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Short Communication

Isolation and structure elucidation of triterpenes from inflorescence of banana (*Musa balbisiana* cv. Saba)

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Abstract

The study aimed to isolate and elucidate the chemical compounds that are found in banana (*Musa balbisiana* cv. Saba) inflorescences. Banana inflorescence buds were extracted using methanol and the resulted methanolic extract was partitioned using chloroform, ethyl acetate and butanol against deionized water. The chloroform partition was further separated into fractions using column chromatography assisted by thin layer chromatography. The structure elucidation was performed using nuclear magnetic resonance spectrometry (NMR). Three triterpenes were isolated namely 31-norcyclolaudenone (1), cycloartenol (2) and (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3). This is the first report on the isolation of these triterpenes from *Musa balbisiana* inflorescence. The discovery of new triterpenes from banana inflorescence should be further explored to open a new perspective that banana by-products might serve as new source of natural products for food and pharmaceutical applications.

Introduction

In Malaysia, nearly 34 thousand hectares of land had been used for the cultivation of bananas and plantain, the second largest fruit crop, which produced more than 320 thousand tons of commodities for trade (Abdul Khalid *et al*., 2006; DOA, 2007). However, approximately 220 tons per hectare of agricultural waste are composted annually (Shah *et al*., 2005). The current usage of banana by-products in Malaysia is low and under-valued, as some of the by-products are used to produce dried pulps and animal feed with a limited extend (Emaga *et al*., 2007). The banana inflorescence is eaten as vegetable by some ethnic groups in the country. Most of the by-products would end up as agricultural wastes and may cause serious ecological problems due to improper management (Shah *et al*., 2005). Attempts have been carried out in exploring the potential of agricultural by-products such as grape pomace (Oliveira *et al*., 2013) and olive mill waste (Tafesh *et al*., 2011) to serve as a new source of phytochemicals.

Agricultural by-products obtained from banana and plantains are undervalued in most parts of world. Stems and leaves are usually left in plantation as organic materials after the fruits brunch is harvested (Cordiero *et al*., 2004). Leaves are used in food wrapping, clothes and ceremonial occasions based on ethnics (Kennedy *et al*., 2009), although some have suggested they could serve as suitable candidate for bioactive components. Varieties of plant sterols (β-sitosterol, stigmasterol and campesterol) were isolated from *Musa paradisiaca* peels (Dutta *et al*., 1983). Knapp and Nicholas (1969) and Akihisa *et al.* (1986) had discovered several cycloartane type alcohol and ketones from *Musa accuminata* peels. Several antibacterials including malic acid, ß-sitosterol, succinic acid, palmatic acid, 12-hydroxystrearic acid were also isolated from *Musa Cavendish* peels (Mokbel and Hashinaga, 2005). Similarly, ß-sitosterol was reported as the most abundant sterols present in banana pulps obtained from 10 different cultivars of banana in Portugal (Vilela *et al.*, 2014). Recently, Pereira and Maraschin (2014) proposed that peel and pulp of banana are source of carotenoids, phenolics and amine compounds, which could be potentially developed into phytomedicines. However, most of the bananas studied are limited to internationally importance banana cultivars such as *Musa sapientum* and Cavendish cultivar.

Organic solvent extracts (hexane, petroleum ether, chloroform as well as ethyl acetate) obtained from Malaysia banana (*Musa paradisiaca* cv.

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Mysore) inflorescences were recently reported to exhibit antibacterial activity against several pathogenic bacteria (Padam *et al*., 2012). Meanwhile, Jawla *et al.* (2014) also reported that banana *(Musa paradisiacal)* flower extracts posed antibacterial activity against nine bacteria such as *Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium* and *Streptococcus pneumoniae*. The aim of the current study is to isolate and elucidate the chemical compounds that are present in the non-polar extracts of *Musa balbisiana* cv. Saba.

Materials and Methods

Materials

Optical rotations were measured on an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, USA) while fourier transform infrared data were obtained using Thermo Nicolet (Thermo, USA). High-resolution mass spectrums (HR-ESI-QToF) were obtained using UPLC-Synapt QToF (Waters, USA) with leucine enkephaline as lock mass reference. The1H-NMR (600 MHz) and 13C-NMR (150 MHz) spectra were recorded with a JEOL ECA 600, with TMS as internal standard (JEOL, Japan). Preparative TLC was performed with silica gel plates (Merck, Kieselgel 60 F254). Silica gel (Merck, Kieselgel 60, 70-230 mesh) was used for column chromatography (Merck, Darmstadt). Analytical TLC was performed on Merck Kieselgel 60 F254. Spots were visualized by UV light or by spraying with a 5% phosphomolybdic acid-ethanol solution.

Sample preparation

The banana inflorescences (8 kg) were purchased from local traditional (Tamu) market. The samples were separated into buds and bracts, cleaned and dried at 50°C under constant ventilation using a factory-scale dehydrating oven (Memmert, Germany) for 72 hours until the sample's moisture content remains stable at 10.0 ± 0.5%. The moisture content of the dried banana buds was determined using a moisture analyser (Metler Toledo, Switzerland). Samples were ground into powder using an electric blender (Panasonic, Japan) to achieve mesh size at approximately 1.0 mm. The powder was kept in airtight plastic bags at 4°C until further use.

Extractions

Banana inflorescence buds were extracted using 94% methanol by direct solvent infusion in a thermostated water bath (Wisebath, Korea) at 35°C for 6 hours with constant shaking. Then, the samples were filtered using Whatman filter paper No. 1 and rotary evaporated under reduced pressure. The extract was successively partitioned with chloroform, ethyl acetate and butanol against water. Chloroform partition (1.0291 g) was fractionated by Silica gel (Merck, Kieselgel 60, 70-230 mesh) column chromatography with a step gradient of hexane and ethyl acetate (EtOAc) in the ratio of 9: 1, 8: 2, 7: 3, 6: 4, 1: 1 and EtOAc. The fraction (411.3 mg) eluted with hexane/ EtOAc (9:1) was further separated by a combination of preparative TLC (Merck, Kieselgel 60 F254) with benzene to yield 7 fractions with cycloartenol (2) (6.2 mg) and (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3) (4.4 mg). Fraction 4 (8.0 mg) from the elution of benzene was further purified to yield 31-norcyclolaudenone (1) (5.6 mg).

31-norcyclolaudenone (1). Crystallized white colour needle. [α]28D -270.00° (c 0.08; $CHCl_3$); IR Vmax ($CHCl_3$) cm^{-1} 3044, 2917, 1715, 1463, 888; HR-ESI-QToF m/z 425.3806 $[M+H]^+$ (calcd. for $C_{30}H_{48}O$, 425.3783); NMR δC and δH: C-1 [33.5; 1.65, 1.66 *dd*], C-2 [41.7; 2.40, 2.43 *dd*], C-3 [214.1], C-4 [50.7; 2.23 *m*], C-5 [46.8; 1.56 *dd*], C-6 [26.6; 0.73, 1.71 *m*], C-7 [25.9; 1.10, 1.36 *dd*], C-8 [47.8; 1.65 dd], C-9 [25.7], C-10 [30], C-11 [27.9; 1.23; 2.04 *m*], C-12 [33.6; 1.55, 1.84 *m*], C-13 [46.0], C-14 [49.5], C-15 [36.1; 1.29, 1.30 *m*], C-16 [28.7; 1.31, 1.85 *m*], C-17 [52.9; 1.59 *s*], C-18 [18.6; 0.99 *s*, C-19 [26.6; 0.39, 0.61 *d*], C-20 [36.7; 1.35 *m*], C-21 [19.0; 0.86 *d*], C-22 [34.6; 0.91, 1.36 *dd*], C-23 [32.2; 1.15, 1.47 *m*], C-24 [42.3; 2.10 *m*], C-25 [150.9], C-26 [110.1; 4.46, 4.47 *s*], C-27 [19.3; 1.64 *s*], C-28 [20.9; 1.01 *d*], C-29 [19.8, 0.88 *s*], C-30 [11.4; 0.98 d]. The 31-norcyclolaudenone (1) was elucidated and compared with literature (Akihisa *et al*., 1998; Ragasa *et al*., 2007) to confirm the structure.

Cycloartenol (2). Crystallized white colour needle. [α]28D +36.33° (c 0.60; CHCl3); IR Vmax ($CHCl_3$) cm^{-1} 3322, 3037, 2928, 1644, 887; HR-ESI-QToF m/z 427.3958 $[M+H]^+$ (calcd. for $C_{30}H_{51}O$, 427.3940); NMR δC and δH: C-1 [31.1; 1.50, 1.75 *dd*], C-2 [28.8; 1.29, 1.90 *m*], C-3 [79.6; 3.28 *t*], C-4 [41.2], C-5 [47.8; 1.31 *m*], C-6 [21.8; 0.77, 1.58 *d*], C-7 [27.2; 1.07, 2.00 *m*], C-8 [48.7; 1.50 *dd*], C-9 [20.7], C-10 [26.8], C-11 [26.7; 1.03, 1.33 *m*], C-12 [36.3; 1.28, 1.29 *d*], C-13 [46.0], C-14 [49.5], C-15 [32.7; 1.25, 1.58 *d*], C-16 [25.6; 1.04, 1.85 *m*], C-17 [53.0; 1.56 *t*], C-18 [26.1; 0.89 *s*], C-19 [30.6; 0.33, 0.55 *t*], C-20 [36.6; 1.36 *m*], C-21 [18.9; 0.88 *d*], C-22 [37.0; 1.05, 1.43 *m*], C-23 [33.6; 1.63, 1.63 *d*], C-24 [126.0; 5.10 *t*], C-25 [131.6], C-26 [26.4;

1.68 *s*], C-27 [18.3; 1.60 *s*], C-28 [20.0; 0.88 *s*], C-29 [14.7; 0.80 *s*], C-30 [18.7; 0.96 *s*]. The structure of cycloartenol (2) was elucidated and compared with literature (Akihisa *et al.*, 1986) to confirm the structure.

(24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3). Crystallized white colour needle. [α]28D +55.13° (c 0.78; $CHCl_3$); IR Vmax ($CHCl_3$) cm^{-1} 3351, 2988, 1644, 1454, 886; HR-ESI-QToF m/z 427.3965 [M+H]+(calcd. for C30H51O, 427.3940); NMR δC and δH: C-1 [35.7; 1.20, 1.77 *dd*], C-2 [28.8; 1.30, 1.90 *dd*], C-3 [77.2; 3.09 *m*], C-4 [37.0; 1.37 *m*], C-5 [47.8; 0.95 *m*], C-6 [21.4; 1.24, 1.77 *m*], C-7 [31.5; 1.16, 1.59 *dd*], C-8 [134.3], C-9 [135.4], C-10 [37.1], C-11 [22.5; 1.03, 2.01 *dd*], C-12 [26.2; 1.71, 2.09 *dd*], C-13 [45.2], C-14 [50.5], C-15 [31.9; 1.75, 1.85 *dd*], C-16 [31.7; 1.48, 1.69 *dd*], C-17 [51.1; 1.49 *m*], C-18 [16.4; 0.70 *s*], C-19 [19.3; 1.64 *s*], C-20 [42.3, 2.09 *m*], C-21 [19.4; 0.89 *d*], C-22 [34.6; 0.88, 1.33 *m*], C-23 [32.1; 1.14, 1.41 *m*], C-24 [42.3; 2.09 *m*], C-25 [150.9], C-26 [110.1; 4.66, 4.71 *s*], C-27 [18.9; 0.97 *s*], C-28 [20.9; 1.00 *d*], C-29 [25.1; 0.87 *s*], C-30 [15.8, 0.99 *d*]. The structure of (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3) was elucidated and compared with literature (Dutta *et al*., 1983) to confirm the structure.

Results and Discussion

The chloroform partition of the methanolic extract of *Musa balbisiana* cv. Saba buds has undergone separation using column chromatography on silica gel followed by normal phase TLC isolation and purification, yielded with a cycloartane-type triterpene ketone, a cycloartane-type triterpene alcohol and a tetracyclic triterpene alcohol. Three known compounds were isolated from chloroform fraction based on 1H-NMR chart and their structures were elucidated based on 2D-NMR data as well as comparison with existing literature. The 1H NMR spectrum of 31-norcyclolaudenone (1) (Figure 1) indicated resonances for germinal olefinic protons at δ 4.46 and 4.67, a cyclopropyl at δ 0.39 and 0.61, an allylic methyl at δ 1.65 and 5 other methyl group. These data was further supported 13C NMR spectrum which showed a total spectrum of 30 carbons with corresponding functionalities of ketone carbonyl at δ 214.1 and olefinic carbons at δ 110.1 and 150.9, as well as other carbons of methyl, methylene and methine. These resonances indicated the presence of triterpene with cyclopropyl, a ketone carbonyl and olefinic functionalities. The connectivity of 1H and 13C were verified with HMBC and the structure was elucidated with HMBC and COSY 2D NMR data

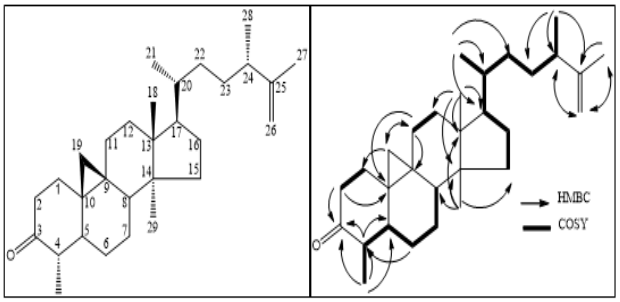

Figure 1. Structure and 1H-1H COSY and 1H-13C HMBC for of 31-norcyclolaudenone (1) from *Musa* balbisiana cv. Saba

with both of the key correlations showed in Figure 1. Ketone carbonyl was placed at C-3 due to its long-range correlation with C-2 and C-4 while methyl peak C-30 showed long-range correlation to ketone carbonyl as well. The 6-member carbon ring was verified with COSY correlation between 1H spectrum methine C-4 as well methylene C-5, C-6, C-7 and C-8. Cyclopropylene was placed at C-19 with the long range correlation between proton of methylene C-1, C-11, which showed response of attachment at protons C-9 and C-10. Olefinic exo-methylene was placed at C-25 and C-26 with its correlation with methyl C-27 and the correlation of C-25 quaternary carbon with methyl C-28. The alkyl chain was confirmed with COSY correlations between C-20/ C-22, C-22/ C-23, and C-23/ C-24, which proton duplets observed for methyl C-21 and C-28. Additionally, methyl C-18 showed long-range connectivity of 1H and 13C with C-12, C13, C-14 and C-17 while methyl C-29 showed long-range correlation with C-8, C-13, C-14 and C-15. With the COSY correlation C-15/ C-16, C-16/ C-17 and C-17/ C20, the substructure of alkyl chained attached on the position of C-17 of 5-member ring was confirmed. COSY correlation between 1H spectrum of methylene C-11 and C-12 confirm the tetracyclic structure of 31-norcyclolaudenone (1). Thus, the 31-norcyclolaudenone (1) was elucidated and compared with the literature (Akihisa *et al*., 1998; Ragasa *et al*., 2007) to confirm the structure.

Cycloartenol (2) is found very similar to 31-norcyclolaudenone (1) after comparing both spectrums while structure elucidation was done upon the correlations of HMBC and COSY (Figure 2). At 1H NMR spectrum found germinal olefinic protons resonances at δ 5.10, a cyclopropyl at δ 0.33 and 0.55, two allylic methyl at δ 1.60 and 1.68 with 5 other methyl groups as well as hydroxyl methine at δ 3.28. A 30 peak carbon spectrum indicated two olefinic carbons at δ 131.6 and 126.0. The chemical shift of these two carbons, which are lower suggested that the presence of exo-methylene is rare and most probably an endo-methylene constructing a double

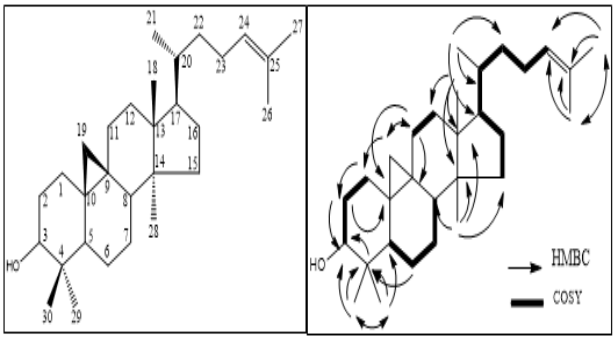

Figure 2. Structure and 1H-1H COSY and 1H-13C HMBC for of cycloartenol (2) from *Musa balbisiana* cv. Saba

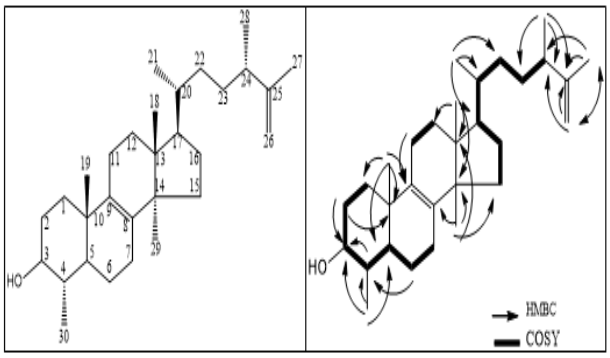

Figure 3. Structure and 1H-1H COSY and 1H-13C HMBC for of (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3) from *Musa balbisiana* cv. Saba

bond (Akihisa *et al.*, 1986). Besides, the presence of hydroxyl group as well as cyclopropylene was agreeable in 13C spectrum. Hydroxyl group carbon was placed at C-3 as its proton showed long-range correlations with methylene protons of C-2 and C-4. This was further confirmed with the correlation of allylic methyl proton at C-29 and C-30. Similar to 31-norcyclolaudenone (1), cyclopropylene was placed at C-19 through their correlation with proton of C-1 and C-11 and structure of rings; it was confirmed with COSY correlation of those methylene and methine, discussed in the elucidation of 31-norcyclolaudenone (1). Since the presence of olefinic exo-methylene was excluded, the functionality present would be double bond. It was placed in C-24 and C-5 since the proton was observed to have correlation with proton of two methyls of C-26 and C-27. These two methyls were found to be allylic methyl with singlet proton chemical shifts of δ 1.60 and 1.68. The alkyl chain was confirmed with COSY correlations. Thus, the structure of cycloartenol (2) was elucidated and compared with the literature (Akihisa *et al.*, 1986).

(24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3) which was isolated from the same chloroform partition of 31-norcyclolaudenone (1) and cycloartenol (2), found to pose different skeleton. As compared to 31-norcyclolaudenone (1) and cycloartenol (2) reported in the previous section, two olefinic protons were observed δ 4.66 and 4.71 (Figure 3). This chemical shift was found similar to the 31-norcyclolaudenone (1), which indicate the presence of exo-methylene olefinic functionalities. Besides, a hydroxyl was found on δ 3.09 while no allylic methyl was found. These data were further supported with 13C NMR spectrum. However, there was found four olefinic carbons existed in this structure, which are δ 150.9, 134.3, 135.4 and 110.1. The carbon of δ 150.9 and 110.1 is exo-methylene, as reported in the previous sessions and another two olefinic resonances indicated the presence of double bond. Since there is no exist of allylic methyl, it was suggested that the double bond appear further from methyl group in this compound. Elucidation was done upon the correlations of HMBC and COSY as well as compared with spectrum of 31-norcyclolaudenone (1). At 1H NMR spectrum, hydroxyl group containing carbon was placed at C-3 as its proton showed correlations with methylene protons of C-2 and C-4. Additionally, it posed similar correlation with methyl C-30 with proton chemical shift of δ 0.99, indicating the attachment close to carbonyl or hydroxyl functionalities. The α and β ring were further confirmed with COSY correlation between C-1/ C-2, C-2/ C-3, C-3/ C-4, C-4/ C-5, C-5/ C-6 and C-6/ C-7. Double bond was placed at C-8 and C-9 by their long-range correlation with methyl C-19 and C-29. Long alkyl side chain was confirmed as compared to chemical shift of 31-norcyclolaudenone (1). This structure was further confirmed with the comparison of its chemical shift reported by Dutta *et al.* (1983).

Plants do produce bioactive secondary metabolites, which are lipophilic. Five cycloartane triterpenes have been identified in Musa sapientum peels by Akihisa *et al*. (1998) while four tetracyclic triterpenoid were isolated from the flower of *Musa* paradisiaca (Dutta *et al.*, 1983). Recently, three cycloartane type triterpenes exhibiting leishmanicidal activity was found in *Musa paradisiaca* fruit peel (Silva *et al*., 2014). However, *Musa* spp. contain high amount of wax as well as fatty acids that are also non-polar substances (Padam *et al.*, 2012). Thus, crude chloroform extract gives low bioactivity since the total amount of secondary metabolites might be very low within the crude chloroform partition itself. To further speculate into the finding, chloroform partition undergone a series of TLC and PTLC purification to yield only three purified triterpenes, labelled as 31-norcyclolaudenone (1), cycloartenol (2) and (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3).

Plants, algae and some protists synthesize their sterols through a biosynthetic route that contains a pentacyclic steroidal cyclization product of 2,3-oxidosqualene, namely, cycloartenol (2) the product of the cycloartenol synthase (CAS1, EC 5.4.99.8) (Benveniste, 2004). Cycloartenol (2) can be commonly found in many plants such as pecan nut (Bouali *et al*., 2014), breadfruit (Tsai *et al*., 2013) and villous deadly carrot (Drew *et al.*, 2013). The 31-norcyclolaudenone (1) was first described by Knapp and Nicholas (1969) from banana peel *(Musa sapientum)*. This compound was subsequently isolated from another cultivar of banana *(Musa errans)* in Philippines (Ragasa *et al*., 2007) as well as banana peel *(Musa sapientum)* by Akihisa *et al*. (1986; 1998). Thus, 31-norcyclolaudenone (1) synthesised through this cycloartenol synthase pathway in banana as proposed by Akihisa *et al.* (1986). The first record of (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3) was described by Dutta *et al.* (1983) from the flower of banana *(Musa paradisiaca)* and it was proposed to be synthesised through banana steroidal pathway.

The 31-norcyclolaudenone (1) was reported to possess inhibitory activity against *Candida albicans*, *Escherichia coli, Pseudomonas aeruginosa* while inactive against gram-positive bacteria *(S. aureus and Bacillus subtilis)* as well as *Aspergillus niger* (Ragasa *et al.*, 2007). This finding somehow contradictory against the common fundamental in which gram positive bacteria are generally more susceptible against antimicrobial substances as compared to gram negative bacteria. Kikuchi *et al.* (2007) demonstrated 31-norcyclolaudenone (1) and cycloartenol (2) are inhibitory to 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells as anti-tumor promoters (IC_{50} 9.9 and 8.8 nM respectively) as well as inhibitory to the activation of (±)-(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide (NOR 1), a nitric oxide (NO) donor in tumor initiating activity using rat skin model.

Although the three compounds isolated are known compounds, but there is lack of information on their biological activity. Thus, further analysis and exploration on their application in food and human health, post-modification of triterpenes could serve as a major interest of the natural products sector. Post-modification of triterpenes can be achieved through the enzymatic biosynthesis such as P450 monooxidases (Seki *et al*., 2008; Zhu *et al*., 2011), glycosyl transferases (Zhu *et al.*, 2011) as well as chemical synthesis (Yu *et al*., 2013; Huang *et al*., 2014). Oxygenated and glycosylated triterpenes often associated with improved functionality in biological mediums as well as foods (Agustin *et al*., 2011). This can be seen on the post-oxidation as well as post-glycosylation of ß-amyrin (common phytosterols precursors) which produced a world-wide sweetener and flavouring additives, glycyrrhizin with extensive biological activities such as anti-inflammatory (Man *et al*., 2013), anti-ulcer (Ranade *et al*., 2014) as well as immunomodulatory effect (Jia *et al.*, 2014). Conjugation of functional group on triterpenes contributed to the advancement of pharmaceutical industry. Yu *et al.* (2013) developed a new anti-hepatitis C active compound based on inactive triterpene through conjugation of disaccharides onto triterpenes, which increase the activity by five folds. Antibacterial and anti-tumor activity of triterpenes also increased through the modification of exocyclic α,ß-unsaturated ketones moiety in ring A (Huang *et al*., 2014). Thus, with the recent advancement of bioengineering and chemical synthesis by the enzymes, post-modification of triterpenes opening up the room for the rapid access and exploration of these biologically important and commercially potential compounds.

Conclusion

Extraction, isolation and purification of chloroform partition from the banana inflorescence had yielded three triterpenes which are confirmed as 31-norcyclolaudenone (1), cycloartenol (2) and (24R)-4a,24-trimethyl-5a-cholesta-8,25(27)-dien-3b-ol (3). The isolation of triterpenes from banana by-products opened a new perspective in conversion and management of waste into a potential source of high value phytochemicals for nutraceutical, pharmacological or food additive applications.

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