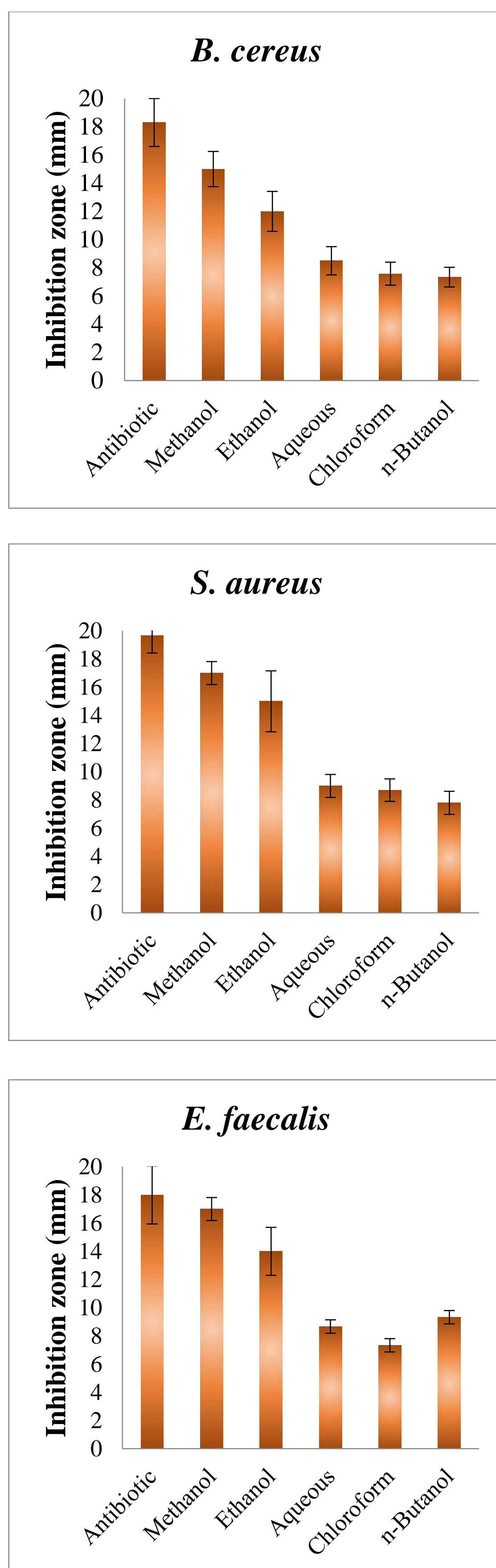


Figure 3a. Antibacterial activity of extracts against gram positive bacteria.

4.4. Antibacterial activity of extracts against gram negative strains

Antibacterial effect of *Corchorus depresssus* compounds against gram negative strains is also determined. As shown in Figure 3b, methanolic extract displayed remarkable inhibition of zones, which was noted to be 10.0, 11.0 and 12.0 mm against *E. coli*, *P. aeruginosa* and *S. typhi*, respectively. Ethanolic extracts showed relatively lower effect against all gram negative strains (8.0 - 10.mm). Aqueous extract was observed to be less effective with inhibition of zones up to 8.0-9 mm. n-butanolic and chloroformic extracts showed similar effects against *E. coli* (9.3 mm), however, effect of chloroformic extract was less (8.3 mm) against *S. typhi*. Chloroformic and butanolic extracts showed inhibition of zone up to 7.0 mm and 8.0 mm with extracts against *P. aeruginosa*, respectively.

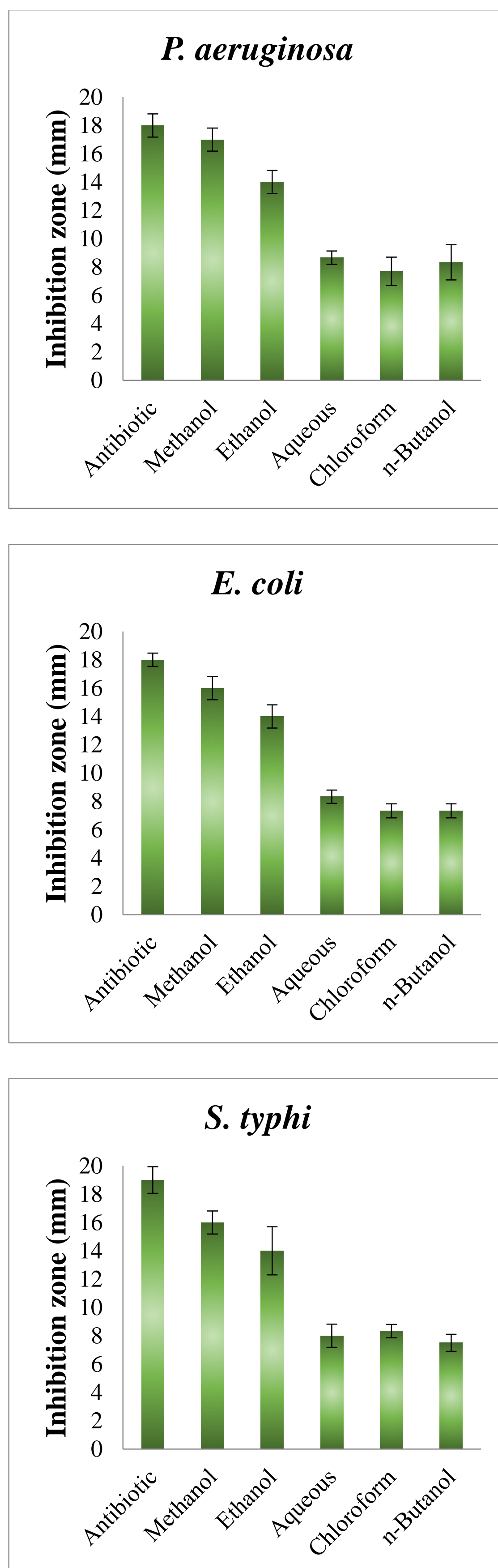


Figure 3b. Antibacterial activity of extracts against gram negative bacteria

4.5. MICs of extracts against gram positive bacteria

MIC for each extract was determined by conducting disc diffusion tests with various concentrations (100mg/ml, 75mg/ml 50 mg/ml, 25mg/ml, 10mg/ml, 5mg/ml, 1mg/ml) of each extract. Our results revealed that methanolic, ethanolic and chloroformic extracts are efficiently effective against *B. cereus,* with MIC value of 5mg/ml, whereas aqueous and butanolic extracts showed MIC value of 10mg/ml (Table 3a). Also, methanolic and chloroformic extracts showed MIC value of 5mg/ml against *S. aureus*, whereas ethanolic, aqueous and butanolic extracts gave 10mg/ml. In case of *E. faecalis,* methanolic, ethanolic and chloroformic extracts showed MIC of 10mg/ml, aqueous 25 mg/ml and butanolic extracts 5mg/ml.

Table 3a. MICs of extracts against gram positive bacteria

Bacteria	***Methanol mg/ml***	***Ethanol mg/ml***	***Aqueous mg/ml***	***Chloroform mg/ml***	***n-Butanol mg/ml***
B. cereus	*5*	*5*	*10*	*5*	*10*
S. aureus	*10*	*10*	*25*	*10*	*5*
E. faecalis	*5*	*10*	*10*	*5*	*10*

4.6. MICs of extracts against gram negative bacteria

In gram negative strains, methanolic and ethanolic extracts displayed MIC of 5mg/ml, aqueous and chloroformic extracts 10mg/ml, whereas butanolic extracts showed 25mg/ml against *E. coli* (Table 3b). Against *S. typhi* Methanolic, ethanolic, chloroformic and butanolic extracts showed 10mg/ml, whereas aqueous extracts exhibited 25mg/ml. ethanolic, aqueous and butanolic extracts gave 10mg/ml whereas methanolic, ethanolic, aqueous and butanolic extracts exhibited MIC of 5mg/ml against *P. aeruginosa,* whereas chloroformic extracts gave 10mg/m.

Table 3b. MICs of extracts against gram negative bacteria

Bacteria	*Methanol mg/ml*	*Ethanol mg/ml*	*Aqueous mg/ml*	*Chloroform mg/ml*	*n-Butanol mg/ml*
E. coli	*5*	*5*	*10*	*10*	*25*
S. typhi	*10*	*10*	*25*	*10*	*10*
P. aeruginosa	*5*	*5*	*5*	*10*	*5*

4.7. Chromatograms of crude saponins from TLC method

Two clearly identified spots on the TLC plate were visualized. Saponins were detected as purple-black zone approximately at Rf =0.63 and the other spot nearer to the baseline considered as sugars (fig. 4).

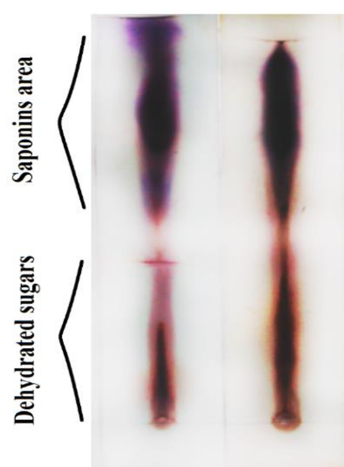


Figure 4. Chromatogram of crude saponins obtained by TLC

4.8. Antibacterial activity of crude saponins against gram positive and gram negative pathogens

Crude saponins were tested against gram positive and gram negative pathogens and showed significant results. Against *B. cereus, E. faecalis and S. aureus,* crude saponins exhibited 16.5 ±0.5, 19±1.5mm and 17.5±0.5 mm zone of inhibition, respectively (fig. 5a), which is very close to the standard (chloramphenicol). Crude saponins showed zones of inhibitions of 17±0.5mm, 20±1.5mm and 19.0±1.0mm against gram negative pathogens *E. coli, P. aeruginosa and S. typhi,* respectively (fig. 5b).

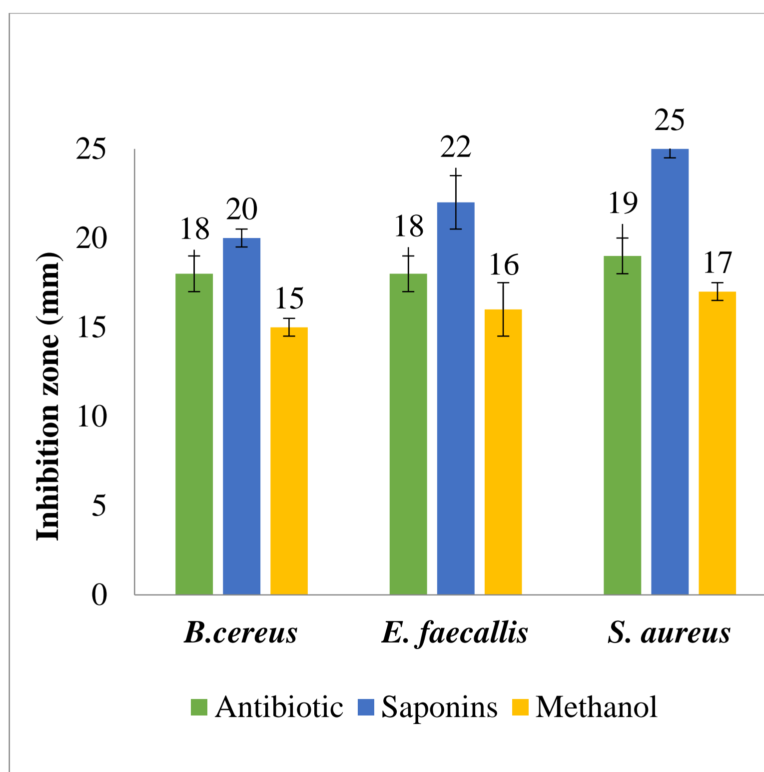


Figure 5a. Antibacterial activity of crude saponins against gram positive pathogens

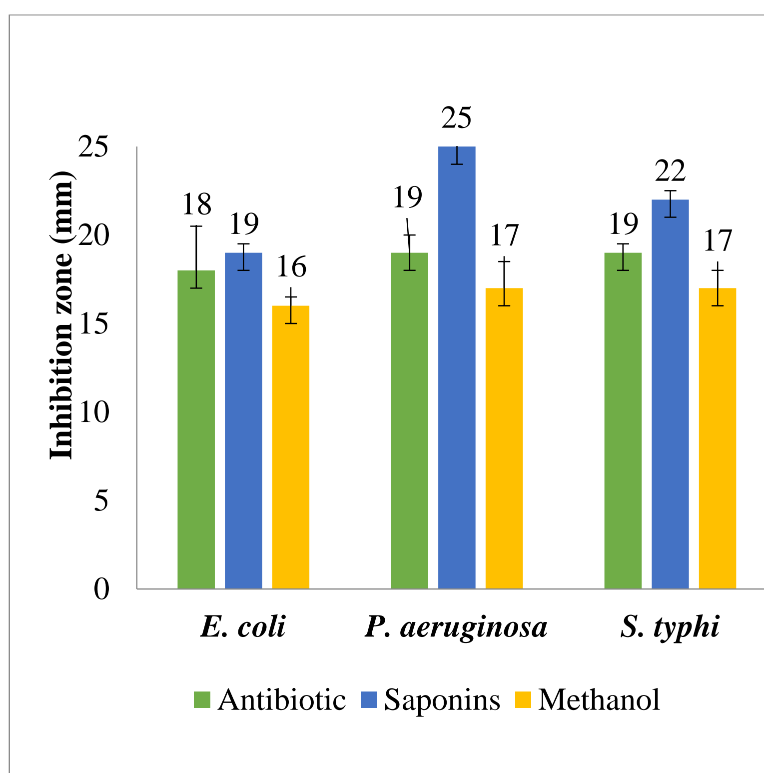


Figure 5b. Antibacterial activity of crude saponins against gram negative pathogens

4.9. MIC of crude saponins against gram positive and gram negative pathogens

MIC of crude saponins extract was determined by conducting disc diffusion tests with various concentrations (100mg/ml, 75mg/ml 50 mg/ml, 25mg/ml, 10mg/ml, 5mg/ml, 1mg/ml). Results revealed that crude saponins showed low MIC values of 2.5 mg/ml against *B. cereus, S. aureus, P. aeruginosa* and *E. coli,* whereas 5mg/ml against *E. faecalis* and *S. typhi* (table 4).

Table 4. MIC of crude saponins against gram positive and gram negative pathogens

Bacteria	***MIC (mg/ml)***	**Concentration (µg/disc)**
B. cereus	*2.5*	25
S. aureus	*2.5*	25
E. faecalis	*5*	50
E. coli	*2.5*	25
S. typhi	*5*	50
P. aeruginosa	*2.5*	25

4.10. Fourier Transform Infra-Red (FTIR) absorption spectral data of crude saponins

Crude saponins were also confirmed by infrared absorption spectrum obtained by FTIR analysis. Characteristic infrared absorbance for hydroxyl group (OH) was recorded at 3416.70 cm^{-1}. Absorption of carbon-hydrogen (C-H) was observed at 2928.14 cm^{-1}. Carbon-oxygen

(C=O) absorption was displayed at 1688.07 cm^{-1}. Absorbance of carbon-carbon (C-C) bond and (C-O-C) glycosidic linkages were observed at 1624.10 cm-1 and 1032.04 cm-1 respectively (fig. 6).

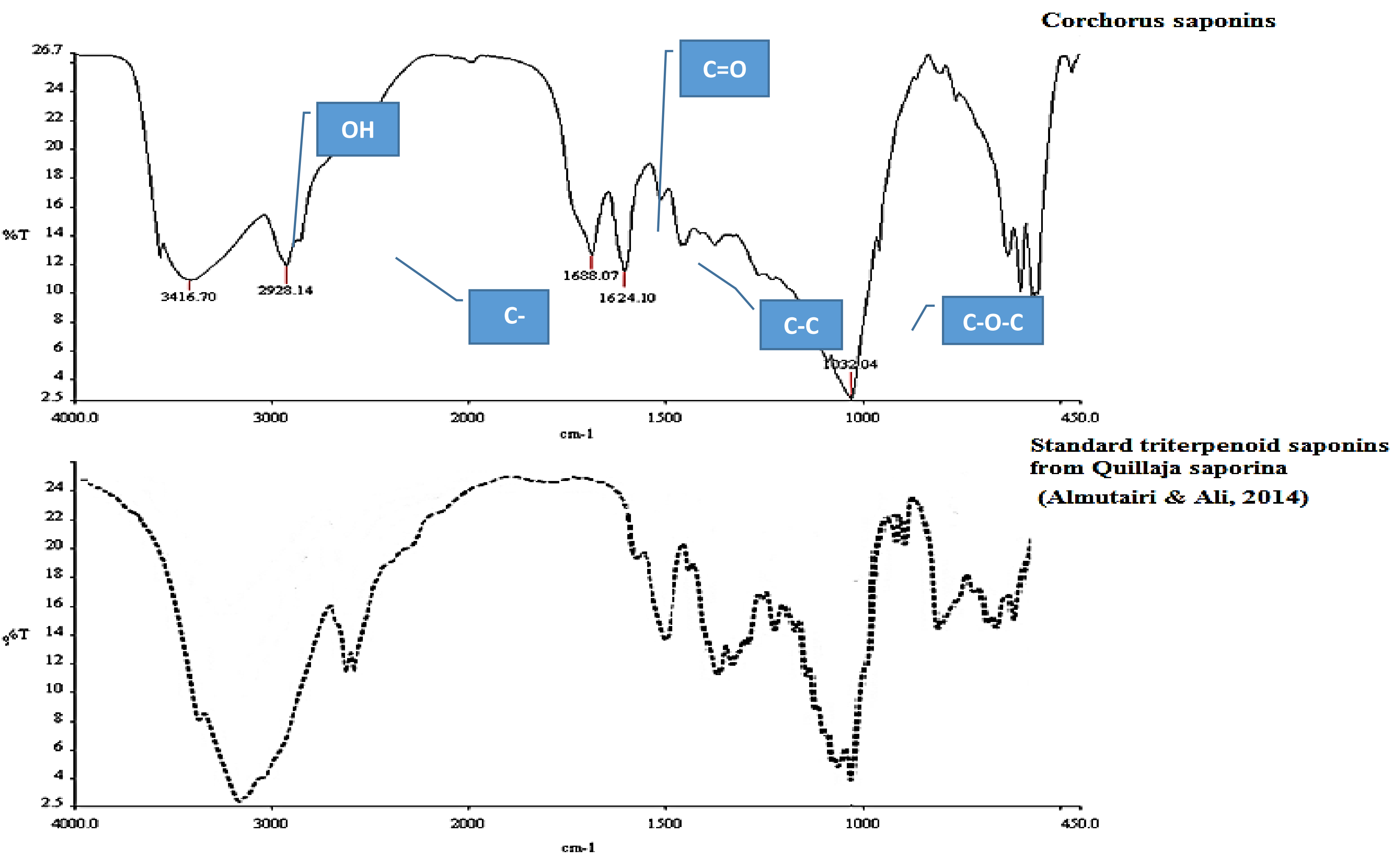


Figure 6. FTIR analysis of crude saponins

4.11. Mode of action of *Corchorus* saponins

The cfu/100µl values of cultures treated with saponins and untreated cultures were calculated. *S. aureus, B. cereus* and *E. faecalis* showed values of 10 cfu/100µl, 16 cfu/100µl and 15 cfu/100µl, whereas untreated cultures showed values of 65 cfu/100µl, 55 cfu/100µl and 50 cfu/100µl respectively. Cultures of *E. coli, P. aeruginosa* and *S. typhi* treated with saponins showed values of 16 cfu/100µl, 25 cfu/100µl and 20 cfu/100µl, whereas untreated cultures showed values of 50 cfu/100µl, 80 cfu/100µl and 70 cfu/100µl, respectively (fig. 7).

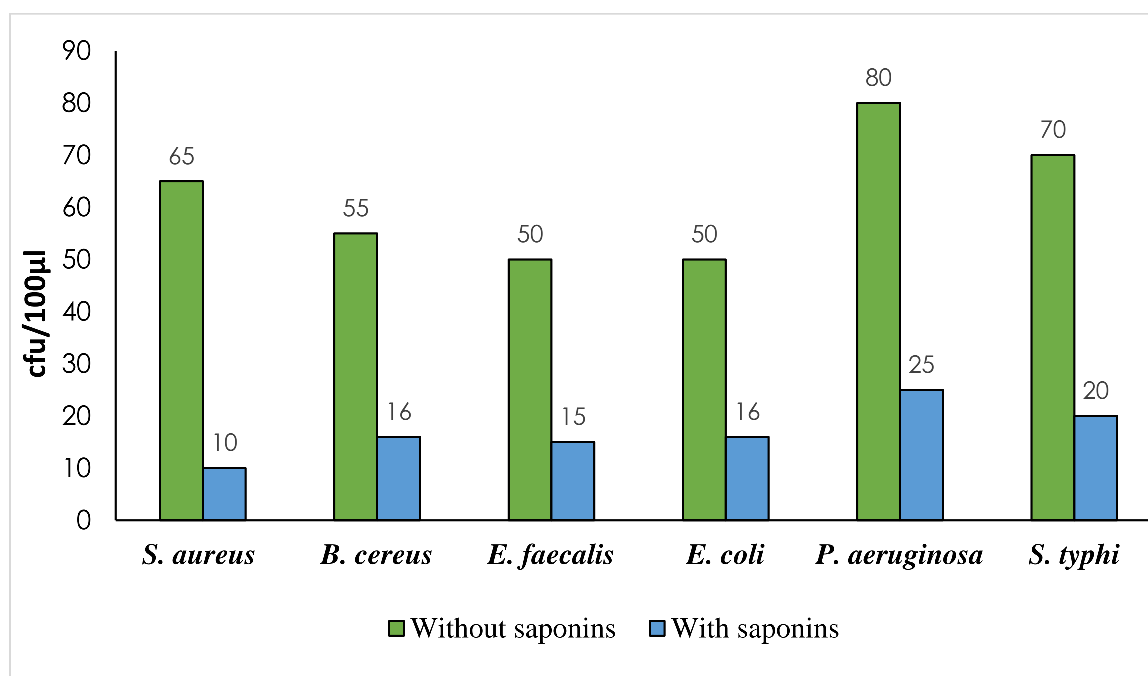
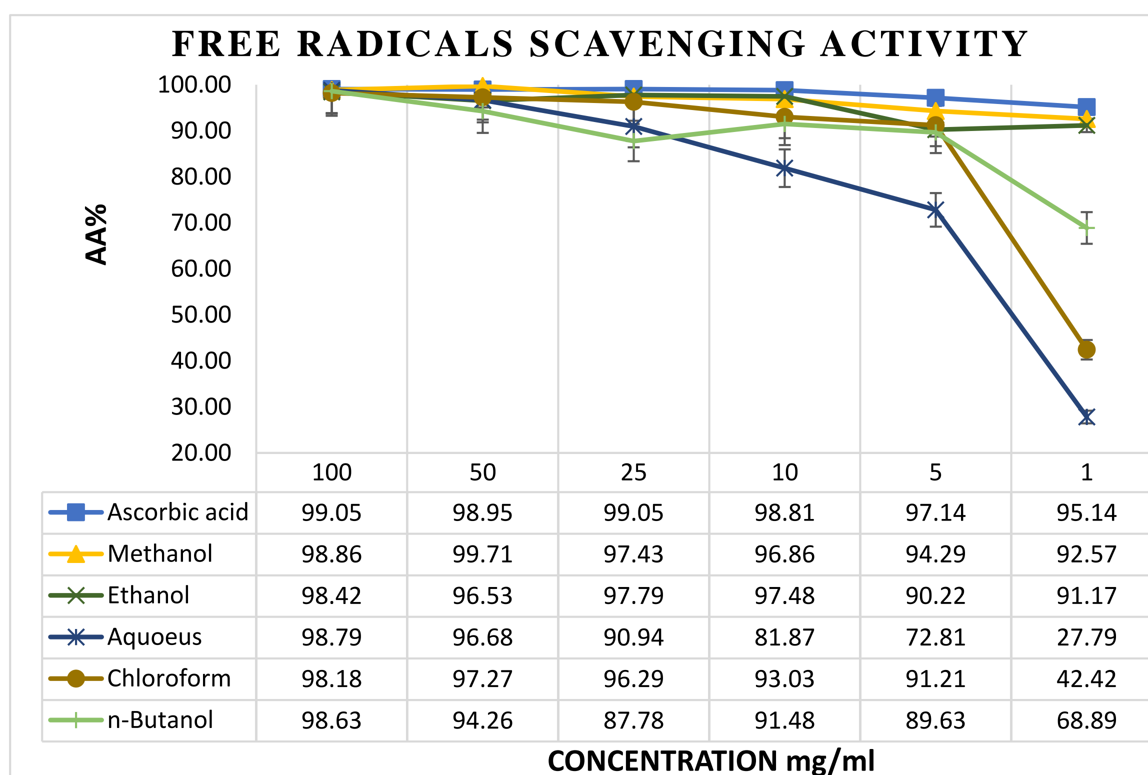


Figure 7. Colony-forming unit (cfu) values of saponins-treated and untreated cultures for the determination of the mode of action of *Corchorus* saponins

4.12. Free radicals scavenging activity of extracts

DPPH assay was performed to assess free radicals scavenging activity of *C. depresssus* extracts. Results in fig. 8 show that ascorbic acid showed 99.05 - 97.14% of DPPH free scavenging at the conc. range of 100 - 1mg/ml. Methanolic and ethanolic extracts showed 98.86 - 92.57.0 % and 98.42 - 91.17 % of DPPH free scavenging at the conc. range of 100 - 1mg/mL, respectively. Aqueous extracts exhibited activity 98.79 - 27.79%, chloroformic extracts 98.18 - 42.42% and butanolic extracts 98.63 - 68.89%. Extracts showed higher values of AA% in regards to ascorbic acid at their high concentrations (100 mg/mL, 50 mg/mL and 25 mg/mL). Methanolic and ethanolic extracts exhibited significant values in comparison to the standard.

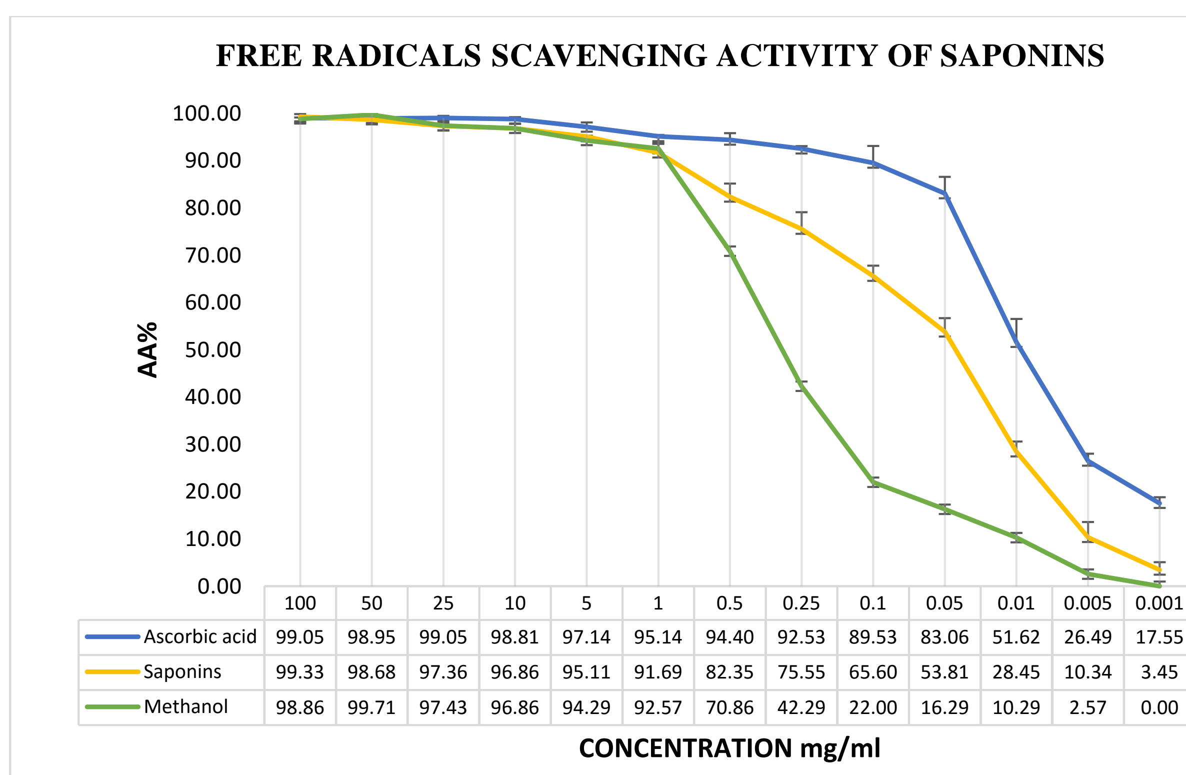


	100	50	25	10	5	1
Ascorbic acid	99.05	98.95	99.05	98.81	97.14	95.14
Methanol	98.86	99.71	97.43	96.86	94.29	92.57
Ethanol	98.42	96.53	97.79	97.48	90.22	91.17
Aquoeus	98.79	96.68	90.94	81.87	72.81	27.79
Chloroform	98.18	97.27	96.29	93.03	91.21	42.42
n-Butanol	98.63	94.26	87.78	91.48	89.63	68.89

Figure 8. Free radicals scavenging activity of *Corchorus* extracts.

4.13. Free radicals scavenging activity of crude saponins

DPPH assay was performed to assess free radicals scavenging activity of saponins extracted from branches of *C. depressus* in comparison to ascorbic acid and methanolic extracts. Crude saponins showed significant free radicals scavenging activity as compared to methanolic extracts. They followed the standard (ascorbic acid) up to the concentration of 0.001mg/ml (1µg/ml), whereas methanolic extracts showed no activity at this concentration (fig. 9).



	100	50	25	10	5	1	0.5	0.25	0.1	0.05	0.01	0.005	0.001
Ascorbic acid	99.05	98.95	99.05	98.81	97.14	95.14	94.40	92.53	89.53	83.06	51.62	26.49	17.55
Saponins	99.33	98.68	97.36	96.86	95.11	91.69	82.35	75.55	65.60	53.81	28.45	10.34	3.45
Methanol	98.86	99.71	97.43	96.86	94.29	92.57	70.86	42.29	22.00	16.29	10.29	2.57	0.00

Figure 9. Free radicals scavenging activity of *Corchorus* saponins

5. Discussion

Pathogens infection is alarming risk to human health and a notable increase is seen in the bacterial diseases globally. Bacterial pathogens utilize different mechanism in order to survive in a variety of host lesions and escape immune system. Meanwhile some bacteria have changing genomes due to presence of transposons that help them in survival by giving them resistance to antibiotics (Khan, Bano et al. 2006, Akinpelu, Aiyegoro et al. 2010). Therefore, it is highly desirable to look for more effective drugs against the challenging microorganisms.

Phytochemicals such as saponins, terpenoids, glycosides, tannins and phenolic compounds are secondary metabolites of plants, which contribute in their defense mechanisms against various contagious microorganisms (bacteria, fungi, and insects). Plant decoctions or extracts have been used for centuries to treat and prevent various ailments and diseases including arthritis, constipation, microbial infections, intestinal disorders and ulcers (Craig 1999, McGaw, Lall et al. 2008). *C. depresssus* branches contain bioactive compounds, which show potential effect against both gram positive and gram negative bacterial strains. Based on the solubility of solvents, various extracts contained different phytochemicals. Results revealed that *C. depresssus* contain high amount of saponins and other glycosides in methanolic and ethanolic extracts. Data generated by antibacterial assay showed that methanolic and ethanolic extracts produced significant inhibition against both gram positive and gram negative bacteria. Chloroformic, aqueous and butanolic extracts displayed relatively lesser antibacterial activity. Certainly saponins and glycosides actively participated in antibacterial activity of *C. depresssus* as they showed remarkable antibacterial activity with low MIC against all pathogens.

This idea is supported by MIC values of crude saponins extracts (2.5 mg/mL). *E. faecalis* have the potential resistant to antibiotic, however the extracts showed inhibition against it (Amyes 2007). Aqueous and n-butanolic extracts contained lesser amount of bioactive compounds as compared to other extracts and showed higher MIC especially against *E. coli* and *S. typhi* (25 mg/mL).

Naturally plant-isolated phytochemicals have gained huge attention as modulator of ROS generations as well as a treatment strategy for inflammatory and aging diseases (Beatty, Koh et al. 2000). Free oxygen radicals are essential components of cell signaling and can cause cancer, autoimmune disorders, neurodegenerative diseases and cardiovascular disorders. ROS generation causes oxidative damage by reacting with biomolecules such as proteins, lipids, enzymes and DNA/RNA. Different parts of plant contain antioxidants such as ascorbic acid, phenolics and Vitamin E that are capable of curing cardiac diseases, diabetes, arthritis, Alzheimer's disease, cancer and immune deficiency disorders (Frank and Gupta 2005, Pham-Huy, He et al. 2008).

Study suggested that the mode of action of *Corchorus* saponins is bactericidal. The cfu/100µl values of saponins-treated cultures are less than 50% of the values of untreated cultures. These low cfu/100µl values denoted the bactericidal mode of action of saponins.

Clinical data support the notion for search of novel and potent antioxidant compounds from natural resources including plants. *Corchorus depresssus* saponins displayed comparable antioxidant activity to ascorbic acid even at the low concentrations.

6. Conclusion

The work demonstrated the antibacterial efficacy as a platform for further studies to explore the therapeutic and pharmacologic potentials of *Corchorus depresssus*. A few studies have been done for the saponins use as antibacterial agents. The functional group absorbance data generated by FTIR have been considered as characteristics of triterpenoid saponins. Therefore it is required to further explore the saponins and glycosides of this plant as antibiotic candidate.

This study also show the effectiveness of extracts isolated from branches of *Corchorus depresssus* especially methanolic and ethanolic extracts as significant source of natural antioxidant to prevent cell damage and numerous oxidative stress-induced diseases. Studies have been conducted for use of saponins anti-inflammatory effects. Further and detailed investigation of *C. depresssus* saponins is however required.

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