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RESEARCH ARTICLE

Antibacterial Activity of Long-Chain Primary Alcohols from *Solena amplexicaulis* Leaves

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Abstract Extraction, thin layer chromatography and gas chromatography–mass spectrometry of *Solena amplexicaulis* (Lam.) Gandhi, commonly known as creeping cucumber, (Cucurbitaceae) leaves revealed 21 long-chain primary alcohols, and 100 g leaves indicated presence of 3651.59 ± 327.18 SE µg long-chain primary alcohols. 1-Heptadecanol and 1-triacontanol were the predominant and least abundant primary alcohols, representing for 780.44 ± 42.59 and 3.28 ± 0.55 SE µg, respectively. Antibacterial property of the complete synthetic blend (0.1%), comparable to long-chain alcohols as detected by GC-FID of 100 g *S. amplexicaulis* leaf extracts was evaluated on the pathogenic bacteria *Salmonella gallinarum* by agar well diffusion method, and exhibited 20.4, 26.7 and 38.2 mm zone of inhibition at 25, 50 and 100 µl doses, respectively. One hundred µl dose of 6 individual pure synthetic compounds, 1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol comparable to the amounts present in 0.1% solution of pure isolated alcohols from *S. amplexicaulis* leaves displayed 16.2, 17.7, 18.6, 22.8, 15.8 and 14.5 mm zone of inhibition against this bacterium, respectively. Hundred µl dose from a synthetic blend of above 6 compounds (comparable to the proportions as present in 0.1% solution of pure isolated alcohols from 100 g *S. amplexicaulis* leaves) exhibited 38.1 mm zone of inhibition against this bacterium. Furthermore, 100 µl dose from a mixture (1:1) comprising of chloramphenicol (1 µg/ml) and a synthetic blend of above 6 compounds displayed 38.8 mm inhibition zone against *S. gallinarum*, and hence, this combination might be used against this pathogenic bacteria.

Keywords *Solena amplexicaulis* · Long-chain primary alcohols · 1-Pentadecanol · 1-Heptadecanol · 1-Nonadecanol · 1-Eicosanol · 1-Tricosanol · Antibacterial activity · *Salmonella gallinarum*

Introduction

Plants act as an indigenous source of traditional medicines in developing countries. Therefore, new compounds possessing therapeutic value might be used in drug development. Furthermore, natural compounds possess minimal toxicity as well as cost effective remedy for various diseases than that of synthetic drugs, which might have side effects. At present, an increase of infection by antibiotic-resistant microorganisms has led to search for compounds, which have potential antimicrobial activity. Plants synthesize numerous compounds as products of secondary metabolism, and some of these compounds have antimicrobial potential, which makes plants as a valuable source of pharmaceutical and therapeutic products. A number of studies revealed the effectiveness of plant extracts on microorganisms.

Solena amplexicaulis (Lam.) Gandhi (syn, *Melothria heterophylla*) (Cucurbitaceae), commonly known as creeping cucumber, is a promising fruit in India, Bangladesh, Pakistan and China (Karmakar and Barik 2016; Karmakar et al. 2016a, b; Sarkar et al. 2016). The young

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leaves of this plant are also consumed as vegetable (Parameshwar et al. 2010; Nagarani et al. 2014), and the whole plant (tubers, leaves and seeds) is used in traditional medicine as hepatosplenomegaly, spermatorrhoea, appetizer, cardiotonic, diuretic and thermogenic, haemorrhoids and invigorating (Kirtikar and Basu 1988; Parameshwar et al. 2010; Arun et al. 2011; Venkateshwarlu et al. 2011; Karthika and Paulsamy 2012; Karthika et al. 2012; Karthika and Paulsamy 2014). Increasing scientific evidences have proved that the whole plant is one of the most promising plant parts for diabetes today with some other pharmacological activities such as antioxidant, antibacterial and antifungal properties (Venkateshwarlu et al. 2011; Karthika and Paulsamy 2012; Karthika et al. 2012; Karthika and Paulsamy 2014; Kabir et al. 2014).

Empirical evidences suggest that long-chain alcohols have antibacterial activities (Mates 1974; Hattori et al. 1987; Kabelitz et al. 2003; Kato and Shibasaki 1980; Kubo et al. 1993a, b; McDonnell and Russell 1999; Tanaka et al. 2002; Dengle-Pulate et al. 2013). But till date, there is no information on the antibacterial activity of long-chain primary alcohols from *S. amplexicaulis* leaf extracts. Hence, the objectives of this study were to extract and identify long-chain primary alcohols by using non-polar solvent (chloroform) from *S. amplexicaulis* leaves, and to identify antibacterial property of long-chain alcohols against a pathogenic bacteria *Salmonella gallinarum*. We further studied the role of individual synthetic alcohols, followed by a combination of synthetic compounds (the individual compounds that showed antibacterial property) to determine zone of inhibition and minimum inhibitory concentration (MIC) against *S. gallinarum*.

Materials and Methods

Plant Materials

Seeds of *S. amplexicaulis* were germinated on moist filter papers. Each seed with cotyledon was planted in a pot containing ca. 1500 cm^3 of sterilized soil [organic matter 5.3 ± 0.2% (±Standard Error), pH 7.7, collected from the Crop Research Farm, University of Burdwan (23°16′N, 87°54′E), West Bengal, India]. One hundred grams of mature leaves were harvested from at least 20 plants.

Extraction, Identification and Quantification of Long-Chain Alcohols

One hundred grams of mature leaves (number of leaves 133 ± 4; mean ± standard error) were harvested randomly from the crop field. Leaves were initially rinsed with distilled water and dried in room temperature. The dried leaves were extracted with chloroform in a soxhlet extractor at 50 °C for 72 h. A total of three crude extracts were collected. Each crude extract was then passed through Whatman No. 41 filter paper, and the solvent was removed under reduced pressure. The residue was fractioned by preparative thin-layer chromatography (TLC) on silica gel G (Sigma, St Louis, MO, USA) layers (thickness 0.5 mm), which had been prepared using a Unoplan (Shandon, London, UK) coating apparatus, with benzene: ethyl acetate (95: 5) as the mobile phase (Mukherjee and Barik 2016). The plate was air-dried under laboratory conditions and then placed in an iodine chamber for 1 min, which produced a deep yellowish band with an Rf (Retardation factor) value of 0.645 to detect the band of primary alcohols on other TLC plates and this plate was not used for collection of samples. The Rf value (0.645) was compared with the Rf value of a mixture of synthetic primary alcohols between C10 and C30. The hydrocarbon band produced in each TLC plate was eluted from the silica gel layer with chloroform. A total of three purified primary alcohol samples were produced for gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS) and antibacterial assay. Each sample was equally divided into three fractions. First, 2nd and 3rd fractions of each sample were used for quantification and identification by gas chromatography–flame ionization detector (GC-FID), for further confirmation of the identification of primary alcohol compounds by GC–MS and antibacterial assay, respectively. All solvents used were of GR grade and purchased from E. Merck, India Pvt. Ltd (Mumbai, India).

Three separate extracts of *S. amplexicaulis* leaves were analysed by a Techcomp GC (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 fitted with an SE-30 capillary column (Agilent, Santa Clara, CA, USA; length: 30 m × 0.32 mm × 0.25 µm film thickness) and a flame ionization detector. The oven temperature program was initially 120 °C held for 3 min, then raised at 3 °C/min to 280 °C and finally held for 5 min (Mukherjee and Barik 2016). The carrier gas was nitrogen with a flow rate of 18.5 ml/min. The injector port temperature was 280 °C. One µl sample was injected with a split ratio of 1: 5. Components were characterized and quantified against the retention times of authentic standards, which were purchased from Sigma Aldrich. The peaks were identified by comparison of their retention times with those of standard primary alcohols from C10 through C30, and the areas of each peak were converted into quantities of primary alcohols based on internal standard tricosane (n-C_{23}).

For further confirmation of identifications, the extracts were analysed with an Agilent 6890 GC coupled to a 5973 Mass Selective Detector, which was analyzed under the same temperature conditions as mentioned under GC

analysis and using an SE-30 column. Helium was the carrier gas. The MS parameters were 280 °C at the interface, ionization energy 70 eV, scan speed approximately 1 s, and scanned over the mass range 40–600 mass units. The identity of the compounds was confirmed by injection of a mixture of synthetic primary alcohols (C10–C30). All primary alcohols were verified by comparison of the diagnostic ions and GC retention times with those of respective authentic standards.

Agar-Well Diffusion Method for Antibacterial Susceptibility Test

Maintaining the proportions and combinations of long-chain primary alcohols as detected by GC-FID from 100 g *S. amplexicaulis* leaf extracts, a synthetic blend of 0.1% solution was prepared, and from this solution 25, 50 and 100 µl were used for antibacterial assays. Individual pure synthetic compounds present in 0.1% solution of pure isolated alcohols from *S. amplexicaulis* leaf extracts were also tested using 25, 50 and 100 µl doses to find role of individual compounds on the pathogenic bacteria, *S. gallinarum*. Furthermore, a synthetic blend comprising of 1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol (as these individual synthetic compounds exhibited zone of inhibition against *S. gallinarum*), comparable to the amounts as present in 0.1% solution of pure isolated alcohols of *S. amplexicaulis* leaves, using 25, 50 and 100 µl were also tested against this bacterium. Application of (a): chloramphenicol (1 µg/ml), (b): a mixture (1:1) of chloramphenicol (1 µg/ml) and crude extract (0.1%), (c): a mixture (1:1) of chloramphenicol (1 µg/ml) and a complete synthetic blend (comparable to 0.1% primary alcohols as detected by GC-FID from 100 g *S. amplexicaulis* leaf extracts), and (d): a mixture (1:1) of chloramphenicol (1 µg/ml) and a synthetic blend of above 6 compounds (comparable to 0.1% primary alcohols as detected by GC-FID from 100 g *S. amplexicaulis* leaf extracts) were tested using 25, 50 and 100 µl doses to observe the effect of antibiotic only, and any synergistic effect of antibiotic and primary alcohols on the bacterium.

The antibacterial susceptibility assay of the crude extract (0.1%) and synthetic blends against the pathogenic bacteria *S. gallinarum* were conducted by agar well diffusion method (Perez et al. 1990). The bacterial strains were grown on nutrient broth at 37 °C for 18 h having to obtain $\approx 10^8$ cfu/ml. The bacterial suspension was used to spread in Petri plates of 90 mm diameter containing Muller-Hinton agar. Wells (6 mm diameter) were made in the plates with a cork borer and filled with the different doses (25, 50 and 100 µl) of crude extract, individual synthetic compounds and synthetic blends. The dissolution of the organic extracts was aided by 0.1% (w/v) Methanol, which had no bactericidal activity against the selected pathogenic microorganism as shown in our control experiments. The Petri plates were incubated at 37 °C for 24 h in a BOD incubator. Antibacterial activities of different solutions were assessed by measuring diameter of inhibition zone. Each experiment had five replicates. Minimum inhibitory concentration (MIC) of 6 compounds (i.e. 1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol) and a synthetic blend of above 6 compounds were also determined against *S. gallinarum* following standard methodology of Clinical and Laboratory Standards Institute (2009).

Statistical Analysis

Data on zone of inhibition produced by application of crude extract of *S. amplexicaulis* leaves, individual synthetic compounds, synthetic blends and antibiotics against *S. gallinarum* were subjected to Kruskal–Wallis nonparametric ANOVA followed by Steel–Dwass–Critchlow–Flinger multiple pair wise comparisons test. using XLSTAT software (SPSS 16.0; SPSS Inc., Chicago, IL, USA) (Adhikary et al. 2015; Sarkar et al. 2015).

Results

Long-Chain Primary Alcohols in *S. amplexicaulis* Leaves

Twenty-one long-chain alcohols were identified between C10 and C30 alcohols (Fig. 1, Table 1). 1-Heptadecanol was the predominant alcohol followed by 1-pentadecanol, 1-nonadecanol and 1-tridecanol, accounting for 780.44 ± 42.59, 737.45 ± 76.73, 559.33 ± 43.47 and 448.93 ± 40.79 µg, respectively. 1-Triacontanol was least abundant representing 3.28 ± 0.55 µg. The remaining individual alcohols displayed difference in the pattern with other alcohols in the leaf extracts.

Antibacterial Activity

The methanolic crude extract (0.1%) using 25, 50 and 100 µl doses showed 25.4, 27.9 and 39.8 mm inhibition zone, respectively (Table 2). The complete synthetic blend (0.1%), comparable to long-chain alcohols as detected by GC-FID of 100 g *S. amplexicaulis* leaf extracts, exhibited 20.4, 26.7 and 38.2 mm inhibition zone at 25, 50 and 100 µl doses, respectively (Table 2). Among the 21 identified primary alcohols in the *S. amplexicaulis* leaves, 6 compounds (1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol) comparable to 0.1% primary alcohols as detected by GC-FID from

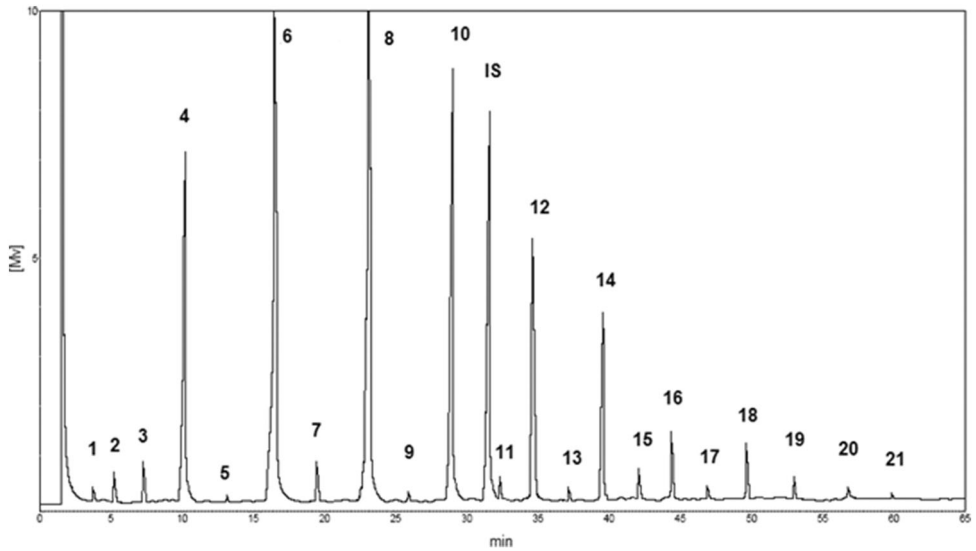

Fig. 1 Example GC-FID chromatogram (SE-30) of long-chain primary alcohols extracted from leaves of *Solena amplexicaulis* plants. Identification of peaks: *1*. 1-decanol, *2*. 1-undecanol, *3*. 1-dodecanol, *4*. 1-tridecanol, *5*. 1-tetradecanol, *6*. 1-pentadecanol, *7*. 1-hexadecanol, *8*. 1-heptadecanol, *9*. 1-octadecanol, *10*. 1-nonadecanol, *11*. 1-eicosanol, *12*. 1-heneicosanol, *13*. 1-docosanol, *14*. 1-tricosanol, *15*. 1-tetracosanol, *16*. 1-pentacosanol, *17*. 1-hexacosanol, *18*. 1-heptacosanol, *19*. 1-octacosanol, *20*. 1-nonacosanol, and *21*. 1-triacontanol

Table 1 Amount of long-chain primary alcohols (μg/100 g leaf) in *Solena amplexicaulis* leaves

Peak	Compound	Amount (mean ± SE, $N = 3$)
1	1-Decanol	24.20 ± 3.28
2	1-Undecanol	40.63 ± 3.99
3	1-Dodecanol	53.12 ± 5.76
4	1-Tridecanol	448.93 ± 40.79
5	1-Tetradecanol	4.68 ± 0.84
6	1-Pentadecanol	737.45 ± 76.73
7	1-Hexadecanol	52.56 ± 5.19
8	1-Heptadecanol	780.44 ± 42.59
9	1-Octadecanol	12.60 ± 1.42
10	1-Nonadecanol	559.33 ± 43.47
11	1-Eicosanol	35.24 ± 3.48
12	1-Heneicosanol	338.69 ± 36.77
13	1-Docosanol	24.89 ± 2.92
14	1-Tricosanol	245.07 ± 29.86
15	1-Tetracosanol	41.69 ± 5.25
16	1-Pentacosanol	94.57 ± 8.17
17	1-Hexacosanol	23.47 ± 2.68
18	1-Heptacosanol	78.58 ± 7.73
19	1-Octacosanol	35.13 ± 3.91
20	1-Nonacosanol	17.06 ± 1.51
21	1-Triacontanol	3.28 ± 0.55
	Total	3651.59 ± 327.18

For explanation of peaks, see Fig. 1

100 g *S. amplexicaulis* leaf extracts showed antibacterial activity against *S. gallinarum* at 25, 50 and 100 μl doses (Table 2). Furthermore, a synthetic blend of above 6 compounds exhibited 10.5, 15.4 and 38.1 mm inhibition zone at 25, 50 and 100 μl doses, respectively (Table 2). Chloramphenicol (1 μg/ml) showed 26.4, 28.4 and 38 mm inhibition zone at 25, 50 and 100 μl doses, respectively. Application of a mixture (1:1) comprising of chloramphenicol and crude extract produced 36.2, 39.6 and 42.2 mm inhibition zone at 25, 50 and 100 μl doses, respectively; whereas a mixture (1:1) comprising of chloramphenicol and a complete synthetic blend showed 30.6, 39 and 40.2 mm inhibition zone at 25, 50 and 100 μl doses, respectively. Furthermore, a mixture (1:1) comprising of chloramphenicol and a synthetic blend of 6 compounds displayed 30, 36.6 and 38.8 mm inhibition zone at 25, 50 and 100 μl doses, respectively (Table 2). This study indicated that a mixture (1:1) of chloramphenicol and the above 6 compounds synergistically produced more effect than individual chloramphenicol at 25, 50 and 100 μl doses against the bacterium *S. gallinarum* (Table 2).

The minimum inhibitory concentration (MIC) of 1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol against *S. gallinarum* were 5.5, 20, 15.08, 15.06, 10.08 and 15.07 μg/ml, respectively, while the application of the mixture of the above 6 compounds exhibited 5.2 μg/ml as MIC against this pathogenic bacterium (Table 3).

Table 2 Zone of inhibition (mm) produced by application of crude extract (0.1%) of *Solena amplexicaulis* leaves, individual synthetic compounds, synthetic blends comparable to the amounts as present in isolated pure alcohols (0.1%) from 100 g of *S. amplexicaulis* leaf extracts and antibiotics against *Salmonella gallinarum*

	Dose (µl)			$\chi^2_{0.05,2}$	*P*
	25	50	100		
	Zone of inhibition (mean ± SE, $N = 5$)				
Crude extract (0.1%)	25.4 ± 0.2[a]	27.9 ± 0.1[b]	39.8 ± 0.2[c]	13.084	0.001
Complete synthetic blend	20.4 ± 0.4[a]	26.7 ± 0.3[b]	38.2 ± 0.2[c]	12.868	0.002
1-Tridecanol (a)	5.2 ± 0.4[a]	13.7 ± 0.2[b]	16.2 ± 0.2[c]	12.750	0.002
1-Pentadecanol (b)	5.0 ± 0.5[a]	15.7 ± 0.2[b]	17.7 ± 0.3[c]	12.868	0.002
1-Heptadecanol (c)	3.2 ± 0.1[a]	14.0 ± 0.3[b]	18.6 ± 0.5[c]	12.727	0.002
1-Nonadecanol (d)	3.6 ± 0.2[a]	20.2 ± 0.2[b]	22.8 ± 0.4[c]	12.868	0.002
1-Eicosanol (e)	3.6 ± 0.1[a]	13.6 ± 0.3[b]	15.8 ± 0.2[c]	12.868	0.002
1-Tricosanol (f)	3.2 ± 0.3[a]	10.8 ± 0.2[b]	14.5 ± 0.3[c]	13.011	0.001
a + b + c + d + e +f	10.5 ± 0.2[a]	15.4 ± 0.4[b]	38.1 ± 0.5[c]	12.658	0.002
Chloramphenicol (1 µg/ml)	26.4 ± 0.3[a]	28.4 ± 0.4[b]	38.0 ± 0.3[c]	12.374	0.002
Chloramphenicol + crude (1:1)	36.2 ± 0.2[a]	39.6 ± 0.3[b]	42. 2 ± 0.4[c]	12.891	0.002
Chloramphenicol + complete synthetic blend (1:1)	30.6 ± 0.3[a]	39.0 ± 0.4[b]	40.2 ± 0.4[c]	12.297	0.002
Chloramphenicol + a synthetic blend of 6 compounds (1:1)	30.0 ± 0.5[a]	36.6 ± 0.3[b]	38.8 ± 0.4[c]	12.704	0.002

$\chi 2$ value is for Kruskal–Wallis test. Within the row means followed by different letters are significantly different by Steel–Dwass–Critchlow–Flinger multiple pair wise comparisons test with $\alpha = 0.05$

Table 3 Minimum inhibitory concentration (MIC) (µg/ml, mean ± SE, $N = 5$) of individual synthetic alcohol compounds and their blend

Compounds	MIC (µg/ml)
1-Tridecanol (a)	5.50 ± 0.02
1-Pentadecanol (b)	20 ± 0.03
1-Heptadecanol (c)	15.08 ± 0.04
1-Nonadecanol (d)	15.06 ± 0.04
1-Eicosanol (e)	10.08 ± 0.05
1-Tricosanol (f)	15.07 ± 0.03
a + b + c + d + e + f	5.2 ± 0.04

Discussion

The present study demonstrated 21 long-chain primary alcohols among the *S. amplexicaulis* leaf extract, and 1-heptadecanol was the predominant compound among the alcohols. The crude extract from *S. amplexicaulis* leaves, a complete synthetic blend of 21 compounds and a synthetic blend of 6 compounds (1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol) comparable to 0.1% long-chain alcohols as detected by GC-FID of 100 g *S. amplexicaulis* leaves showed antibacterial activity at lower concentrations; whereas single pure synthetic compounds were found to be effective against bacterial pathogen at higher doses. This observation implicates that the synthetic compounds in the blend acted synergistically and thus produced higher inhibition zone against the bacterium.

A number of compounds present in different plant extracts were previously investigated for their bactericidal activities, and among them alcohols were proved to be the potent antibacterial agent against several Gram positive and Gram negative bacteria (Kubo et al. 1993a, b; Kabelitz et al. 2003; Mbosso et al. 2010; Mukherjee et al. 2013). The antibacterial efficacy of *Aegle marmelos* was investigated against pathogenic bacterial strains and the methanolic extract was found to be active against *Staphylococcus aureus* at 40 mg/ml concentration (Mujeeb et al. 2014). The methanolic extracts of *Argemone mexicana* leaves and seeds exhibited potential antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *S. aureus* (Bhattacharjee et al. 2006). The antibacterial activity of l-tridecanol, 1-pentadecanol and 1-eicosanol was assessed against *Streptococcus mutans*, and the MIC of l-tridecanol was 6.25, and 1-pentadecanol and 1-eicosanol were >800 µg/ml (Kubo et al. 1993a); whereas this study revealed the MIC of l-tridecanol, 1-pentadecanol and 1-eicosanol was 5.5, 20 and 10.08 µg/ml, respectively against *S. gallinarum*. This observation implicates that these three alcoholic components, l-tridecanol, 1-pentadecanol and 1-eicosanol could also act on *S. gallinarum* like *S. mutans*. Kubo et al. (1993b) demonstrated that 1-dodecanol had the antibacterial activity against *S. aureus* with the MIC of 6.25 µg/ml, but in the present study this alcohol did not display any antibacterial

activity against *S. gallinarum*. 1-Decanol has been reported to act as antimycobacterial agent (Mukherjee et al. 2013), while in the present study this alcohol did not act as bactericidal agent against *S. gallinarum*. Furthermore, 1-dodecanol and 1-tridecanol exhibited the antibacterial activity against *S. aureus* (Togashi et al. 2007). These observations conclude that the antibacterial activity of alcohol compounds might vary in accordance with different bacterial species, and the carbon chain length of the alcohols is one of the major factors contributing antibacterial activities (Kato and Shibasaki 1980; Kubo et al. 1993a, b; Tanaka et al. 2002; Kabelitz et al. 2003; Togashi et al. 2007). Furthermore, the occurrence and stereochemistry of double or triple bonds within the alcoholic molecules can also be a determining factor in their antibacterial potency (Kabelitz et al. 2003).

Synergistic activities of antibiotics and plant extracts against several pathogenic bacteria have been proved as a new foundation for the control of multi drug resistant bacteria (Chattopadhyay et al. 2009; Zhang et al. 2013; Yildirim et al. 2013). The present investigation demonstrated that a mixture (1:1) comprising of a synthetic blend of 6 compounds (1-tridecanol, 1-pentadecanol, 1-heptadecanol, 1-nonadecanol, 1-eicosanol and 1-tricosanol) comparable to the amounts as present in 0.1% solution of the isolated pure alcohols as detected by GC-FID from 100 g of *S. amplexicaulis* leaf extracts and chloramphenicol antibiotic (1 µg/ml) could act as potent antibacterial agent against the pathogenic bacteria *S. gallinarum*, which might be used as a new approach for controlling this pathogenic bacterium. However, it remains to be seen the other compounds in the *S. amplexicaulis* crude extract showing antibacterial activity against the pathogenic bacteria *S. gallinarum* as 0.1% crude extract from *S. amplexicaulis* leaves was found to be more potent than a complete synthetic blend and a synthetic blend of 6 compounds comparable to 0.1% long-chain alcohols as detected by GC-FID of 100 g *S. amplexicaulis* leaf extracts.

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