VOLUME - 12, ISSUE - 05, MAY - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Clinical Microbiology

IN-VITRO ANTI-MICROBIAL AND ANTIOXIDANT PROPERTIES: SAPONINS FRACTION OF SAPINDUS MUKOROSSI AGAINST PREVALENT PATHOGENS

Dr. Srinivasa Rao Meesala*	Department of Agriculture and Biotechnology, K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115 *Corresponding Author
Sanjeev Kumar	Department of Entomology, K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115
Rahul Kumar Gupta	Department of Horticulture (Vegetable & Floriculture), K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115
Nikhil Kumar	Department of Soil Science and Agricultural Chemistry, K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115
Dr. Priya Bhargava	Department of Plant Pathology K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115
Supriya Kumari	Department of Soil Science and Agricultural Chemistry, K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115
Rupam Bharti	Department of Horticulture, K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115
Sanjeev Kumar	Department of Plant Breeding & Genetics, K.K. University, Nepura, Bihar Sharif, Nalanda, Bihar-803115

ABSTRACT Developing new antibiotics, it is becoming increasingly difficult, with greater time-consuming and more expensive to develop new antibiotics, especially because emerging strains are becoming increasingly resistant to existing antibiotics. Phytochemically, saponins are plant secondary metabolites with powerful therapeutic effects and there are several scientific evidences that plant saponins are potent pharmacological agents. In this study, Effect of Saponins derived from the leaves of *Sapindus mukorossi* on prevalent bacterial pathogens growth and efflux-pump activity were tested on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. An antibiotic standard ampicillin was comparable to the inhibitory effects of saponins at 1.80 mg/mL of concentration on bacterial growth. The saponins from *Sapindus mukorossi* had showed a MIC of 0.0036 mg/mL and an MBC of 0.96 mg/mL, making them the most effective against *S. aureus* and *P. aeruginosa*. It was proven that plant saponins had bacteriostatic effects on *P. aeruginosa* and *S. aureus*. *Sapindus mukorossi* saponins inhibited the drug efflux pump susceptible in *P. aeruginosa*, which resulted in a 132% increase in Rhodamine-6G accumulations compared to the control. As a result, Leaf saponins from *Sapindus mukorossi* exhibited antibacterial activity as well as the ability to inhibit the compounds energy dependent transport across cell membranes, and these saponins can be used as potential principal composites for the development of herbal based anti-pathogenic bacterial agents and their adjunct composites.

KEYWORDS : *Sapindus mukorossi*, CFU-Colony forming unit, MIC-Minimum inhibitory concentration, MBC-Minimum bactericidal concentration.

INTRODUCTION

One of main factors contributing to causes of communicable diseases in India is prokaryotic microbial infections. On worldwide, immune compromised convalescents in the emerging nations are dying from life-threatening illnesses caused by pathogenic bacterial species. A concerted effort to fight infectious diseases is made more challenging by the fact that bacteria are continually acquiring resistance to a wide variety of medicines, despite their availability. Since from the advent of antimicrobials/antibiotics were used to treat bacterial illnesses, bacteria have retorted by developing a variety of resistance mechanisms. Treatment failures may be caused by the level and complexity of bacterial infectious resistance mechanisms, which are evolving with time and becoming more sophisticated.

Gram-positive and Gram-negative microorganisms that are characteristically comprehended in community and hospital acquired infections, respectively; *Staphylococcus aureus* and *Pseudomonas aeruginosa* [1]. More critically, *P. aeruginosa* and *S. aureus infections* are potentially fatal because the exotoxins and endotoxins they release continue to induce inflammation and injury even after the bacteremia has been treated with antibiotics [2].

The global escalation in each community and hospital acquired antimicrobial microbes is intimidating the potent remedy of patients, underscoring the necessary for sustained reconnaissance, prudent disease control, and innovative treatment options.[3]. New antimicrobials are required to control the increasing number of antibiotic-resistant infections, yet resistance development is inevitable because it is a fundamental key element of microbial evolution [4].

Hence, there is an exigent necessity for progression of novel unique anti- bacterial agents that targets and combat bacterial resistance mechanisms. Herbal remedy use, whether in traditional medicine or complementary and alternative medicine (CAM) practices, is common in Asia with as claimed to have slight undesirable side effects [5]. Moreover, with concerns of escalating cost of drug discovery, globally majority of plants have proven to be some of the greatest cost-effective and cheap alternative sources of medicine [6].

The *Sapindus mukorossi* belongs to Malvaceae family, which is widely available in all parts of India's tropical and subtropical regions. Conventionally It is well known that plant has medicinal potentials. The *S. mukorossi* commonly known as kanghai in Hindi. The plant has historically been used as

an anti-inflammatory, anthelmintic, and is useful in the treatment for jaundice, ulcers, leprosy, piles, and lumbago [7]. The leaves of *S. mukorossi* are used to treat liver diseases, toothaches, lumbago, piles, and anti-fertility [8]. Root and bark are used as diuretics, Nervine tonics, aphrodisiacs, and anti-diabetics [9]. Higher plants may be a source of novel antibiotic prototypes, as evidenced by the antibacterial activity of plant extracts and their derivatives [10]. The major goal was to assess the efficacy of *S. mukorossi* against *S. aureus* and *P. aeruginosa*.

MATERIALS AND METHODS

Microorganisms and Plant Provenance

The plant material was collected from different regions of India and was authenticated by a taxonomist at the Botanical Department of K.K. University Nalanda, India (Voucher. No: K.K.U-4026). The test microorganisms were obtained from Department of Microbiology, K.K. Medical College, Nalanda, Bihar, India.

Extraction, Isolation and standardization of the *Sapindus mukorossi* saponins

One kilogram (1 kg) of dried coarsely powdered fruits of *S. mukorossi* was extracted with cold ethanol (70%) by maceration. Dried coarsely powdered drug has been taken in a closed container with 70% ethanol and permitted to stand at room temperature 35°C for 7-days with frequent agitation till the soluble material has been dissolved. On 8th day, combined liquid was decanted and clarified by filtration and concentrated by removing solvent under reduced pressure and temperature. The residue was freeze-dried and stored at -10°C in airtight container. The crude ethanolic extract of S. mukorossi was resuspended in H_2O and chloroform in HCl (50%v/v) was added to perform acidic hydrolysis of sapogenin to isolate saponins. Chloroform phase was separated and concentrated up to 1/3rd of its original volume at 40°C. Concentrated chloroform phase was extracted 3-4 times with water-saturated n-butanol and solvent was removed under reduced pressure. Dried brown colored powder with 2.41% of yield, represented as crude saponin mixture and was named as SMSF (*S. mukorossi* saponin fraction).

The isolated saponin fraction was standardized qualitatively by using Thin Layer Chromatography (TLC) profile with known markers. All standard markers (sapindoside-A, B, C & E and M, saponin E, Y) procured from Avra Synthesis Private Limited, India.

Microbial Susceptibility Test:

S. mukorossi saponins extraction, prepared at a concentration of 25 mg/ml, and was distinctly added to 96-well plates in a volume of 20 μl in order to pervade the tested cell cultures with 500 μg each 1.67 mg /ml extract in every well. *P. aeruginosa and S. aureus* at 1×10^6 cfu/ml and wells of microtiter plate were diluted with broth made total volume of 300 μL per well. A progressive (+ve) control containing ampicillin was also prepared, with 500 μg added to each well containing bacterial culture medium and broth. Appropriate destructive (-ve) control containing medium only/medium with extract were also prepared. Absorbance measurements at 600nm were determined before incubation using a microplate reader. Post-incubation in a Lab Companion incubator for 24 hours at 37°C cell density was determined.

Determination of MIC and MBCs:

MICs and MBCs of S. mukorossi's saponin extracts were measured. In order to create 10 different diluted concentrations, the saponins extracts were serially diluted two-fold in a 96-well polystyrene microplate from 1.67 mg/ml to 0.0032 mg/ml. 20μl of each dilution were successively transferred in triplicate into the wells of a separate 96-well microplate. In each species microplate wells,100 μl of *P. aeruginosa* and *S. aureus* broth cultures 1×106 were added. Then, in each well 180 μl of broth was added to built a total volume of 300 μl. As a positive control, rows of wells with media containing 100 μl of cell cultures as well as extract-only and extract-free control were employed while broth medium without extract was used as negative control for each well. Using a microplate reader, preincubation absorbance values were recorded. After24-hour incubation at 37°C in a Lab-Companion incubator, absorbance values were read and recorded. The MIC values in the well with the least noticeable purple coloration in each test row were found using chemical 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide The experiment was carried out three times.

In vitro antioxidant properties of *S. mukorossi*

The DPPH radical assay is a suitable model for estimating the total antioxidant potential of antioxidants (Huang *et al*., 2005). The assay was carried out in 96 well microtitre plate. In 200μl of DPPH solution, 10μl of each of the test sample or the rutin standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions were 1000, 500, 250, 125, 62.5, 31.25, 15.625 μg/ml, respectively. The plates were incubated at 37°C for 30 min and the absorbance of each solution was measured at 517 nm, using a microplate reader.

Rhodamine 6G: Drug Efflux and Accumulation

In an incubator, *P. aeruginosa* and *S. aureus* were incubated in separate culture flasks for an overnight at 37°C with proper agitation at 120rpm. Subsequently, the bacterial cultures were transferred into centrifuge tubes (50 mL), centrifuged for 10min at 3000 rpm and the supernatents were discarded.

The pellet was re-suspended in the buffer after being washed twice with phosphate buffered saline. The revived cells were poured into centrifuge tubes that had been pre weighed and spun over again for 5min at 4,000rpm. After decanting the supernatents, the cells were again was hed in PBS, centrifuged once more for five minutes at 4,000rpm. The pellet was weighed and the supernatant was decanted. *P. aeruginosa* and *S. aureus* pellets were suspended in PBS containing 10 mM NaN_3 at a concentration of 40 mg/mL. In a Lab Companion Incubator, pellets were stirred and at a speed of 90 rpm and agitation by adding a final concentration of 10M of rhodamine-6G immediately. After that, with pellets were divided into two centrifuge tubes i.e., A and B for each species in the ratio of 1:3.

The tubes were spun for 5 min at 4000 rpm in a Centromix-BLT. Cells from tube A of the each bacterial species were resuspended in PBS, unaided at a concentration of 40 mg/mL after the supernatant was discarded. Cells from tube B of the two species were re-suspended in PBS containing 1M glucose. The cells from tube B of both bacteria were separated into two 5 mL quantities and placed in six distinct falcon tubes, two of which were used for glucose alone, twofor reserpine + glucose and two for saponins derived from the leaves of *S. mukorossi* extracts + glucose, and cells from tube A.

This was carried out for each of the *S. aureus* and *P. aeruginosa* pellets. The Saponins were added to their respective tubes at an absolute concentration of 61μg /mL, and reserpine was added to tubes labelled reserpine + glucose at an absolute concentration of 61μg/mL. The tubes were shaken on a Vortex mixer for proper mixing and put in a shaking incubator at 37°C with proper agitation at 90 rpm for 30 minutes.

To determine how much R6G was pumped out of the cell after 30 minutes, tubes were spun in a centrifuge-5425 (Sigmaaldrich) for 10 minutes at 4000 rpm. The supernatant was then collected to quantitate the amount R6G pumped. Each tube's pellet was lysed by being reconstituted in 5 mL of 3M glycine pH 3.

The tubes were shaken together on a vortex mixer before being incubated at 37°C for overnight. Subsequently, spun at 4000 rpm for 10 minutes and the amount of R6G that had accumulated in the cells was measured in the supernatant. After evaluating the absorbance in 96-well microplates at 527 nm using a microplate reader, R6G was calculated from the samples using a standard R6G calibration curve.

Statistical Analysis:

Graph Pad Instant software was used for data analysis. ANOVA with Dunnett's post test was used to establish levels of significance when all columns of treatments were compared to the control. All data were presented in the form of mean standard deviation. $P \leq 0.05$ or less was regarded to be statistically significant.

RESULTS

Microbial Susceptibility Test:

The effects of *S. mukorossi* saponins extract on bacterial species were assessed by measuring absorbance values at 600nm before and after incubation. *S. mukorossi* saponins extract significantly reduced bacterial growth at an initial screening concentration of 1.80 mg/mL (P=0.001, Figure 1). The mean absorbance for the cells exposed to the *S. mukorossi* saponins extract had 0.028 AU, making it the most effective at slowing the growth of *P. aeruginosa*. As the recorded mean absorbance was 0.0062 AU, ampicillin as expected nearly killed all the cells in the positive controls. In comparison to *S. aureus, P. aeruginosa* was generally more resistant to the antibacterial effects of the saponins and ampicillin.

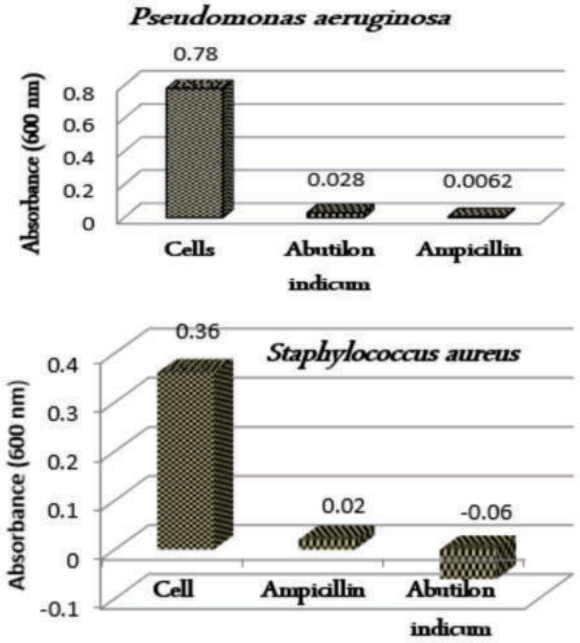


Figure. no.1: The effect of saponins extract of the *S. mukorossi*, ampicillin on growth of *S. aureus* & *P. aeruginosa*. Concentrations of 1.80 mg/mL & 1×10^{6} cfu/mL of the extract and bacteria, Values are expressed as mean absorbance at 600nm wavelength.

Determination of MIC and MBCs:

The MIC was determined for the well with least discernible MTT color. The *Sapindus mukorossi* Saponins extract was most effective, with MIC as low as 0.0036 mg/mL against *S.aureus* and 0.32 mg/mL against *P.aeruginosa*.

The *S. mukorossi* saponins extract demonstrated bactericidal activity against *S. aureus* (Table.no1), with an MBC of 0.96 mg/mL, whereas ampicillin had an MBC of 0.008mg/mL [25]. Saponins extract of the *S. mukorossi* were bacteriostatic against both bacterial species.

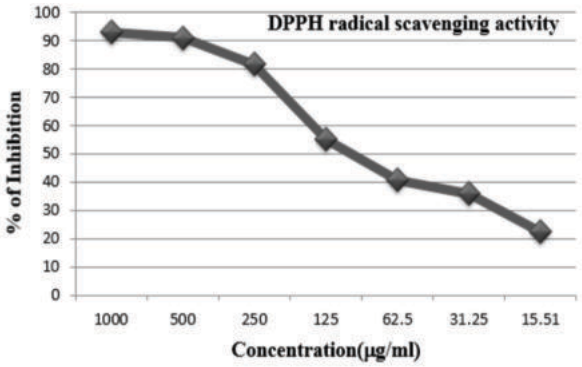
Table-1: Determination of Minimum inhibitory concentration and Minimum bactericidal concentration of *S. mukorossi* against *S. aureus* and *P. seudomonas aeruginosa*.

Table.no1: Assay of MIC and MBC of *S. mukorossi* against *S. aureus* and *P. aeruginosa*.

Name of Micro-Organism	G+ve/G-ve	Extract	MBC (mg/mL)	MIC (mg/mL)
Pseudomonas aeruginosa	Gram-ve	Sapindus mukorossi	-	0.32
Staphylococcus aureus	Gram+ve	Sapindus mukorossi	0.96	0.0036

In vitro antioxidant activities of *S. mukorossi*

DPPH radical scavenging activity has been widely used to evaluate the antioxidant activity of plant extracts and foods. The presence of antioxidant in the sample extract react with DPPH, which is a stable free radical, and convert it to 1,1 diphenyl 2 (2,4,6 trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds which can be detected spectrophotometrically at 517nm. Figure no 2 shows the DPPH radicals scavenging capacity of extracts *S. mukorossi* with reference to rutin. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC50) under the experimental condition was calculated. Therefore, lower value indicates a higher antioxidant activity. The experimental data indicated that extracts of *S. mukorossi* displayed the highest DPPH scavenging effect 96%.



Sample	DPPH
Extract	Sapindus mukorossi
	32.46±0.2
Standard	Rutin
	3.89±1.08

Figure. no.2: DPPH radicals scavenging capacity of extracts *S. mukorossi* with reference to rutin.

Rhodamine-6G: Drug Efflux and Accumulation

Fluorescent dye Rhodamine-6G, dynamically pumped out by the ATP-dependent efflux pumps of both species in this investigation, was used to measure the effects of the extracts on drug accumulation [26].

With a 132% of glucose increase above only in control, *S. mukorossi* saponins extract showed the highest accumulation of Rhodamine-6G against *P. aeruginosa* Due to a 116% increase in accumulation, *S. aureus* was found to be less vulnerable to efflux pump inhibition. *S. mukorossi* saponins extract blocked efflux pumps to a greater extent. The conventional inhibitor reserpine's efficacy was more perceptible against *P.aeruginosa* than against *S. aureus*, which is a notable finding because it showed that *P. aeruginosa* was more vulnerable to efflux pump inhibition than *S. aureus* (Figure.no.3 and Table.no.2).

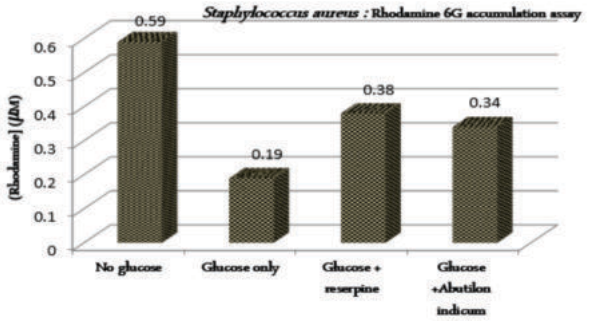


Figure. no.3: The effect of saponins extract of the *S. mukorossi* on accumulation of Rhodamine-6G over time and ATP-dependent efflux pumps.

Table no. 2: Determination of the R-6G concentration that accumulated in the bacterial cells after exposure to saponins extract of the *S. mukorossi*.

S.No	Name of Micro-Organism	Glucose	Reserpine	Sapindus mukorossi
i	Pseudomonas aeruginosa	0.19 ± 1.36	0.64 ± 1.09 (268%)	0.42 ± 2.28 (132%)
ii	Staphylococcus aureus	0.19 ± 0.42	0.45 ± 1.21 (140%)	0.40 ± 1.56 (116%)

DISCUSSION

The intrinsic potential of pathogens to generate and adopt antibiotic resistance mechanisms necessitates the search for antimicrobial substances for the benefit of humans. The emphasis is gradually turning to plant-derived phyto chemicals with therapeutic value as a result of potentially harmful side effects connected with the use of new chemical entities created artificially and the unacceptably high costs of drug development [11]. An innovative strategy for combating antibiotic resistance is the co-formulation of naturally derived antimicrobial adjuvants, such as efflux pump inhibitors, with both old and new generation antibiotics remains a novel antimicrobial resistance mechanism. Antibacterial susceptibility tests, minimum inhibitory concentration (MIC) analyses, and minimum bactericidal concentration (MBC) determinations were used to investigate the effects of saponins extract of *S. mukorossi*. The *S. mukorossi* extract exhibited strong antibacterial activity, as conforming according to the results of antibacterial susceptibility tests.

This study's findings revealed that *S. aureus* was more sensitive to saponins extract than *P. aeruginosa*, which was consistent with earlier research that indicated Gram^{+ve} strains are more vulnerable to harmful xenobiotics than Gram^{ve} microorganisms [12]. In this regard, the saponins extract showed efficacy against Gram^{+ve} and Gram^{-ve} bacteria, implying broad range activity.

Saponins from *S. mukorossi* were more potent against *S. aureus* which had a 0.0036mg/mL value of MIC and 0.96 mg/mL of MBC, according to the MIC and MBC assay. The The MBC of common conventional antibiotics was 0.008mg/mL [13], which means that activity of the saponins is much outweighed by ampicillin. Saponins from *S. smukorossi* exhibited bactericidal activity only against *S. aureus*. All plant saponins extracts may have a bacteriostatic impact on *P. aeruginosa* because of the pathogen's thick, highly hydrophobic outer membrane, which may have a permeability barrier to extract [14].

The major primary mechanisms for stopping the accretion of an effective concentration of antibiotics at molecular target locations within the bacterial cell is the use of ATP-dependent efflux pumps. Mex(XY)-Opr(M) or Mex(CD)-Opr(J) are the main efflux pump systems investigated in Gram^{-ve} organism like *P. aeruginosa* and have been linked to acquire multidrug resistance [15]. MFS transporters Qac-A, Qac-B, Nor-A, and Nor-B have been discovered in strains of Gram^{+ve} bacteria like *S. aureus* that cause hospital-acquired infections and confer resistance to puromycin and fluoroquinolones [16]. One feasible strategy that can be used to successfully battle the effects of resistance is to inhibit efflux pumps.

Additionally, it was discovered that inhibiting efflux pumps was also reported to reduce the MICs for bacteria that were both susceptible and resistant to antibiotics, as well as reversed acquired resistance in certain strains of *P. aeruginosa* [17]. Rhodamine-6G could accumulate in both as a result of plants' saponins extract able to cause accumulation. Hence, saponins from *S. mukorossi* were powerful inhibitor and increases in Rhodamine-6G accumulation in S. aureus and P. aeruginosa by 116% and 132%, respectively. According to the findings of this investigation, cells exposed to *S. mukorossi* induced the greatest accumulation of R6G in *P. aeruginosa*. As a result, less sensitive to growth suppression compare to *P. aeruginosa* was more responsive to phytoconstituent induced efflux pump inhibition than Gram^{+ve} bacteria. The study detection that was made when crude extracts showed a strong suppression of Rhodamine-6G drug accumulation in *P. aeruginosa* cells by 64 –100% [18].

It is significant to recognize the substances that prevent bacteria from extruding medications, as these substances could serve as sources of adjuvants in formulations that combine them with traditional antibiotics [19]. An effective co-formulation of amoxicillin, a traditional beta-lactam antibiotic, and clavulanic acid, a penicillinase inhibitor, in the coamoxiclav generic serves as a clinical illustration. In vitro studies have showed considerable synergistic effects when an MDR pump inhibitor is used with combinations of antimicrobial phyto-constituents and conventional antibiotics [20]. The efficiency of *S. mukorossi* extract for in vitro antibacterial activity has already been established by prior studies [21-23].

It is critical to identify the substances that prevent drug efflux from bacteria because they are potential sources of adjuvants in co-formulated antibiotics [24]. In the coamoxiclav generic, clavulanic acid, a penicillinase inhibitor, was successfully coformulated with amoxicillin, an old traditional beta-lactam antibiotic. In vitro studies of the synergistic effects of MDR pump inhibitors combination with other antimicrobial phytoconstituents as well as conventional antibiotics have yielded remarkable findings [8]. Previous research has demonstrated the efficacy of *S. mukorossi* extract for in vitro antibacterial activity [25-27].

In conclusion, *S. aureus* and *P. aeruginosa* have exhibited potent antibacterial efficacy when exposed to the saponins extract from *S. mukorossi*. The saponins extract from *S. mukorossi* exhibited more growth-inhibitory action. However, compared to ampicillin, the extract's activity was much, much lower. The action of drug efflux pump was more susceptible in P. areuginosa by the treatment with *S. mukorossi* saponins, which also induced significant accumulation in *S. aureus*. For the reason that *P. aeruginosa* has been shown to be particularly sensitive to efflux pump inhibition, pharmaceutical formulations containing EPI activity may be more effective than expected against this particular bacterial species. To identify the precise compounds that have antibacterial activity, more research must be done.

REFERENCES

1. M. Stavri, L. J. V. Piddock, and S. Gibbons, "Bacterial efflux pump inhibitors fromnatural sources," *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 6, pp. 1247–1260, 2007.
2. R. T. Irvin, "Attachment and colonization of *Pseudomonas aeruginosa*: role of the surface structures," in *Pseudomonas aeruginosa As an Opportunistic Pathogen*, InfectiousAgents and Pathogenesis, pp. 19–42, Springer, New York,

NY, USA, 1993.
3. Okwu DE. Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J. Pure Appl. Sci. 7: 455-459, 2001.
4. M. Simoes, L. C. Simoes, and M. Lemos, "Phytochemicals against drug-resistant microbes," in *Dietary Phytochemicals and Microbes*, pp. 185–205, Springer, Berlin, Germany, 2012.
5. J. H. Doughari, "Antimicrobial Activity of *Tamarindus indica* Linn," *Tropical Journal of Pharmaceutical Research*, vol. 5, no. 2, pp. 597–603, 2007.
6. T. Ibrahim, L.Ajala, F.Adetuyi, and B. Jude-Ojei, "Assessment of the antibacterial activity of *Vernonia amygdalina* and *Occimum gratissimum* leaves on selected food borne pathogens," *The Internet Journal of Third WorldMedicine*, vol. 8, no. 2, pp. 1–3, 2009.
7. H. Nikaido, "Multidrug resistance in bacteria," *Annual Review of Biochemistry*, vol. 78, pp. 119–146, 2009.
8. Arora, S., Singh, D. P., Saini, A., and Kumar, A. Pharmacognostic investigation on roots & leaves extract of *Sapindus mukorossi linn*. International Journal of Research and Development in Pharmacy and Life Sciences, Vol. 2, No. 5, pp 567-573, 2013.
9. J. H. Doughari, "Antimicrobial Activity of *Tamarindus indica* Linn," *Tropical Journal of Pharmaceutical Research*, vol. 5, no. 2, pp. 597–603, 2007.
10. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A. Antimicrobial activity of saponins extracted from bergamot (Citrus bergamia Risso) peel, a byproduct of the essential oil industry. J. of App. Microb., 103(6): 2056-2064, 2007.
11. E. N. Scott, A. J. Gescher, W. P. Steward, and K. Brown, "Development of dietary phytochemical chemo preventive agents: biomarkers and choice of dose for early clinical trials," *Cancer Prevention Research*, vol. 2, no. 6, pp. 525–530, 2009.
12. Baharaminejad S, Asenstorfer RE, Riley IT, Schultz CJ. Analysis of the antimicrobial activity of saponins and saponins isolated from the shoots of Oats (Avena sativa L.). J. of Phytopathology, 156(1): 1–7, 2007.
13. T. A.Chitemerere and S.Mukanganyama, "*In-vitro* antibacterial activity of selected medicinal plants from Zimbabwe," *African Journal of Plant Science and Biotechnology*, vol. 5, pp. 1–7, 2011.
14. Sofowara, A..Screening plants for bioactive agents. In: medicinal plants and traditional medicine in Africa. (2nd edn.)Spectrum books Ltd. Sunshine house, Ibadan; Nigeria, pp 81-93,1993.
15. H. P. Schweizer, "Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions," *Genetics and Molecular Research*, vol. 2, no. 1, pp. 48–62, 2003.
16. H. Nikaido, "Multidrug resistance in bacteria," *Annual Review of Biochemistry*, vol. 78, pp. 119–146, 2009.
17. O. Lomovskaya, A. Lee, K. Hoshino et al., "Use of a genetic approach to evaluate the consequences of inhibition of efflux pumps in *Pseudomonas aeruginosa*," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 6, pp. 1340–1346, 1999.
18. T. A.Chitemerere and S.Mukanganyama, "*In-vitro* antibacterial activity of selected medicinal plants from Zimbabwe," *African Journal of Plant Science and Biotechnology*, vol. 5, pp. 1–7, 2011.
19. Y. C. Fiamegos, P. L. Kastritis, V. Exarchou et al., "Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from *Artemisia absinthium* against gram-positive pathogenic bacteria," *PLoS ONE*, vol. 6, no. 4,Article ID e18127, 2011.
20. M. Stavri, L. J. V. Piddock, and S. Gibbons, "Bacterial efflux pump inhibitors fromnatural sources," *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 6, pp. 1247–1260, 2007.
21. S. M. Seyydnejad, M. Niknejad, I. Darabpoor, and H. Motamedi, "Antibacterial activity of hydroalcoholic extract of *Callistemon citrinus* and *Albizia lebbeck*," *American Journal of Applied Sciences*, vol. 7, no. 1, pp. 13–16, 2010.
22. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol. 36: 493–496, 1966.
23. P. R. Shinde, P. S. Patil, and V. A. Bairagi, "Pharmacognostic, phytochemical properties and antibacterial activity of *Callistemon citrinus* viminalis leaves and stems," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 4, no. 4, pp. 406– 408, 2012.
24. Y. C. Fiamegos, P. L. Kastritis, V. Exarchou et al., "Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from *Artemisia absinthium* against gram-positive pathogenic bacteria," *PLoS ONE*, vol. 6, no. 4, Article ID e18127, 2011.
25. Parekh, J., Darshana, J. and Sumitra, C., 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Journal of Biology, 29: 203-210.
26. K. V. V. S. Krishna, G. Surendra, M. Anjana, and K. S. K. Nagini, "Phytochemical screening and antimicrobial activity of *Callistemon citrinus* (L.) leaves extracts," *International Journal of Pharmaceutical and Technology Research*, vol. 4, no. 2, pp. 700– 704, 2012.
27. P. R. Shinde, P. S. Patil, and V. A. Bairagi, "Pharmacognostic, phytochemical properties and antibacterial activity of *Callistemon citrinus* viminalis leaves and stems," *International Journal*