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Antimicrobial and antiquorum-sensing activity of *Ricinus communis* extracts and ricinine derivatives

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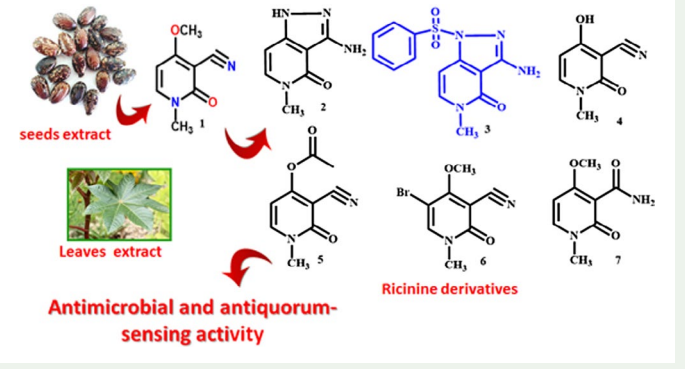
ABSTRACT

Ricinine (**1**), a known major alkaloid in *Ricinus communis* plant, was used as a starting compound for the synthesis of six ricinine derivatives; two new and four known compounds. The new derivatives; 3-amino-5-methyl-1*H*-pyrazolo[4,3-c]pyridin-4(5*H*)-one (**2**), and 3-amino-5-methyl-1-(phenylsulfonyl)-1*H*-pyrazolo[4,3-c]pyridin-4(5*H*)-one (**3**), as well as the previously prepared derivatives (**4**–**7**) were subjected for antimicrobial and antiquorum-sensing evaluation in comparison to different *R. communis* extracts. Acetyl ricininic acid derivative (**5**) showed the highest antimicrobial activity among all tested derivatives against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeuroginosa* and *Candida albicans*. However, compound **7** (4-methoxy-1-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide) showed the highest antiquorum-sensing activity among all tested compounds and extracts. These findings proved the usefulness of ricinine as a good scaffold for the synthesis of new antimicrobial and antiquorum-sensing derivatives in spite of its poor contribution to the antimicrobial activity of the plant extracts.



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1. Introduction

The aggravating global threat of the development of antimicrobial resistance is leading to a continuous demand for the discovery of new antimicrobial drugs and anti-virulence agents. Bacterial cell to cell communication termed as 'quorum-sensing' represents a signalling system that is important for the regulation of several cellular processes such as virulence factors expression, biofilm formation and competence (Bassler 1999; Miller and Bassler 2001; Kalia 2013). Therefore, targeting the bacterial pathogenic potential through quorum-sensing inhibition is one of the new strategies that are used to combat the microbial resistance (Bhardwaj et al. 2013).

Plants represent a valuable sustainable source of antimicrobial drugs and resistance modifying agents (Cowan 1999; Abreu et al. 2012). *Ricinus communis* L. (Castor oil plant) is an important industrial crop belonging to the spurge family, Euphorbiaceae. It is a fast-growing plant with a great environmental and economic value and it was reported to have different biological activities (Ribeiro et al. 2016). The antimicrobial activity of *R. communis* extracts is one of the most studied activities of this plant. The plant extracts, especially its leaf extract, were reported to have antimicrobial activity against different microorganisms; however, the pure compound(s) responsible for this activity was not identified (Ribeiro et al. 2016). It is important to identify the pure compound(s) responsible for the antimicrobial activity since the use of total plant extracts as antimicrobial drugs is not preferable because of their complicated chemical nature and difficulty of standardisation (Cos et al. 2006; Abreu et al. 2012). Plant alkaloids, either natural or semisynthetic, represent a historical opulent source of antimicrobial and antibiotic enhancing agents (Cushnie et al. 2014). Ricinine, the predominant alkaloid in *R. communis* plant, is a simple and moderately toxic α-pyridone alkaloid. Several α-pyridone nucleus-containing compounds, either natural or synthetic, were reported to have antimicrobial activity (Li et al. 2000; Jessen and Gademann 2010; Wu et al. 2014). Despite the reported antimicrobial activity of *R. communis* extracts and compounds containing the α-pyridone ring, the antimicrobial activity of pure ricinine and most of its derivatives was not previously investigated. It is worth to note that the antimicrobial activity for only the 3-carboxamide derivative of ricinine (**7**) was previously investigated against *Staphylococcus aureus* normal and drug-resistant strains (Swarupa et al. 2017). They reported that the 3-carboxamide derivative of ricinine showed potent activity against *S. aureus* and caused inhibition of biofilm formation in all tested strains (Swarupa et al. 2017).

Castor meal, the residual waste left after defatting the castor seeds, contains about 0.77% w/w of ricinine and it represents a good source for isolation of this alkaloid (De Melo Cazal et al. 2009; Li et al. 2013). Though, castor meal has about 34–36% protein content, it is used with caution as organic fertilizer and cannot be used as an animal feed because of the presence of the extremely toxic protein (ricin), other toxic allergens and ricinine alkaloid (Anandan et al. 2005; Dubois et al. 2013). The production of large amounts of this waste is one of the problems associated with biodiesel industry from castor oil on large scale (Li et al. 2013). Therefore, the use of this waste for isolation of large amounts of ricinine alkaloid may reduce the environmental hazards of biodiesel industry from castor oil.

The aim of this research was the isolation of ricinine (**1**) and testing its antimicrobial and antiquorum-sensing activities in comparison to different *R. communis* extracts and investigation of its contribution to the antimicrobial activity of these extracts. Furthermore, the antimicrobial and antiquorum-sensing activities of some synthesised ricinine derivatives

(**2**–**7**) were determined and compared to ricinine activities in an attempt to study their structure–activity relationship.

2. Results and discussion

2.1. Chemistry

Ricinine (**1**) was isolated in a high yield from *R. communis* seed extract (0.11% w/w). Its structure was identified by comparing its spectroscopic data with that reported in literature (Sule and Sani 2008; De Melo Cazal et al. 2009; Li et al. 2013). Several studies reported the isolation of ricinine from *R. communis* leaves and seeds (Kang et al. 1985; De Melo Cazal et al. 2009; Li et al. 2013). However, this method of isolation of ricinine showed to be more suitable for isolation of ricinine from large quantities of the castor meal that is left after obtaining the castor oil for biodiesel production.

Compounds **2**–**7** (Figure 1) were prepared via chemical derivatization of ricinine and subjected to antimicrobial and antiquorum-sensing evaluation. Compound **2** was obtained by the reaction of ricinine with hydrazine hydrate. ^{1}H-NMR spectrum (Table S1) of **2** showed close resemblance to that of ricinine except for the presence of two additional proton signals at δ 5.13 (2H, br. s, NH_2) and δ 11.69 (1H, br. s, 1-NH) and the absence of the methoxyl group signal. The ^{13}C-NMR spectrum (Table S2) revealed the presence of only seven carbon signals instead of eight and showed the absence of the methoxyl group and the nitrile carbon signals. In addition, the upfield shift of the carbon signal at δ 144.3, C-4c and the appearance of imine carbon at δ 153.2, C-3 indicated reaction of ricinine with hydrazine hydrate and formation of pyrazolo-pyridine structure. Therefore, **2** was concluded to be a new compound (3-amino-5-methyl-1*H*-pyrazolo[4,3-c]pyridin-4(5*H*)-one).

Compound **3** was obtained by reaction of **2** with benzene sulfonyl chloride. Analysis of its HR-FAB^{+}-MS data suggested the molecular formula $C_{13}H_{12}N_4O_3S$ with 10 degrees of

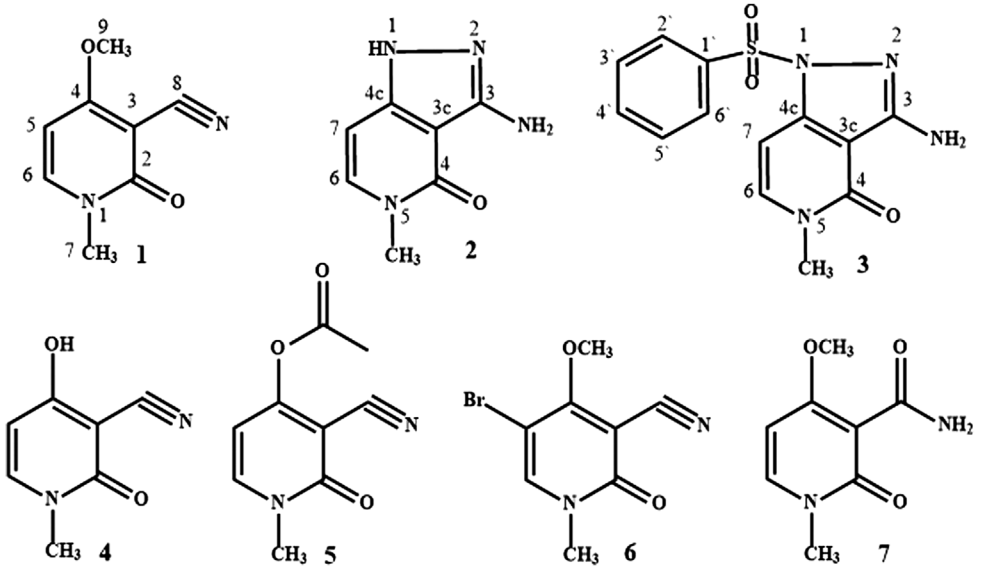

Figure 1. Structures of compounds **1**–**7**.

unsaturation indicating presence of extra phenyl moiety. This was confirmed by the presence of extra signals for 5 aromatic protons at δ 7.83 (2H, d, J = 7.6 Hz, H-2′/6′), 7.73 (1H, overlapped dd, H-4′) and 7.61 (2H, dd, J = 8.0, 7.6 Hz, H-3′/5′) in its ^{1}H-NMR spectrum (Table S1). Its ^{13}C-NMR spectrum (Table S2) also showed the presence of extra signals for 6 aromatic carbons at δ 136.9 (qC, C-1′), 135.2 (CH, C-4′), 130.2 (CH, C-3′/5′) and 127.5 (CH, C-2′/6′). The assignment of the carbons of this compound was confirmed by HMBC spectrum. Consequently, compound **3** was concluded to be the new compound, 3-amino-5-methyl-1-(phenylsulfonyl)-1*H*-pyrazolo[4,3-c]pyridin-4(5*H*)-one and confirmed the incidence of a reaction between benzene sulfonyl chloride and the NH group of (**2**).

The known compounds **4–7** were identified as ricininic acid (**4**), the acetyl derivative of ricininic acid (**5**), 5-bromoricinine (**6**), 3-carboxamide derivative of ricinine (**7**) when compared to their reported spectroscopic data (Robinson and Hook 1964; Yuldashev 2001; Zhao et al. 2015).

2.2. Antimicrobial activity

Ricinine (**1**), its derivatives (**2–7**), total MeOH extracts of leaves and seeds of *R. communis*, EtOAc and aqueous fractions of the seed extract and castor oil were subjected for *in vitro* agar disc-diffusion antibacterial assay. In this assay, the MICs were determined for the most active compounds and/or extracts using Gram-positive bacteria (*S. aureus*), and Gram-negative bacteria (*E. coli*, *K. pneumoniae* and *P. aeuroginosa*) as presented in Table S3. Ampicillin and gentamicin were used as standard antibacterial drugs (positive control), while DMSO alone was used as a negative control. The obtained results indicated that **5** showed the highest activity among the tested ricinine derivatives and *R. communis* extracts. It showed moderate activity against *K. pneumoniae* and *P. aeruginosa*, while, strong activity against *E. coli* and *S. aureus*. However, ricinine itself was found to be completely inactive against any of the tested bacterial strains. Derivative **2** showed moderate activity against *E. coli* and weak activity against *K. pneumonia*e. Derivative **3** showed moderate activity against *E. coli*, *K. pneumonia*e, *P. aeruginosa* and *S. aureus*. In addition, derivative **4** showed moderate activity against *K. pneumonia*e and *P. aeruginosa*, while, weak activity against *E. coli*. Moreover, derivative **6** exhibited moderate activity against *K. pneumonia*e. In accordance with the results of Swarupa et al. (2017), derivative **7** showed antibacterial activity against *S. aureus*. Although, its potency against *S. aureus* was found to be moderate, and lower than that reported by Swarupa et al. (2017). In accordance with literature (Ribeiro et al. 2016), the leaf extract of *R. communis* showed reasonable antibacterial activity against all tested bacterial strains. The seed extract showed strong activity against *P. aeruginosa* and moderate activity against *E. coli* and *S. aureus*. Moreover, the castor oil and the EtOAc fraction of the seed extract showed reasonable antibacterial activity against *P. aeruginosa* bacterial strain and to lesser extent towards *E. coli*.

The antifungal activity of the same compounds and extracts was also tested against the yeast-like pathogenic fungus *C. albicans* and fluconazole was used as a standard antifungal drug and DMSO as a negative control. Leaf extract showed the highest antifungal activity among all tested compounds and extracts. In addition, MeOH extract of the seed, **4** and **5** showed potential antifungal activity. However, the parent compound, ricinine, showed no antifungal activity. In all antimicrobial assays, the negative control, DMSO, did not show any noticeable activity.

2.3. Antiquorum-sensing activity

Besides the antimicrobial testing, all compounds and extracts were further evaluated for their antiquorum-sensing activity against *Ch. violaceum* using catechin as a positive and DMSO as a negative controls. The antiquorum-sensing activity of the compounds and extracts was determined by testing their ability for inhibiting the release of a violet pigment (violacein), which is released in response to acyl homoserine lactones signals (McLean et al. 2004; Chu et al. 2011). The pigment inhibition radius in this assay, revealed that **7**, showed the highest antiquorum-sensing activity among the tested compounds and extracts. On the other hand, **5** showed moderate activity, and **2** and **3** exhibited weak activity as shown in Table S4. However, the parent compound, ricinine showed no activity at all. Furthermore, the leaf extract showed moderate and much better activity than that of the total seed extract.

2.4. Structure–activity relationship of ricinine derivatives as antimicrobial agents

Based on the obtained results of antimicrobial and antiquorum-sensing activities for ricinine derivatives (**1–7**), it could be inferred that conversion of the cyano-pyridone nucleus of ricinine into a pyrazolo-pyridone nucleus imparted moderate and weak antibacterial activities to **2** against Gram-negative bacteria *E. coli* and *K. pneumonia*, respectively, and weak antiquorum-sensing activity. Interestingly, the presence of the benzene sulfonyl moiety in **3** increased the antibacterial activity of **2** against *E. coli* and *K. pneumonia* and improved its activity against *P. aeuroginosa* and *S. aureus*. Meanwhile, the removal of the *O*-methyl group of ricinine and the presence of a phenolic group at position 4 as in ricininic acid (**4**) caused moderate antimicrobial activity against *P. aeuroginosa*, *K. pneumoniae* and *Candida albicans*, while weak antibacterial activity against *E. coli*. The replacement of the *O*-methyl group of ricinine with acetyl group greatly affected its antimicrobial activity. The presence of the acetyl group imparted antimicrobial activity to the most active ricinine derivative (**5**) against all tested bacterial strains and the pathogenic fungus, *C. albicans*. The presence of this group also caused reasonable inhibition of quorum-sensing in comparison to the *inactive* parent compound (ricinine). Bromination of ricinine at position 5 as in derivative **6** did not greatly affect its antimicrobial activity and only caused moderate antibacterial activity against *K. pneumonia*. The conversion of the cyanide group of ricinine into a carboxamide group as shown in **7** caused weak antibacterial activity against *E. coli* and *K. pneumoniae*, while, moderate antibacterial activity against *S. aureus*. However, the presence of the carboxamide group imparted the highest antiquorum-sensing activity to compound **7** among the other derivatives.

3. Conclusion

This study described a simple method for isolation of ricinine alkaloid in high yield from *R. communis* seeds. The isolated ricinine was used as a synthon for production of valuable compounds with better and diverse biological activities. Six compounds; two new and four known were prepared from ricinine. The antimicrobial testing of the prepared compounds showed that although ricinine does not contribute to the antimicrobial activity of the leaf and seed extracts of *R. communis* plant, it represents a good scaffold for antimicrobial and antiquorum-sensing derivatives. Castor oil and the components of the EtOAc fraction may

partially contribute to the antimicrobial activity of the seed extract and they may exhibit a synergistic effect.

It is worth to be noted that chemical treatment of castor meal with NaOH is one of the most efficient methods used for detoxification of castor meal (Anandan et al. 2005). Treating castor meal with NaOH may be used for its detoxification to be used as an animal feed and for preparation of ricininic acid (**4**) (Yuldashev 2001) to be used as a synthon for preparation of ricinine derivatives. This may contribute to minimise the environmental hazards associated with biodiesel industry from castor oil and providing additional medical and economic benefits for *R. communis* plant via preparing new antimicrobial and antiquorum-sensing derivatives.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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