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Research Article

MODULATION ACTIVITY OF *SIDA CORDIFOLIA* L. AND *SIDA RHOMBIFOLIA* L. IN *STAPHYLOCOCCUS AUREUS* SA-1199 B

Pallavi Ahirrao [1,2]*, Rushikesh Tambat [3], Anjoo Kamboj [2], Upendra K Jain [2], Hemraj S Nandanwar [3]
[1] Research scholar of IK Gujral Punjab Technical University, Kapurthala; Department of Pharmaceutical Chemistry, Chandigarh College of Pharmacy, CGC Landran, Mohali, Punjab, India
[2] Department of Pharmaceutical Chemistry, Chandigarh College of Pharmacy, CGC Landran, Mohali, Punjab, India
[3] CSIR-Institute of Microbial Technology, Sector-39 A, Chandigarh, India
*Corresponding Author Email: pallavi.ccp@cgc.edu.in

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for several infections in humans. The infections caused by this bacterial strain are difficult to treat due to resistance of MRSA to clinically used antibiotics. Over past two decades medicinal plant extracts and their phytoconstituents have been reported to possess modulation and efflux pump inhibitory (EPI) activity against MRSA strains. *Sida cordifolia* aerial parts, roots and *Sida rhombifolia* aerial parts have been reported to be used in Ayurveda for several ailments including bacterial/fungal infections. Based on this information and in continuation of our efforts to discover EPIs from Indian medicinal plants, we describe modulation activity of methanol extracts of aerial parts and roots of *S. cordifolia* and *S. rhombifolia* and alkaloid-rich fraction prepared from methanol extracts. Methanol extract of *S. cordifolia* and *S. rhombifolia* aerial parts showed 4-fold reduction each in MIC of norfloxacin at 125 µg/mL concentration whereas alkaloid-rich fraction of both the plants did not show any reduction in MIC of norfloxacin. Fractionation of bioactive MeOH extract of *S. cordifolia* aerial resulted in isolation and characterization of 8 compounds including 2 alkaloids, 5 flavonoids and 1 coumarin compound whereas LC-MS analysis of alkaloid-rich fraction of *S. rhombifolia* aerial parts revealed presence of alkaloids including cryptolepine and 11-methoxyquindoline. Although the methanol extracts of aerial parts of *S. cordifolia* and *S. rhombifolia* showed weak modulatory activity in NorA over expressed *S. aureus* 1199B strain, the present study helped to assess EPI activity potential of these plants.

Keywords: MRSA, *Sida cordifolia*, *Sida rhombifolia*, modulation, efflux pump inhibition

INTRODUCTION

The multidrug efflux pumps are responsible for self defence mechanism to bacteria by eliminating antibiotics from the cell. At present, efflux pumps are the major antibacterial target. Therefore, there is an urgent need to develop and identify potent efflux pump inhibitors[1]. The combination of an efflux pump inhibitor (EPI) and the antibiotic is the strategy which prevents efflux of antibiotic and recovers its intracellular concentration. Alternatively, these compounds can act as regulators of efflux systems, namely as efflux pump inhibitors (EPI) to block the activity of drug efflux pumps[2]. Thus, EPIs help in restoring the susceptibility of resistant strains to antibacterial agents[3]. *Staphylococcus aureus* is a major pathogen in both hospital and the community, responsible for a wide range of infections from uncomplicated skin and soft tissue infections to more serious illnesses like pneumonia, endocarditis, and sepsis[4]. Methicillin-resistant *S. aureus* (MRSA) strains are of particular concern among MDR microorganism and are responsible for both community and hospital acquired infections[5]. In *S. aureus,* NorA is predominant efflux protein and belongs to major facilitator superfamily. NorA – over expressed strains have been shown to be the most common among MRSA strains[6]. However, NorA efflux pump provides resistance or less susceptibility to quinolones, quaternary ammonium compounds, reserpine, verapamil and the dyes ethidium bromide (EtBr), rhodamine and acridines. Verapamil and reserpine are commonly used as positive controls in efflux pump inhibitory assays but these are not clinically relevant due to neurotoxicity.[7]

The *Sida* species belonging to Malvaceae, is one of the most important Genera of medicinal plants in India. *Sida cordifolia* L. (Malvaceae) is an herbaceous plant that grows to a height of 3–5 ft and is used as a common herbal drug in the Indian subcontinent. The roots, leaves, stem, and seeds of *S. cordifolia* are used in Ayurveda against chronic dysentery, asthma, and gonorrhoea and it is popularly known as Bala *S. cordifolia* showed anti-inflammatory, anti-cancer, antibacterial activities and its aqueous extract has been investigated for liver regeneration activity.[8,9] *S. cordifolia* is a part of important Ayurvedic formulation such as Balarishta, Balataila, Balacurna, Baladyaghrita, Baladyakvatha that are described in Ayurvedic Formulary of India.[10] An aqueous extract of the leaves is reported to exhibit analgesic and anti-inflammatory activity in animal models.[11] *S. cordifolia* showed the presence of ephedrine, pyrroloquinazoline alkaloids, e.g. vasicine, vasicinol, vasicinone, along with N-methyltryptophan, β phenthylamine.[12,13] Further phytochemical investigation revealed presence of flavonoids such as 5,7-dihydroxy-3-isoprenyl flavone, 5-hydroxy-3-isoprenyl flavone and flavonol-C-glycosides from the whole plant[14] and ecdysteroid glycosides.[15]

Sida rhombifolia L. is a perennial herbaceous plant native to the tropic and sub tropic areas. The stems are erect to sprawling and branched growing 50-120 cm in height, with a woody lower section. The leaves are green; diamond-shaped and are arranged

alternately along the stem, 4-8 cm long. In India, infusion of leaves of *S. rhombifolia* showed diuretic and aphrodisiac effects. It is also used for the treatment of tuberculosis, skin, urogenital diseases and also as food. The root and stems of the plant are useful for the treatment of fever, heart disease, piles and inflammation.[16] Various studies reported that *S. rhombifolia* showed cytotoxic, anti-bacterial, anti-inflammatory, antipyretic and antinociceptive activities.[17-19] Phytochemical investigation revealed presence of alkaloids, flavonoids and phenolic compounds in aerial parts of *S. rhombifolia*.[20] Recently four alkaloids viz. quindoline, quindolinone, 11-methoxyquindoline and cryptolepine salt were reported from aerial parts of *S. cordifolia* found in Brazil.[21]

So far there is no report on modulatory and efflux pump inhibitor (EPI) activity of *S. cordifolia* and *S. rhombifolia*. In our efforts to search for EPIs from natural products,methanol extracts of aerial parts and rootsof *S. cordifolia* and *S. Rhombifolia* and alkaloid-rich fraction prepared from methanol extracts were investigated for their modulatory and EPI activity on mutant strain, NorA over expressed strain of *S. aureus* (SA 1199B).

MATERIAL AND METHODS

Chemicals and solvents

All the organic solvents and reagents were purchased from Merck India. HPLC solvents were purchased from Merck, India. Standard antibiotics and EPIs such as norfloxacin, verapamil, reserpine were procured from Sigma-Aldrich, USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) and EtBr were also purchased from Sigma-Aldrich, USA. All the solvents and reagents used in the study were of LR grade.

Plant material

The aerial parts and roots of *Sida cordifolia* were collected from area adjoining Mansa Devi Complex, Panchkulaand voucher specimen (CCP/HB/KPH/08) was deposited in the Herbarium, Chandigarh College of Pharmacy, Landran, Punjab. Similarly the aerial parts and roots of *Sida rhombifolia* were collected from area adjoining Mansa Devi Complex, Panchkula and voucher specimen (CCP/HB/KPH/09) was deposited in the herbarium of the college.

Extraction and isolation

Aerial parts of *S. cordifolia*

Dried aerial parts (1 kg) of *S. cordifolia* were extracted with methanol using Soxhlet apparatus. The obtained methanol extract was concentrated to obtain 40 g of dried extract.

Preparation of alkaloid-rich fraction: 10 g of MeOH extract was suspended in water and acidified with tartaric acid to pH 5.0 and partitioned with ethyl acetate (EtOAc). The acidic aqueous extract was further treated with sodium carbonate solution to pH 11.0 and partitioned with EtOAc. The combined EtOAc layers obtained from basic aqueous extract was passed through anhydrous sodium sulphate and concentrated under vacuum to obtain 0.3 g of alkaloid-rich fraction.

Isolation of alkaloids: 0.3 g of alkaloid-rich fraction was subjected to column chromatography (CC) on basic alumina (#70-230, 50 g) and eluted gradient wise with increasing polarity of MeOH in chloroform (0-20 % MeOH). Fractions eluted with 1-5% MeOH showed presence of alkaloids. The combined fraction (0.35 g) upon repeated CC on basic alumina and sephadex LH-20 gave two compounds (1, 8 mg and 2, 12 mg).

Isolation of compounds from MeOH extract: 30 g of MeOH extract was suspended in water and partitioned with EtOAc (4 x 250 ml). The combined EtOAc layers were concentrated to yield 8.5 g of EtOAc fraction. This fraction was subjected to VLC using TLC grade silica gel (300 g). An elution was carried out in gradient manner starting with hexane to EtOAc and MeOH by 5% increment of polar solvents (0 – 100% EtOAc and 0 – 20% MeOH) to give five pooled fractions, A to E. Fraction C (1 g) was further subjected to CC on silica gel (# 230 – 400, 30 g) and eluted with 0 – 100% EtOAc in hexane to give four subfractions (C1 to C4) with 200, 150, 100 and 80 mg respectively. Fraction C2 on concentration gave precipitate that upon recrystallisation with EtOH gave pure compound 3 (10 mg). From fraction C3 two compounds 4 and 5 were identified co-TLC with reference standards available in the lab. Fraction D (2.5 g) was subjected to CC (silica gel, # 100-200, 200 g) and column was eluted gradient wise with 0-20% MeOH in chloroform to give five pooled fractions D1 to D5. Fraction D4 (0.8 g) was subjected to CC on (silica gel, # 100-200, 50 g) and eluted with 0 – 20% MeOH in chloroform to give six pooled fractions D_{4a} – D_{4f}. Fraction D_{4b} (150 mg) was subjected to repeated CC on sephadex LH-20 eluting with MeOH to obtain compound 6; similarly fraction D_{4d} (80 mg) upon repeated CC on sephadex LH-20 eluting with MeOH to obtain compound **7** (5 mg). Fraction D_{4c} (150 mg) was subjected to repeated CC on sephadex LH-20 eluting with MeOH to obtain compound 8 (15 mg).

Aerial parts of *S. rhombifolia*

Dried aerial parts (1 kg) of *S. rhombifolia* were extracted with methanol using Soxhlet apparatus. The obtained methanol extract was concentrated to obtain 50 g of dried extract.

Preparation of alkaloid-rich fraction: 20 g of MeOH extract was suspended in water and acidified with tartaric acid to pH 5.0 and partitioned with ethyl acetate (EtOAc). The acidic aqueous extract was further treated with sodium carbonate solution to pH 11.0 and partitioned with EtOAc. The combined EtOAc layers obtained from basic aqueous extract was passed through anhydrous sodium sulphate and concentrated under vacuum to obtain 0.8 g of alkaloid-rich fraction.

Preparation of Ethyl acetate fraction and n-butanol fraction from MeOH extract of *S. rhombifolia.*

MeOH extract (20 g) was suspended in water and partitioned with ethyl acetate and n-butanol until the clear organic layer was obtained. Obtained organic layer was concentrated on rotary evaporator to give 5.8 g and 6 g of EtOAc and n-butanol fractions, respectively.

LC-MS analysis

Waters 2767 Sample Manager, 2525 Binary Gradient Pump and coupled to a single quadrupole ZQ mass spectrometer (Micro mass 4000), operating in the ESI –ve modes.LC-ESI-MS analysis was carried out at room temperature. The mass spectra were acquired between 0.00-500.00 *m/z.* The MS parameters were set as: ESI in negative ion mode; sheath gas flow rate: 500 L/hr; aux gas flow rate: 80 L/hr; spray voltage: 5kV; capillary temperature: 250 °C; capillary voltage: 2 V.

Chromatography conditions: RP-18 Sunfire column (4.6 x 250 mm, 5 µ), gradient elution using Water (0.1% Acetic acid, A) and MeOH (B): 0 min 20% B, 7 min 40% B, 12 min 75% B, 18 min

95% B, 23 min 95% B, 25 min 80% B; flow rate- 1 ml/min., detection done at 254 and 366 nm (scanning range, 220-500 nm using photo-diode array detector).

Bacterial strains

In this study three well characterized *Staphylococcus* strains were used. *Staphylococcus aureus* 1199 (SA-1199) is a methicillin and fluoroquinolone susceptible clinical isolate. Fluoroquinolone-resistant mutant of SA-1199 strain is *S. aureus* 1199 B (SA-1199B) that was recovered from the blood and cardiac vegetations of rabbits that had experimental endocarditis with SA-1199.[22] SA-1199 B is also methicillin resistant strain of *S. aureus*.[23] Strains were grown in Mueller–Hinton growth medium II (MHB II; Oxoid, Hampshire, UK) with appropriate antibiotics where applicable. All the strains were obtained from CSIR-Indian Institute of Integrative Medicine, Jammu, India with kind permission of Dr G. W. Kaatz, Wayne State University School of Medicine, Detroit, MI, USA. Cells were stored at -80°C for further use.

Minimum Inhibitory Concentration (MIC) determination

MICs of the isolated flavonoid compounds, 1-4 were determined against *S. aureus* 1199 B using broth micro-dilution method. In brief, according to CLSI guidelines[24], bacterial strains were inoculated in 5 ml MHB independently and kept for incubation at 35 ºC for 6-8 hours (to reach OD_{600} = 0.13). Dilutions were done to make final inocula of 5×10^5 CFU/mL and two-fold serial dilutions of agents were done in 96-well non-treated polystyrene micro titre plates. The MIC of norfloxacin, standard EPI's and test compounds was determined in Ca^{2+} and Mg^{2+} adjusted MHB. Briefly, the compounds were first dissolved in DMSO and then diluted in MHB, to give the starting concentration of 1000 µg/mL which was further diluted across a 96 well micro titer plate in two fold serial dilution to give the final concentration range from 1000 to 0.97 µg/mL. The inoculum (100 µl) was added to all the wells and the micro titer plate was incubated at 37 °C for 24 hours. After incubation, 20 µl of MTT was added at the concentration 10 mg/mL in methanol to each well and incubated at 37 °C for 20 min where bacterial growth was indicated by purple coloration adhered to cells. MIC was interpreted as the lowest concentration that completely inhibited the visible growth of bacteria after 24 hours.[25] The bacterial viability was registered for each well based on the MTT color change. Here the number of viable cells is directly proportional to the amount of purple color generated and in the bottom of the wella precipitate of cells can be observed. Each agent was tested in triplicates, with three different sets of experiments for 48 hours. Wells that contained only DMSO, sterile saline and compounds in MHB served as negative control. Cells and controls were carried out in the same set of experiment.

Modulation assay using norfloxacin as the substrate

The modulatory activity of all the compounds on norfloxacin was studied by replacing EtBr with norfloxacin as the substrate. The rest of the protocol was followed as described above for EtBr modulation. Serially diluted concentration of norfloxacin for different *S. aureus* strains1199 was 0.39 to 0.003 µg/mL; for *S. aureus* 1199 B was 25 to 0.09 µg/mL and for *S. aureus* K1758 was 0.19 to 0.02 µg/mL for MIC determination with and without modulator. MF was used to express the modulating effects of compounds on MIC of norfloxacin. The MF was calculated by the formula:

$$MF = \frac{\text{MIC (norfloxacin)}}{\text{MIC(norfloxacin + modulator)}}$$

RESULTS

LC-MS analysis of alkaloid-rich fraction of *S. rhombifolia*

The TIC (Time Ion-Chromatogram) of alkaloid rich fraction showed a major peak at rt of 10.63 minutes and another peak at rt of 15.98 min. The peak at rt of 10.63 with molecular ion peak of 233 (M+H) was identified as cryptolepine, an indolo-quinoline alkaloid with molecular weight of 232 whereas the peak at rt of 15.98 with molecular ion peak of 249 (M + H) was identified as 11-methoxyquindoline having molecular weight of 248.

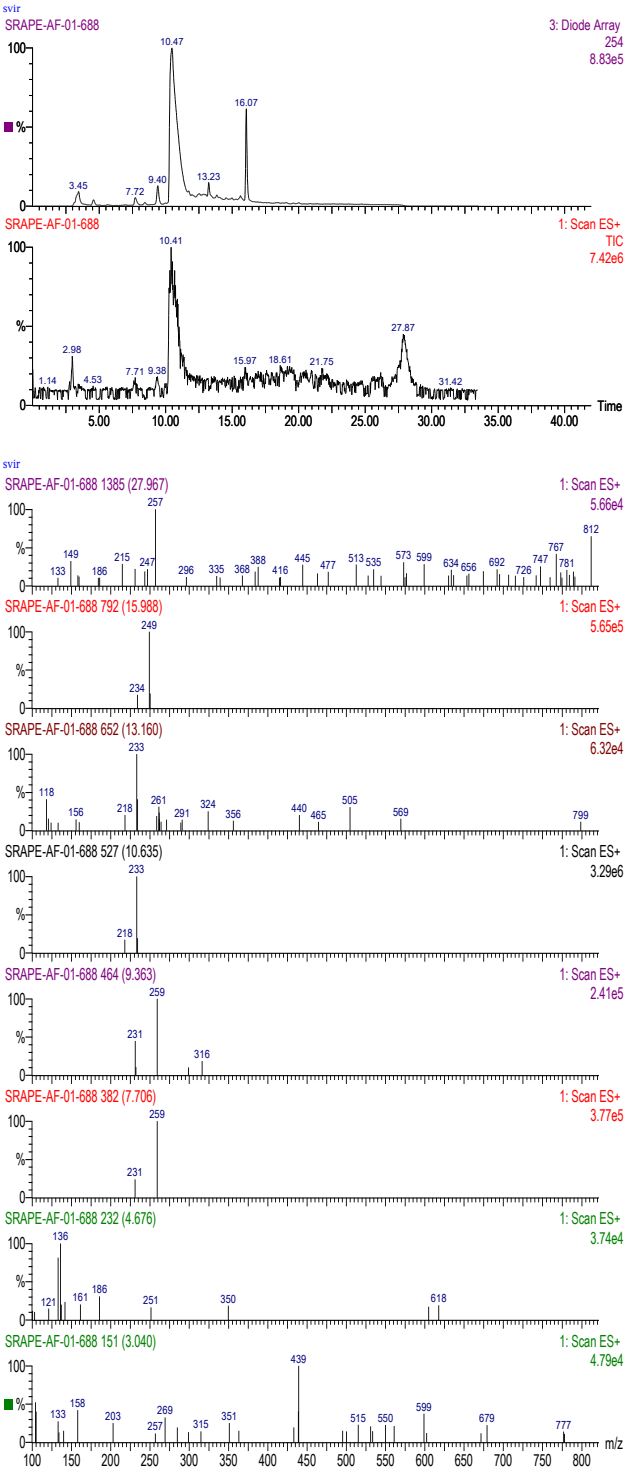
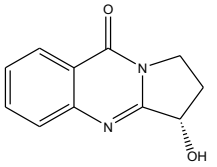


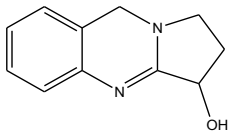
Table 1: Modulation activity of *S. cordifolia* and *S. rhombifolia* in *S. aureus* SA-1199 B

S. No.	Plant Extract	MIC (µg/ml)	Concentration of inhibitor used (µg/ml)	Fold reduction in Norfloxacin MIC
1	*Sida rhombifolia* (aerial Part)			
	Methanolic extract	500	125	4*
	Ethyl acetate fraction	64	16	-
	n-butanol fraction	500	125	-
	Alkaloid rich fraction	128	32	-
2	*Sida rhombifolia* (roots)			
	Methanolic extract	500	125	-
	Methanolic extract	> 500	125	-
4	*Sida cordifolia* (aerial part)			
	Methanolic extract	> 500	125	4*
	Alkaloid rich fraction	500	125	-
5	*Sida cordifolia* (roots)			
	Methanolic extract	500	125	-
	Alkaloid rich fraction	> 500	125	-

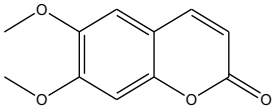
***Strain used:** *Staphylococcus aureus* SA-1199B (Nor A pump over-expressed); MIC of Norfloxacin is 32 µg/ml



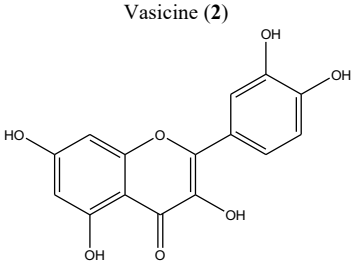
Vasicinone (**1**)



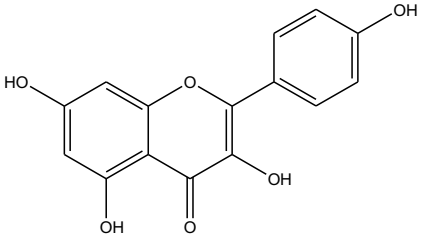
Vasicine (**2**)



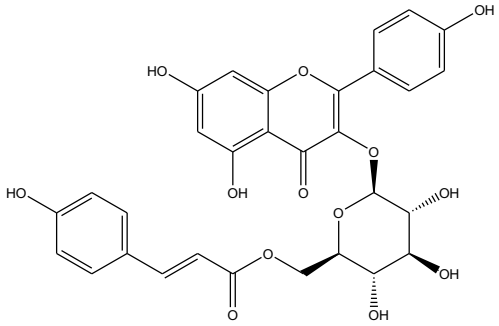
Scoparone (**3**)



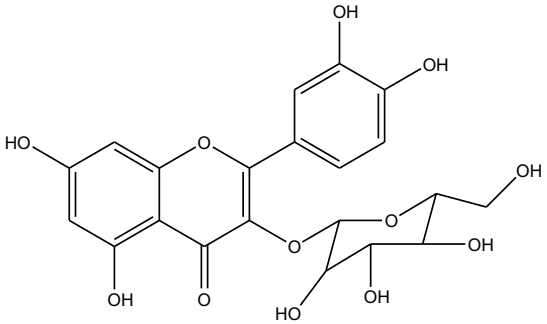
Quercetin (**4**)



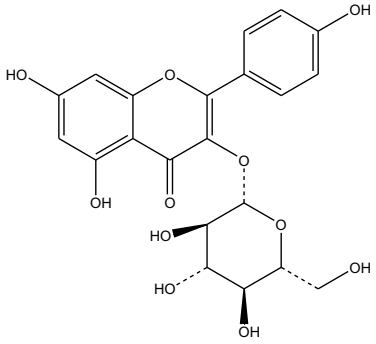
Kaempferol (**5**)



Trans tiliroside (6)

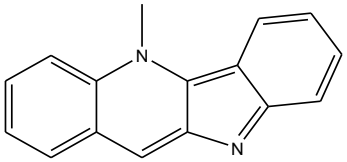


Isoquercetin (**7**)

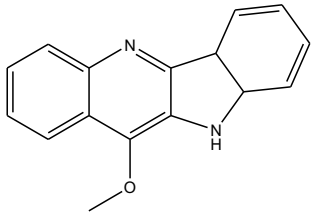


Kaempferol-3-O-glucopyranoside (**8**)

Figure 1: Structures of compounds isolated from *S. cordifiolia* aerial parts



Cryptoleptine



11-methoxyquindoline

DISCUSSION

Characterization of isolated compounds, 1- 8 from *S. cordifolia*

Compound 1 was obtained as yellowish brown solid. It was confirmed as vasicinone by co-TLC with reference standard. Compound 2 was also obtained as yellowish brown solid and confirmed as vasicine by co- TLC with reference standard. Compound 3 was obtained as a white crystalline solid. It showed blue florescence after treatment with alcoholic KOH at 366 nm which is characteristic of coumarins. APCI-MS suggested that its molecular weight is 206. ^{1}H NMR spectrum exhibited resonances typical at δ 6.30 ppm (d, 1H, J = 9.4. H-3) and 7.64 ppm (d, 1H, J = 9.4, H-4) of the coumarin nucleus, two methoxy groups from the signals at δ 3.93 ppm (s, 3H, 7-OCH_3) and 3.96 ppm (s, 3H, 6-OCH_3) corresponding signal in ^{13}C NMR exhibited at δ 113.5 ppm (C-3), 143.2 ppm (C- 4) and δ 56.3 ppm (7-OCH_3), δ 56.3 ppm (6-OCH_3). It also showed signal at δ 161.2 ppm (C-2) for carbonyl group. ^{1}H NMR also showed signal for aromatic region at δ 6.86 ppm (s, 2H, H-8 and H-5). The ^{1}H NMR and ^{13}C NMR data was found in agreement with that reported in literature for 6,7- dimethoxycoumarin (scoparone).[26]

Compound 4 showed orange colour florescence in UV at 366 nm after derivatization with NP reagent. Its R_f value was 0.62 in Toluene: EtOAc: AcOH (5:4:1) and was confirmed as quercetin by co-TLC with reference standard. Compound 5 showed greenish-yellow florescence with NP reagent at 366 nm. Its R_f value was 0.7 in Toluene: EtOAc: AcOH (5:4:1) and was confirmed as kaempferol by co-TLC with reference standard. Compound 6 was isolated as a yellow amorphous solid and gave positive test with NP reagent. Its R_f value was 0.8 in EtOAc: AcOH ; HCOOH : H_2O (100:11:11: 26) suggested that it might be a flavonoid glycoside. ESI-MS suggested that its molecular weight is 594.The ^{1}H NMR spectrum revealed a set of signals of kaempferol, p- coumaroyl and a sugar moiety. The identity of kaempferol was confirmed by two doublets at δ 6.10 ppm and δ 6.25 ppm (J = 1.7 Hz) for H-6 and H-8 respectively and a pair of doublets at δ 7.97 ppm (J = 8.2 Hz) and 6.82 ppm (J = 9.2 Hz) for H-2$^{'}$, 6$^{'}$ and H-3$^{'}$, 5$^{'}$, respectively, corresponding to an AA$^{'}$, BB$^{'}$ system. The p- coumaroyl group was characterized by the presence of A, A$^{'}$, B, B$^{'}$ – type aromatic proton signals at δ 7.29 ppm (2H, d, J = 8.4 Hz) and δ 6.78 ppm (2H, d, J = 8.9 Hz) and two- olefinic *trans* hydrogen signals at δ 6.04 ppm (1H, d, J = 15.9 Hz) and 7.37 ppm (1H, d, J = 15.9 Hz) and corresponding carbon showed signal at δ 113.0 ppm (C-α) and 145.5 ppm (C-β). The identity of a sugar was confirmed by a doublet at δ 5.23 ppm (J = 7.0 Hz) related to the anomeric proton and set of multi pate δ 3.30 – 3.54 ppm (m, 4H, H-2$^{''}$, 3$^{''}$, 4$^{''}$, 5$^{''}$) in the sugar region, anomeric carbon showed signal as δ 102.5. The diaxial coupling (J = 7.0 Hz) between H-1$^{''}$ and H-2$^{''}$ suggested a β-configuration.The linkage of the sugar to the C3 position of kaempferol was deduced on the basis of HMBC spectrum, which showed the cross peak correlation between δ 5.24 ppm (H-1$^{''}$) and δ 133.7 ppm (C-3). The downfield shift of C-6$^{''}$ carbon signal of the glucose moiety compared with kaempferol -3- O- β-D-glucopyranoside indicated that p-coumaroyl group might be attached at C-6$^{''}$ of the glucose. ^{13}C NMR spectrum also showed signals at δ 178 ppm (C-4) for carbonyl group and δ 165.4 ppm for ester carbonyl of p-coumaroyl group. So the observed ^{1}H NMR and ^{13}C NMR data of compound 6 was found in agreement with that reported in literature kaempferol -3- O- β-D- (6–*E*-*p*-coumaroyl)-glucopyranoside (tiliroside).[27] Compound **7** was isolated as a yellow amorphous solid and gave positive test with NP reagent. ESI- MS suggested its molecular weight as 464. The ^{1}H NMR spectrum revealed the set of signals of quercetin and sugar moiety. Two singlets at δ 6.28 ppm (1H, s) and δ 6.08 ppm (1H, s) were due to the protons at C6 and C8 positions, resp. Signals at δ 7.49 ppm (d, 1H, J= 8.4 Hz), δ 6.78 ppm (d, 1H, J= 8.4 Hz) and δ 7.61 ppm (s, 1H) were characteristic for a 3, 4 – disubstituted B ring that confirmed presence of quercetin as an aglycone moiety. Further it showed a set of multi plet at δ 3.21-3.47 ppm for sugar protons and a doublet at δ 5.13 ppm (J = 7.3 Hz) due to an anomeric proton of glucose and corresponding anomeric carbon showed signal at δ 102 ppm in ^{13}C NMR spectrum. The diaxial coupling (J = 7.3 Hz) between H-1'' and H-2'' suggested β-configuration. Based on the comparison of observed spectral data with that reported in literature, compound **7** was identified as quercetin-3-O- β-D-glucopyranoside (isoquercitrin).[28] Compound 8 was isolated as a yellow amorphous solid that gave positive test with NP reagent and its rf value was observed as 0.6 using mobile phase, EtOAc-AcOH-HCOOH-H_2O (100:11:11:26), suggesting it to be a flavonoid glycoside. APCI-MS indicated its molecular weight as 448. The ^{1}H NMR spectrum revealed a set of signals of kaempferol and a sugar moiety. Two meta-coupled doublets observed at δ 6.1 and 6.25 ppm (J = 1.7 Hz) were assigned to H-6 and H-8 protons of A ring. Further a pair of doublets observed at δ 8.01 ((J = 8.6 Hz) and 6.89 ppm were assigned to H-2', H-6' and H-3' and H-5', respectively of B-ring. The presence of sugar was confirmed by a doublet observed at δ 5.24 ppm (J = 7.1 Hz) due to anomeric proton of glucose and a set of multi plets at δ 3.16 to 3.7. Further anomeric carbon observed at δ 102.5 ppm in ^{13}C NMR spectrum, confirmed sugar to be glucopyranoside. The diaxial coupling (J = 7.1 Hz) between H-1" (anomeric proton) and H-2" suggested β-configuration. The linkage of glucose at C-3 position of kaempferol was confirmed by HMBC spectrum that showed the cross-peak between H-1" (δ 5.24 ppm) and C-3 (δ 134 ppm). The above spectral data was in agreement with that reported in literature for kampferol-3-O-β-D-glucopyranoside.[29] Structures of the 8 isolated compounds are presented in Figure 1.

Efflux pumps are believed to be one of the major causes of drug resistance in bacterial diseases and most of the drug resistant bacterial strains are becoming unsusceptible to clinically used antibiotics. Fluroquinolones, commonly used for treating MRSA infections are developing resistance in *Staphylococcal* strains due to active efflux pumps and thus becoming ineffective in treating these infections. The rate of new drug discovery is much lesser than the development of bacterial drug resistance. Thus, there is an urgent need to tackle the drug-resistance by an alternate strategy. Based on literature information that both *S. rhombifolia* and *S. cordifolia* possess anti-bacterial activity, these medicinal herbs were selected to study EPI activity. Methanol extract and

alkaloid-rich fractions were prepared *S. cordifolia* aerial parts and roots as well as from *S. rhombifolia* aerial parts and roots. Methanol extract of *S. cordifolia* and *S. rhombifolia* aerial parts showed 4-fold reduction each in MIC of norfloxacin, at 125 µg/mL concentration whereas alkaloid-rich fraction of both the plants did not show any reduction in MIC of norfloxacin (Table 1). Accordingly the methanol extracts of *S. cordifolia* aerial parts was fractionated for isolation of phytoconstituents. Total 8 compounds including two alkaloids viz. vasicine and vasicinone; 5 flavonoids viz. quercetin, kaempferol, trans-tiliroside, isoquercitrin, and kaempferol-3-O-glucopyranoside; and a coumarin, scaparone were isolated and characterized from bioactive MeOH extract of *S. cordifolia* aerial parts. Vasicine and vasicinone were also identified in alkaloid-rich fraction of *S. cordifolia* roots. Further TLC study, HPLC and LC-MS analysis of bioactive methanol extract of *S. rhombifolia* aerial parts revealed presence of alkaloids and flavonoids. Indolo-quinoline alkaloids viz. cryptolepine and 11-methoxyquindoline (Figure 2) were identified by LC-MS, in alkaloid-rich fraction prepared from MeOH extract of *S. rhombifolia* aerial parts.

CONCLUSION

Although the methanol extracts of aerial parts of *S. cordifolia* and *S. rhombifolia* showed weak modulatory activity in NorA over expressed *S. aureus* 1199 B strain, the present study helped to assess EPI activity potential of these plants. The flavonoids quercitrin, *trans*-tiliroside and kaempferol-3-O-β-D-glucopyranoside are being reported for the first time from *S. cordifolia* aerial parts to the best of our knowledge. In conclusion this study provided insights into bacterial modulation and EPI activity of *S. cordifolia* and *S. rhombifolia* as well as phytochemical investigation of both the species.

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