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Saponins and their synergistic antibacterial activity with traditional antibiotics against Staphylococcus aureus and Escherichia coli: Review

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Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.

Abstract

The rapidly increasing rate of antimicrobial drug resistance requires novel ways of treating infections. Harnessing the synergistic effect of the combined use of conventional antibiotics with naturally occurring antimicrobial substances is an emerging frontier in the fight against the spread of antimicrobial resistance. Synergy is measured by using the fractional inhibitory concentration index (FICI). Saponins are secondary metabolites produced by plants and they help defend the plant against natural stressors. This article aims to review the synergistic activity of saponins with traditional antibiotics. Thirteen plants were included in the final review, out of which eight species showed a FICI score below 0.5 (synergistic). These were *Jatropha curcas*, *Melanthera elliptica*, *Glycine max, Tribulus terrestris, Salvia officinialis, Spergulara marginata, Paromychia argenetea*, and *Syzigium aromaticum*. The highest degree of synergy was observed against S. aureus with the combined use of J*atropha curcas* and rifampicin (FICI 0.04), *Melanthera elliptica* and tetracycline (0.05), and *Glycine max* and benzylpenicillin (0.22). In addition, a high degree of synergy against E. coli was observed with the combined use of *Melanthera elliptica* and tetracycline (0.07), *Jatropha curcas* and rifampicin (0.08), *Salvia officinialis* and amoxicillin (0.38).

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Keywords: Saponin, antibiotic synergy, antibiotic resistance, phytochemicals, fractional inhibitory concentration index.

Introduction

Antimicrobial drug resistance (AMR) has been identified as a major global public health threat of the 21st century and it is placing a rapidly increasing burden on our healthcare system [1]. Over the past few decades, the rate of infections caused by multidrug-resistant bacteria, parasites, viruses, and fungi has been increasing rapidly. The issue of antimicrobial drug resistance appears to be particularly challenging when we consider the growing rate of drug resistance in clinically relevant bacteria. An ever-increasing number of bacteria causing common infections (like tuberculosis and pneumonia) have acquired resistance to most of the currently available antibiotics [2]. Thus, the effective treatment of a quickly growing list of infections is being compromised. According to a report from the US Center for Disease Control and Prevention (CDC), infections caused by drug-resistant bacteria are responsible for more than two million infections annually and around 23,000 deaths in the United States alone [3]. Similarly, the European Center for Disease Prevention and Control estimated that in 2007, around 400,000 infections and 25,000 deaths were caused by a few common bacteria that had developed resistance to most antibiotics [4].

Although it is widely known that antibiotic resistance is on the rise in all parts of the world, we still lack a comprehensive and accurate record of its global impact on human health and the costs for the healthcare sector [1]. The impact of antimicrobial drug resistance extends beyond infectious disease therapy and can affect almost all aspects of healthcare. In the absence of effective antibiotics, most of the medical procedures that we take for granted (e.g. cesarean section, joint replacement surgery, cancer chemo-radiotherapy, etc.) can't be performed because these procedures depend on the presence of effective antibiotics [5]. Throughout the years, numerous strategies have been forwarded in the fight against antimicrobial drug resistance. One of the proposed mechanisms to fight AMR is to combine antibiotics with non-antibiotic drugs or bioactive agents from nature where 40% of our medicines are derived from plants [6][7]. Plants produce two types of metabolites, namely - primary and secondary metabolites. Whereas the function of primary metabolites is largely in promoting plant growth and development, secondary metabolites are useful in defending the plant from environmental factors including herbivore attacks and abiotic stress [8][9]. The ability to synthesize, the quantity yielded, and the type of secondary metabolites produced by plants depends on several factors. Because secondary metabolites are produced only by certain plant groups, these compounds are deemed not to be essential for survival. However, these plant-based bioactive elements have gained the attention of the scientific community because, in addition to their antimicrobial activities, they can also be used in combination with conventional antibiotics to improve the effectiveness of these antibiotics [6].

Saponins are a group of secondary metabolites that are produced by more than 100 families of plants and marine animals. Their soap-like nature was identified early on, in fact, the name saponin originated from the word "*sapo*" (which is the Latin word for soap). This is to denote the stable soap-like foam that saponins form when shaken in water [10]. The type and quantity of saponins contained in a particular plant depend on its genetic profile as well as on a variety of other factors including its age and physiological state [11]. Although some authors add a third group, saponins can broadly be classified into two groups based on the nature of their aglycone skeleton, they are steroidal saponins or triterpenoid saponins. Steroidal saponins are almost exclusively present in the monocotyledonous angiosperms (e.g. in families of *Agavaceae*, *Dioscoreaceae*, and *Liliaceae*). The second group, triterpenoid saponins, are the most common type and occur mainly in the dicotyledonous angiosperms (*Leguminosae*, *Araliaceae*, *Caryophyllaceae*) [12][13]. Saponins provide

selective advantages to the host plant by inhibiting the growth of neighboring plants as well as by providing protection against various forms of plant pathogens and environmental stressors [11]. Numerous types of saponins have been shown to exhibit various degrees of antibacterial properties. In the future, these antibacterial activities may find several applications in the healthcare sector and the potential contribution of saponins in combating antimicrobial drug resistance is under investigation. In addition, saponins consist of compounds with diverse biological effects that may have potential clinical relevance. These include anti-ulcer, hemolytic, anti-inflammatory and hepatoprotective properties (besides their antibacterial, antifungal and antiviral properties) [14][15][16][17]. However, the scope of this paper is to review the antimicrobial activity of saponins and their synergistic potential with traditional antibiotics.

Main text

a. Antimicrobial activity of saponins

Reports indicate that 90–95% of *Staphylococcus aureus* strains worldwide are resistant to penicillin and 70–80% are resistant to methicillin [18][19]. With this rise in bacterial, fungal, parasitic, and viral resistance to antibiotics, plant-derived biologically active antimicrobials have garnered greater attention in the scientific world. The antimicrobial activity of saponins is well documented in the literature [10][12][13][14]. Saponin extract of *Melanthera elliptica* has a bactericidal effect against *Escherichia coli* and *Shigella flexneri* [20] whereas *Medicago* species have a strong inhibitory effect against *Proteus vulgaris, Klebsiella pneumonia, Salmonella typhi, Mucor circinelloides, Rhizopus azygosporus, and Rhizopus microspores* [21][22]. Saponins may exert their antibacterial effect via a variety of mechanisms. Most importantly, they have been shown to interact with the cholesterol of cell membranes creating a pore and eventually forcing the cell membrane to burst [23]. The saponin from *Medicago sativa* has an anti-fungal effect against *Candida albicans* which acts by inhibiting germ tube formation [24]. The antifungal characteristics of steroidal saponins are influenced by two main factors. First, the chemical composition of the particular saponin including its content of aglycone moieties and second the quantity and chemical structure of their monosaccharides [25]. Saponins are also known to have an antiviral activity which has been reported from purified saponin mixtures of *Maesa lanceolate*. Additionally, oleanolic acid, a triterpenoid saponin, inhibits HIV-1 virus replication possibly by inhibiting HIV-1 protease activity[26][27]. The cytopathic effects and the replication of herpes simplex type 1 (HSV-l) and polio type 2 viruses were also shown to be inhibited by glycosides isolated from *Anagallis arvensis* [23]. Anti-herpes virus activity has been observed in triterpenoid saponins from the*Fabaceae* family [13]. There are also reports of the antimicrobial activity of saponins against parasites. In a study conducted by Andrade et al., aqueous extracts of *Ziziphus joazeiro* Mart showed an anti-parasitic effect against *Leishmania braziliensis* and *Leishmania infantum* with an unexplained mechanism of action[28]. Further research is also required to characterize the mechanisms of actions involved in the antimicrobial activities of saponins against a variety of pathogens.

b. Antibacterial activity of saponins

Saponins have been evaluated both as antibiotics and as agents to improve the susceptibility of bacteria to existing conventional antibiotics. The effectiveness of the antimicrobial activity of various forms of saponins has been evaluated for the treatment of a variety of infections in humans as well as in plant agriculture. One potential approach that has been explored to overcome the AMR challenge is to utilize the synergistic effect of combining conventional antibiotic therapy with saponins [29]. In this context, Tagousop et al studied the antimicrobial activities of saponins extracted from *Melanthera elliptica* and proceeded to evaluate the presence of synergistic effect with conventional antibiotic drugs. The investigators extracted and purified four compounds from the plant and tested each of them for antibacterial and antifungal properties. Two of the compounds were shown to have effective antibacterial activity against multidrug-resistant *Escherichia coli* and *Shigella flexneri*. This antibacterial activity was shown to be equivalent to that of vancomycin, which was the drug that was used as the reference antibiotic for comparison in the study [20].

However, the synergism between saponins and other conventional antibiotic drugs may not be easily translated into a clinically useful finding. This is because some studies have found that the antimicrobial activity and synergistic effect of saponins with other conventional antibiotic drugs, may occur at a dose that also causes a cytotoxic effect on human cells. Arabski et al studied the effect of saponins (extracted from *Quillaja saponaria*) on clinically relevant strains of *Escherichia coli* as well as on eukaryotic cell lines at various concentrations. Their experiments indicate that the saponins produced a significant cytotoxic effect on the eukaryotic cell line when they were applied at a concentration greater than 12 µg/mL. The investigators experimented to see if the use of saponin at a safe concentration (12 µg/mL) has a synergistic antibacterial effect with either ampicillin, streptomycin, or ciprofloxacin in the presence. The results of the experiment showed that the application of saponins at this concentration failed to exhibit any antibacterial activity or to facilitate the antibacterial activity of the other antibiotics. On the contrary, in the presence of saponins in the above-mentioned concentration, more CFU/mL E. coli were observed [30]. Thus, further studies need to be performed to identify a clinically applicable approach to harness the synergistic activity of saponins ad conventional antibiotics.

In addition to their potential role as antibiotics in the treatment of human infections, the potential role of saponins as alternative forms of food preservatives has also gained attention. This area has received importance because the current practice of adding synthetic chemical additives in food processing is associated with several health problems [31]. In addition, the increased incidence of food-borne bacteria that are resistant to many of the commonly used preservatives also calls for alternative methods including the use of natural inhibitors of bacterial growth to replace artificial chemical additives to food [32]. Thus, the antimicrobial activity of saponins against food-borne bacteria has been studied extensively. In this context, Dong et al investigated the antimicrobial activity of saponins found in *Chenopodium quinoa*. The investigators managed to extract and purify six different compounds, and they tested the antimicrobial activity of each of these compounds against six types of food-borne bacteria. The results of their experiment indicated that all of the compounds exhibited antimicrobial activity against *S. aureus, S. epidermidis,* and *B. cereus*. The antimicrobial activity was also shown to be correlated with the concentration of the respective saponin. Furthermore, the investigators were able to show that the mechanism of action for the antibacterial effect of the saponins consisted of disruption of the bacterial cell wall and cell membrane, with the consequent release of cellular contents [33].

c. Synergy of saponins with traditional antibiotics

Several studies have shown that saponins demonstrate synergistic activity when used in combination with a variety of conventional antibiotics[30][34][35]. This has given hope to scientists as it may pave the way for the reintroduction into clinical use of old antibiotics that were once deliberated ineffective due to antimicrobial resistance. Thus, harnessing the synergistic properties of the combined use of saponins and conventional antibiotics has the potential to be one of the new frontiers in the fight against rapidly spreading drug-resistant microbes. Two (or more) drugs are said to be synergistic when their combined activities are higher than the sum of their individual separate effects [36]. There are three main ways of assessing synergy; agar diffusion assay (performed in solid media), checkerboard (CBA) assay (performed in liquid media), and time-kill curve. CBA has a higher throughput capacity than gradient diffusion tests including agar diffusion assays and thus it is widely used in synergy studies. The interaction between two antimicrobial substances is estimated by fractional inhibitory concentration (FIC). The FIC of drug A is calculated as the ratio of the Minimum Inhibitory Concentration (MIC) of drug A in the presence of drug B divided by the MIC of drug A alone:

$$\mathrm{FIC}_A = \frac{MIC_A(B)}{MIC_A}$$

and vice versa for drug B:

$$\mathrm{FIC}_B = \frac{MIC_B(A)}{MIC_B}$$

The sum of FIC_A and FIC_B gives the FIC Index [36].

$$\text{FIC Index} = FIC_A + FIC_B$$

When FICI is less than or equal to 0.5, it is termed “synergistic”; FICI between 0.5 and 4 indicates “no interaction”, and when the FICI value is greater than 4 the two compounds are termed “antagonistic” [19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36].

A review by Martinez et al. reported that phytochemicals and beta-lactam antibiotics were the most extensively evaluated form of synergy between a plant extract and a traditional antibiotic [8]. The synergistic mechanism of action of phytochemicals includes blocking multidrug-resistant (MDR) pumps, modification of binding proteins, enzyme inhibitors, and permeability enhancers [18]. The amphiphilic nature of saponins facilitates the interaction with the hydrophobic and hydrophilic parts of the cell membrane.

As shown in Figure 1, the highest synergy between antibiotics and saponins was observed from extracts of eight plants namely, *Jatropha curcas, Melanthera elliptica, Glycine max, Tribulus terrestris, Salvia officinialis, Spergulara marginata, Paromychia argenetea* and *Syzigium aromaticum* in descending order (Figure 1). Although cell membrane disruption has been put as the saponins' bactericidal mechanism of action, the role of this mechanism in synergistic studies is unclear. Moreover, the inhibition of efflux pumps is one of the most common mechanisms that enable a synergistic effect when a combination of antibiotics and phytochemicals is used[6][7][8]. *Fabacea* family which includes *Glycine max* and *Lamiacea* family which includes *Salvia officinialis* are excellent sources of efflux pump inhibitors[6]. It should also be noted that the main saponin type in both families is triterpenoid and further study is needed to confirm the efflux pump inhibitor activity of these plants and whether triterpenoid saponins are more likely to act by inhibiting efflux pumps than disrupting cell membranes. Following these lines, aqueous extraction is reportedly the best way to have the highest yield of saponins per extracted plant material[37][38][39]. However, as can be seen in Table 1, the majority of the reported instances in this review are methanol extracts and they showed promising results (Table 1). The relationship between saponin extraction yield and antibacterial activity could be a topic for further studies.

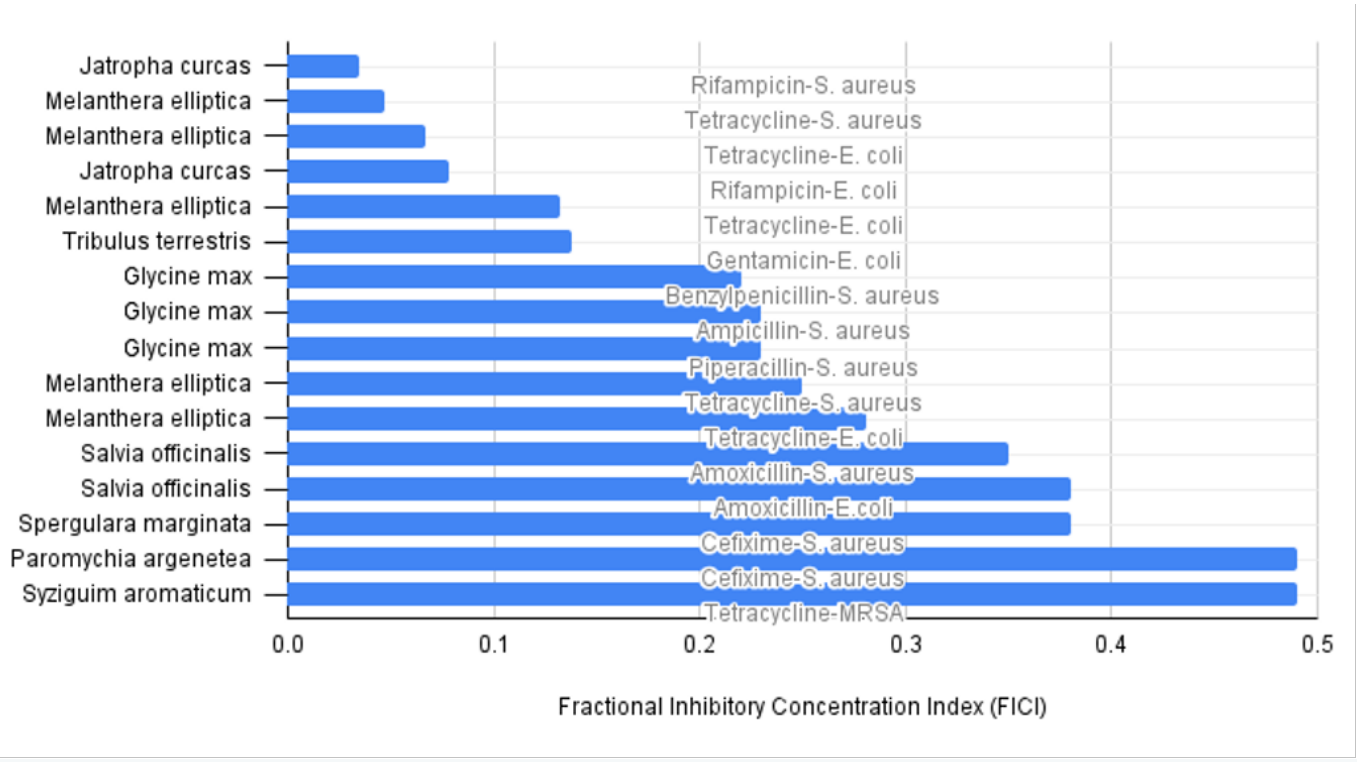

Figure 1. *Synergy of plants with antibiotics against bacteria, measured in fractional inhibitory concentration index (FICI)*

Figure 1 shows that the highest synergy between saponin extracts and antibiotics against S. aureus was reported for Rifampicin + *Jatropha curcas* (FICI=0.04), followed by Tetrcycline + *Mellanthera elliptica*. Two plant extracts, *Jatropha curcas* and *Melanthera ellptica* showed the highest five synergies from the sixteen most promising reported FIC indexes (Figure 1). *Jatropha curcas* belongs to the *Euphrbiacea* family and has been shown to possess antibacterial activity against *E. coli, S. aureus, K. pneumonia, Proteus spp., and P. aueuroginosa* Due to its antimicrobial action, *Jatropha*

curca has played a major role in the treatment of bacterial infections[40]. Saponin extracts from the aerial parts of *Melanthera elliptica* showed high synergy with tetracycline when used against both against*S. aureus and E. coli*. The saponin 3-O-β-d-glucopyranosyl(1→2)-β-d-glucuronopyranosyl oleanolic acid 28-O-β-d-glucopyranosyl ester isolated from the methanolic extracts of *Melanthera elliptica* showed the lowest FICI values (highest synergy) (Table 1).

Table 1. Plant saponins and their synergy with conventional antibiotics when tested against various bacterial species, #MSSA:Methicillin Sensitive S.aureus, $MRSA:Methicillin Resistant S.aureus, @FIC:Fractional Inhibitory Concentration

Plant Source	Antibiotic	Against bacterial (FIC Index)	Part used	Extraction medium	Family	Saponin type	Reference
Acanthopanax henryi (Oliv.) Harms	Oxacillin	MRSA CCARM 3090 (0.53), MRSA DPS-1 (0.53)	Leaf	aqueous	*Araliaceae*	Triterpenoid	[41]
Ficus carica	Ampicilin	MSSA ATCC 25923 (0.5), MRSA ATCC 33591 (0.5)	Leaf	Methanol	*Moraceae*		[42]
	Oxacillin	MRSA ATCC 33591 (0.5)					
Jatropha curcas	Rifampicin	*A. baumannii* (0.15), *E. coli* (0.078), *E. faecalis* (0.09), *S. aureus* (0.0351), *P. aeruginosa* (0.2), *A. baumannii* (MDR strain), *P. chlororaphis*, *E. coli* ATCC25922 (0.1) *and S. aureus* ATCC25923 (0.03)	Cake and seed	Methanol	*Euphorbiaceae*		[43]
Melanthera elliptica	Tetracycline	*E. coli* (0.2812), *S. fexneri* (0.515), *S. aureus* (0.25)	Aerial parts	Methanol		Triterpenoid	[20]
	Tetracycline	*E. coli* (0.132), *S. fexneri* (0.257), *S. aureus* (0.53)					
	Tetracycline	*E. coli* (0.066), *S. fexneri* (0.033), *S. aureus* (0.047)					
Melastoma malabathricum	Amoxicillin	*E. coli* (0.5), *B. cereus* (0.5), *S. aureus* (0.5), *S.aureus* (0.5)	Leaf	Ethanol	*Melastomataceae*		[44]
Paromychia argenetea	Cefixime	*S. aureus* (0.49), *M. luteus* (0.38), *B. cereus* (0.38), *E.coli* (0.52)	Aerial parts	Methanol-water	*Caryophyllaceae*	Triterpenoid	[45]
	Ciprofloxacin	*S. aureus* (0.59), *M. luteus* (0.50), *B. cereus* (0.40), *E.coli* (0.50)					
	Kanamycin	*M. luteus* (0.38), *B. cereus* (0.49)					
Phyllantus muellerianus	Ciprofloxacin	*P. earuginosa* (0.40), *E. faecalis* (0.50)	Leaf	Methanol	*Phyllanthaceae*		[46]
Pitycocapra monilifora	Chloramphenicol	*S. aureus* 02 (0.5)	Leaf	Ethanol-water			[47]
	Erythromycin	*S. aureus* 0687 (0.5)					
	Tetracycline	*S. aureus (0.5)*					
Salvia officinalis	Amoxicillin	*Bacillus subtilis* (0.50), *Enterobacter cloacae* (0.35), *Klebsiella pneumoniae*(0.35), *Staphylococcus aureus* (0.35), *E.coli* (0.38)	Leaf	Acetone	*Lamiaceae*	Triterpenoid	[48]
	Chloramphenicol	*Bacillus subtilis* (0.40)		Ethyl acetate			
Glycine max	Ampicillin	*S. aureus* SA-24 (0.23), SA-69 (0.25), SA-78 (0.24), SA-85 (0.28), SA-91 (0.26)	Stock		*Fabacea*	Triterpenoid	[49]
	Benzylpenicillin	*S. aureus* SA-24 (0.22), SA-69 (0.22), SA-78 (0.23), SA-85 (0.23), SA-91 (0.25)					
	Piperacillin	*S. aureus* SA-24 (0.23), SA-69 (0.24), SA-78 (0.24), SA-85 (0.25), SA-91 (0.26)					
Spergulara marginata	Cefixime	*S. aureus* (0.38), *M. luteus* (0.38), *B. cereus* (0.52)	Roots	Methanol-water	*Caryophyllaceae*	*Triterpenoid*	[45]
	Ciprofloxacin	*S. aureus* (0.50), *M. luteus* (0.40), *B. cereus* (0.50), *E.coli* (0.50)					
	Kanamycin	*M. luteus* (0.38), *B. cereus* (0.49)					
Syzigium aromaticum	Ampicillin	*Staphylococcus aureus*(0.53) , MRSA (0.53)	Buds	Methanol	*Myrtaceae*		[50]
	Tetracycline	*Staphylococcus aureus*(0.50) , MRSA (0.49), *Pseudomonas aeuroginosa* (0.52)					
Tribulus ris	Gentamicin	*Escherichia coli* ATCC 8739 (0.1375)	Aerial parts	Methanol	*Zygophyllaceae*	Steroidal	[51]

The third highest synergy was exhibited by *Tribulus terrestris*, from the *Zygophyllaceae* family which predominantly contains steroidal saponins [52]. The antibacterial activity of T*ribulus terrestris* against both *E. coli* and *S. aureus* has been reported by various authors[53][54][55]. According to Al-Bayti, ethanol extracts seem to have a greater antimicrobial effect than aqueous extracts and their MIC ranged from 0.62 mg/mL to 5 mg/mL [56]. When testing the extract of *Tribuls terrestris* against E. coli along with gentamicin, the combination was highly synergistic with an FIC Index of 0.14.*Glycine max* saponins showed the fourth-highest synergy with beta-lactam antibiotics. The saponin content of*Glycine max* is 0.22–0.49% and the highest activity is against S. aureus similar to the synergy studies by [49][50][51][52][53][54][55][56][57]. When tested against *S. aureus* by combining with ampicillin, benzylpenicillin, and piperacillin and they showed an FIC index of 0.23, 0.22 and 0.23 respectively (Table 1). The antibacterial activity of *Glycine max* against *S. aureus* has also been reported in other literature [58][59][60]. Inhibition of the efflux pump responsible for resistance has been proposed as the mechanism of action of saponins from the *Fabaceia* family [6]. For *E. coli* the FICI values for tetracylcine and rifampicin were similarly reported from *Melanthera elliptica* and *Jatropha curas* (FICI values of 0.07 and 0.08, respectively). Finally, the fifth-highest synergy was reported for saponin extracts of *Salvia officinials* (*Lamiaceae* family). *Salvia officinalis* saponins are triterpenoid and have previously been reported to possess activity against *E. coli, S. aureus, and L. monocytogenes* [61]. The acetone extracts of *Salvia officinalis* showed high synergy with amoxicillin against *S. aureus and E. coli* (Figure 1 and Table 1). Amoxicillin + *Salvia officinalis* against *S. aures* showed higher synergy (0.35) than when tested against *E. coli* (0.38) (Table 1).

Conclusions

The antibacterial, antiviral, antiparasitic, and antifungal activities of saponins are well documented. Although the antibacterial activity of most phytochemicals is deemed to be weak, their synergistic effect when used in combination with conventional antibacterial can enable them to play an important role in the fight against antimicrobial resistance. As evident from their FIC index, saponins isolated from the plants *Jatropha curcas, Melanthera elliptica, Glycine max, Tribulus terrestris, Salvia officinialis, Spergulara marginata, Paromychia argenetea,* and *Syzigium aromaticum* have a synergistic effect when combined with a variety of conventional antibiotics. These plants are a good source of antibacterial phytochemicals particularly against *S. aures* and *E. coli* when used in combination with tetracycline, rifampicin, gentamicin, amoxicillin, ampicillin, piperacillin, cefexime, and benzylpenicillin. More studies are warranted to elucidate the exact mechanism of action of these saponins.

List of abbreviations

AMR: Antimicrobial resistance,

CDC: Center for Disease Control,

MDR: Multidrug resistant,

FICI: Fractional Inhibitory Concentration Index,

CBA: Checkerboard assay

Declarations

Ethical Approval

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received for this work.

Author's contributions

NDA conceived the idea. NDA, SGM, NA conducted the review and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Not applicable

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