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Metabolic disturbance and phytochemical changes in *Andrographis paniculata* and possible action mode of andrographolide

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ABSTRACT

Objective: To explore the effect of gibberellic acid (GA_3) and its inhibitor paclobutrazol (PBZ) on chemical composition and their pharmacological effects on *Andrographis paniculata* (Burm. f.) Wall. ex Nees, and to clarify action mode of andrographolide. **Methods:** The chemical composition was extracted by sequential extraction with hexane, dichloromethane, ethyl acetate and methanol, respectively. Andrographolide and its derivatives were evaluated by HPLC. Moreover, the metabolic profiling was analyzed by GC-MS. Inhibitory effect of crude extracts was tested against *Staphylococcus aureus* using agar well diffusion method. Mode of action was tested against mutant yeast by spotting assay. Andrographolide were tested for their mode of action against eukaryotes. **Results:** Among different solvents, dichloromethane gave the highest yield of crude (3.58% DW), with the highest andrographolide content (8.3 mg/g DW). The effect of plant hormone (10 mg/L GA_3 or PBZ) on phytochemical variations and bioactivity of *Andrographis paniculata* was demonstrated. It was found that PBZ promoted sesquiterpene compounds about 3.5 times over than GA_3 treatment. But inhibitory effect of extracts against *Staphylococcus aureus* was highest in GA_3 treated plants; andrographolide and 14-deoxy-11,12-didehydroandrographolide contents were significantly higher than those of water or PBZ. It was found that there were 11 strains involving in ergosterol biosynthesis, V-ATPase activity and homeostasis, and superoxide detoxification process. In this regard, andrographolide might cause the damage on the lipid bilayer of yeast cell and plasma membrane by interfering ergosterol biosynthesis. **Conclusions:** It is found that GA_3 promotes andrographolide and 14-deoxy-11,12-didehydroandrographolide content while PBZ promotes sesquiterpene content. Andrographolide might cause the damage on the lipid bilayer of yeast cell and plasma membrane by interfering ergosterol biosynthesis. It might also affect mitochondria electron transport chain, leading to the occurrence of ROS, which can further harm cell organelles. However, the library screening is the first step to investigate mode of action of andrographolide.

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1. Introduction

Terpenoids constitute a large group of plant secondary metabolites, many of which are crucially important to plant growth and survival such as α-tocopherol (vitamin E), artemisinin (antimalarial agent), and taxol (anti-carcinogenic agent)[1]. However, as many terpenoids serve biological functions in plants and possess a range of properties, it is crucial to understand the effect of external elicitors on terpenoid variations in plants to better evaluate their modes of formation. In this study, *Andrographis paniculata* (*A. paniculata*) was used as a model to study the effect of terpenoid variations on application of gibberellic acid (GA_3), and its antagonist paclobutrazol (PBZ). *A. paniculata* is an herbaceous plant in the family Acanthaceae. It is generally called 'Fathalaijone' in Thai, and is widely cultivated in many Asian countries apart from Thailand such as China, India, and Malaysia. *A. paniculata* extracts contain a variety of compounds possessing pharmacological properties. For example, andrographolide ($C_{20}H_{30}O_5$) is a major diterpenoid lactone[2] possessing anti-plasmodial activity against *Plasmodium falciparum*, *Plasmodium berghei* ANKA[3], anticancer activity in mice and human cancer cell lines[4] and anti-inflammatory activity[5]. Moreover, its derivatives, 14-deoxy-11,12-didehydroandrographolide exhibit anticancer activities against T-47D breast carcinoma cell[6]. The antibacterial activities of crude extracts have been widely reported[7]. This study aims to evaluate the influence of GA_3 and PBZ on variations of the levels of these compounds, the anti-*Staphylococcus aureus* (*S. aureus*) activity of *A. paniculata* extracts, and to identify the compounds responsible for the activity profile.

2. Material and methods

2.1. Sequential extraction of andrographolide from A. paniculata

The leaves of *ex vitro A. paniculata* wild type (Nan Province, Thailand) were collected and dried in an oven at 50 ℃ for 24 h before extraction. Dry powder (100 g) of leaf sample was extracted sequentially with various solvents in order of low to high polarity (hexane, dichloromethane, ethyl acetate and methanol, respectively) with the ratio 1: 10 (each solvent analytical grade, RCI Labscan Limited, Thailand). After sonicating the mixture for 30 min, it was filtered through Whatman® paper No.1. The crude extract was freeze dried and stored at -20 ℃ for further analysis.

2.2. Plant treatment

A. paniculata seeds were obtained from Nan province, Thailand. They were grown in an open field (temperature 30-35 ℃) at the Faculty of Science, Mahidol University, Bangkok, Thailand. After five months of cultivation, they were treated with 10 mg/L GA_3 (GoldenGib®, S&P formulator Co., Thailand), or PBZ (Predict W. P., Ladda Co., Thailand) by foliar spraying. After 24 h of treatment, the leaves were collected for solvent extraction.

2.3. Sample preparation and extraction

A. paniculata leaves were dried in the oven at 50 ℃ for 24 h. They were then ground prior to extraction with a suitable solvent. Extraction was carried out 3 times, with sonication for 30 min each time. The extracts were then pooled together, evaporated, dried at room temperature and kept at -20 ℃ prior to analysis.

2.4. Anti-S. aureus assay

The agar well diffusion method was performed to study the anti-*S. aureus* activity of extracts, with the procedure modified from previous report[8]. The negative control used was 100% DMSO. After 24 h of incubation, the clear zone diameter was recorded.

2.5. Terpenoid analysis by gas chromatography mass spectrometry (GC-MS)

The dried extracts were re-dissolved in dichloromethane and the concentration to 10 mg/mL was filtered through a Nylon membrane prior to being injected into a HP-5ms capillary column (split ratio 1: 2). The temperatures of the injector and detector were set at 280 ℃ and 250 ℃, respectively. The initial temperature was set at 50 ℃ with a 2 min hold time. The temperature was programmed to increase at a rate of 20 ℃/min until reaching 180 ℃, then a further increase of 10 ℃/min to 280 ℃, and a final increase to 300 ℃ over 2 min. The total run time was 35.5 min. The MS scan range was from 35 to 600 Da. Methyl heptadecanoate (C_{17}) was used as an internal standard.

2.6. Andrographolide and 14-deoxy-11,12-didehydroandrographolide analysis by HPLC

The crude extracts were analyzed using a HPLC equipped with a photodiode array detector[9]. A Luna RP18 reverse phase column (150.0 mm×4.6 mm. *i.d.*, Phenomenex®) was used for separation. For AD analysis, a mobile phase consisting of 30: 70 acetonitrile-dilute formic acid (0.1% in double distilled water, 10 μL) was injected onto the column with a flow rate of 1 mL/min. The runtime for analysis was 15 min, and UV detection wavelength was set to 223 nm.

2.7. Statistical analysis

Raw data GC-MS was processed by Hystar 3.1 (Agilent). Mass peak of all chromatograms and total mass spectra was exported. Hierarchical cluster analysis was applied to calculate the distance between groups of sample using Multi experiment Viewer program version 4.8.1[9,10].

2.8. Identification of Saccharomyces cerevisiae mutant strains hypersensitive to andrographolide

Saccharomyces cerevisiae haploid strain BY4742 (*MAT α his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and a collection of its isogenic deletion mutants were maintained on YPD (1% yeast extract, 2% peptone, 2% dextrose) agar medium, prior to testing andrographolide sensitivity. Andrographolide was purchased from Tokyo Chemical Industry Co., LTD. To prepare YPD with andrographolide supplementation, the concentration was dissolved and adjusted with the analytical grade of methanol (RCI Labscan Limited, Thailand). The YPD medium with 3.5 mM andrographolide in methanol supplementation was prepared for the test. The YPD medium with the same volume of methanol was prepared to determine the effect of methanol which was used as the solvent for andrographolide dissolution, while YPD was used as the control. Each well of the 96-well microtiter plate was then filled with 100 μL of sterile distilled water. The yeast were then inoculated into 96-well microtiter plates using 48-pin replicator and stamped onto the medium. Finally, andrographolide-sensitive strains were identified and selected for the serial dilution spotting assay to confirm the primary phenotype. The experiment was modified from Li *et al*., 2005 and Shima *et al*., 2008[11].

2.9. Andrographolide sensitivity and resistance assay

Spotting assay was performed according to the modified method[12]. The yeast was cultured overnight in 0.5 mL of YPD liquid medium at 30 ℃. After that, OD_{600} was measured and the dilution of OD_{600} equal to 1 was prepared with sterile distilled water. Then 10-fold serial dilutions of yeast culture were made from 10^{-1}-10^{-5}. Next, 3 μL of each dilution was dropped on the agar plate supplemented with 3.5 mM andrographolide. The agar plates were incubated at 30 ℃ for 2-3 d to observe the susceptibility of yeast strains to andrographolide.

3. Results

Andrographolide (AD) is one of the most biologically active diterpenoids present in *A. paniculata*. The sequential extraction was used to extract chemical compounds in *A. paniculata*. The crude extract from dichloromethane (3.86% g DW) was the most suitable solvent followed by methanol, ethyl acetate and hexane (0.53%, 1.15%, 3.09% g DW respectively).

Moreover, the AD content of each extract was measured using HPLC, and the results indicated that AD levels were highest in extracts from dichloromethane [(8.31±0.03) mg/g DW] in comparison to other solvents, methanol [(0.06±0.00) mg/g DW], ethyl acetate [(3.09±0.10) mg/g DW] and hexane [(0.01±0.00) mg/g DW]. While the crude extract yields from methanol and dichloromethane were not appreciably different (3.26% DW and 3.87% DW, respectively).

The result from GC-MS was reported in Table 1. GA_3 could trigger *A. paniculata* to produce compounds that have not been detected in control treatment such as 4-methylheptadecane, 9-n-Octleicosane, globulol, endesma-4(14),11-diene, Caryophylla-3,8(13)-dien-5 β -ol and 9,12-Octadecadien-1-ol,(Z,Z). There were 7 compounds that were detected only PBZ treatment such as perhydrobenzo[a]cycloheptene-6,8-decarboxylic acid, perhydro-7-oxo-,diethyl ester, globulol, methyl(Z)-5,11,14,17-eicosatetraenoate, 7,10,13-Hexadecatrienoin acid, methyl ester, 2,3,6,7-tetramethyl-1,4,4 α ,5,8,8a β ,9 β ,9a α ,10 β ,10a β -decahydroanthracene-9,10-diol and patchoulane. Terpenoid compounds were grouped and their variations after treated with GA_3 and PBZ were demonstrated in Figure 1. Those compounds could be classified into 6 groups: sesquiterpenes, diterpenes, triterpenes, fatty acids and conjugates, phenolics, and aliphatic/aromatic hydrocarbons. The major components in extracts from material subjected to water treatment were fatty acids and conjugates (8 compounds amounting to 42.91% identified), followed by terpenes (8 compounds, 41.26%), hydrocarbons, and phenolic compounds (1 compound, 3.11%). Extracts from material subjected to GA_3, or PBZ treatment exhibited the same trend with terpene-based compounds predominating, followed by fatty acids, hydrocarbons, and phenolic compounds. Considering the terpene-based compounds alone, PBZ treatment resulted in remarkably enhanced levels of sesquiterpenes in the extract, with levels 24 times higher than extract from water treatment, and 4 times higher than extract after GA_3 treatment.

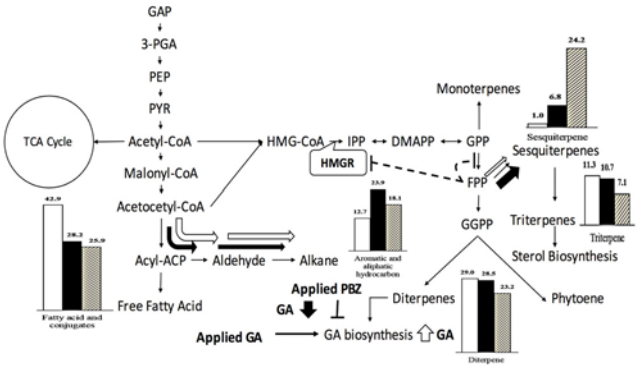

Figure 1. Schematic representation of mevalonate pathway divided into sterol and non-sterol biosynthesis, fatty acid biosynthesis and alkane biosynthesis pathway.

The variations of phytochemicals regulated differently by GA_3 (filled white arrows) or PBZ (filled black arrows), size of arrows represented the different in effect of GA_3 and PBZ treatments, and ⋯indicates feed-back inhibition. The proportion of plant secondary metabolites extracted by dichloromethane after treated with water (▢), 10 mg/L GA_3 (■) or 10 mg/L PBZ (▨) were presented as bar graph, the number above each bar graph indicate the proportion percentages.

The principle component analysis showed that after treatment with PBZ for 24 h, the chemical profiling was different from control and GA_3. On the other hand, biplot principle component analysis revealed that the *Artemisia annua* (*A. annua*) plant sprayed

with GA_3 and water showed no difference in metabolomics pattern. It means that PBZ could interfere metabolite of *A. paniculata*, and result in the metabolic flux to sesquiterpene production. There were 6 compounds that manifested for different effect between each treatment (Table 1): neophytadiene (Ⅱ), palmitic acid, oleic acid, (+-)-2,3,6,7-tetramethyl-4,4a α,5,8,8a α,9a β,10,10a β-octahydroanthra cen-9(1H)-one (Ⅱ), patchoulane and γ-sitosterol. In PBZ treatment, the highest relative content and the most different from others was patchoulane (110.5%) (Table 1). It was interesting to note that two sesquiterpenoid compounds, globulol and patchoulane were found only in PBZ treated plants, which have not been reported to be detected in *A. paniculata*.

AD content of each treatment was measured by HPLC (Table 2). The result showed that AD content obtained from 10 mg/L GA_3 treatment was 37% higher than that of the control. Moreover, the content of DDA after GA_3 treatment was 46% higher than in the control. These results showed that AD content increased by 6.6% after treatment with 1.73 mg/L GA_3 compared to non-treated controls.

The antimicrobial activities of the dichloromethane extracts were evaluated against *S. aureus* (Table 2). The results showed that crude dichloromethane extract following GA_3 treatment had the highest activity followed with water and PBZ treatment, respectively.

Since the extracts from plant material after water, GA_3, and PBZ treatments exhibited different chemical compositions, the constituents shown in Table 1 were further analyzed to examine correlations between extract composition and anti-*S. aureus* activity using Multi experiment Viewer program version 4.8.1[13,14]. The

Table 1

Chemical constituents of *A. paniculata* dichloromethane extract as analyzed by GC-MS.

No	RT	Compounds	Relative average (%)		
			Water	GA_3	PBZ
1	9.65	*n*-Tetradecane	4.21	-	-
2	9.65	4-Methylheptadecane	-	3.75	-
3	9.65	9-*n*-Octyleicosane	-	4.64	-
4	9.78	Phenol, 2,4-bis(1,1-dimethylethyl)-	13.53	11.16	7.75
5	12.35	Phytene	-	3.48	-
6	12.40	Neophytadiene (Ⅰ)	69.88	103.91	73.17
7	12.44	Acetic acid, trifluoro-,3,7-dimethyloctyl ester	5.09	-	-
8	12.44	*Trans*-phyt-2-ene	5.44	6.68	4.41
9	12.59	Neophytadiene (Ⅱ)	8.07	11.28	8.23
10	12.60	Phytol (Ⅰ)	-	-	7.06
11	12.75	Phytol (Ⅱ)	17.60	-	-
12	12.76	Neophytadiene (Ⅲ)	15.40	23.98	17.17
13	13.44	Palmitic acid	49.04	50.29	26.47
14	14.14	Stearic acid	46.71	-	-
15	14.76	Phytol (Ⅲ)	9.67	9.39	5.63
16	14.98	1,2-Epoxy-1-vinylcyclododecene	41.08	-	-
17	14.98	Oleic acid	26.60	-	32.51
18	15.00	Perhydrobenzo[a]cycloheptene-6,8-dicarboxylic acid, perhydro-7-oxo-, diethyl ester	-	-	22.61
19	16.29	Palmitic acid β-monoglyceride (Ⅰ)	2.77	-	-
20	17.06	Caryophyllene oxide	4.44	8.17	-
21	17.06	Trilostane	4.97	-	-
22	17.06	Globulol	-	-	5.17
23	17.07	Eudesma-4(14),11-diene	-	14.34	-
24	17.07	Caryophylla-3,8(13)-dien-5 β-ol	-	15.33	5.55
25	17.79	9,12,15-Octadecatrienal	9.83	7.78	6.51
26	17.79	Methyl-11,14.17-eicosatrienoate	-	8.56	-
27	17.79	Methyl (Z)-5,11,14,17-eicosatetraenoate	-	-	7.17
28	18.00	Palmitic acid β-monoglyceride (Ⅱ)	13.35	15.66	6.44
29	19.48	Methyl hexadecadienoate	10.76	-	-
30	19.48	9,17-Octadecadienal, (Z)-	10.89	12.89	7.14
31	19.48	9,12-Octadecadien-1-ol, (Z,Z)-	-	14.18	10.11
32	19.56	Linolenic acid, methyl ester	16.70	24.07	17.19
33	19.57	Ethyl linoleolate	-	23.75	-
34	19.57	7,10,13-Hexadecatrienoic acid, methyl ester	-	-	15.60
35	25.32	(+-)-2,3,6,7-tetramethyl-4,4a α,5,8,8a α,9a β,10,10a β-octahydroanthracen-9(^{1}H)-one (Ⅰ)	-	124.97	-
36	25.33	2,3,6,7-tetramethyl-1,4,4 α,5,8,8a β,9 β,9a α,10 β,10a β-decahydroanthracene-9,10-diol	-	-	49.78
37	25.36	(+-)-2,3,6,7-tetramethyl-4,4a α,5,8,8a α,9a β,10,10a β-octahydroanthracen-9(^{1}H)-one (Ⅱ)	-	-	18.09
38	25.44	Patchoulane	-	-	110.05
39	29.85	γ-Sitosterol	26.94	31.92	18.37
40	29.85	Stigmasterol, 22,23-dihydro-	22.06	27.61	17.18

The compounds with more than 70% of quality when blast with Wiley No.7 Library (John Wiley&Sons, Inc., USA) were demonstrated as the average of relative percentage compared to the internal standard (Methyl heptadecanocate, C_{17}).

Table 2

Effect of GA_3 and PBZ on AD and 14-deoxy-11,12-didehydroandrographolide content, and antimicrobial activity against *S. aureus*.

Treatments	14-deoxy-11,12-didehydroandrographolide content (mg/g DW)	AD content (mg/g DW)	Antimicrobial activity (mm)[2]
Water	0.52±0.19[b]	22.09±4.16[b]	13.20±0.80[a]
GA_3 (10 mg/L)	0.76±0.04[a]	30.47±2.87[a]	14.40±1.40[b]
PBZ (10 mg/L)	0.41±0.06[b]	24.86±2.72[b]	11.80±0.70[c]

Different letters within columns indicate significant differences. Data were expressed as mean±SD (tested by DMRT at $P<0.05$). Inhibition zones (mm) were evaluated from 9 individual replications of the well (8 mm Ø size) filled with plant extracts.

heat map obtained from hierarchical clustering analysis using the Pearson's correlation was highlighted (Figure 2). The compounds which possibly had anti-*S. aureus* activity were 14-deoxy-11,12-didehydroandrographolide, 22,23-dihydro-stigmasterol, caryophyllene oxide, and *trans*-phyt-2-ene. These results were consistent with the anti-*S. aureus* activity of extracts following GA_3 treatment, which had significantly higher levels of DDA (Table 2) than others. Moreover, 22,23-dihydro-stigmasterol and caryophyllene oxide are putative compounds that also play roles in anti-*S. aureus* activity.

To investigate the action mode of AD, the deletion mutant collection of budding yeast *Saccharomyces cerevisiae*, one of major eukaryotic model organisms, was used to screen for genes required for AD resistance (Figure 3). The spot dilution assay of yeast mutants revealed 11 strains sensitive to AD, *i.e.* Δ*sod1*, Δ*ccs1*, Δ*erg6*, Δ*erg2*, Δ*vma4*, Δ*vma3*, Δ*vma22*, Δ*ykl118w*, Δ*vph2*, Δ*ypr123c* and Δ*ycl007c* mutants.

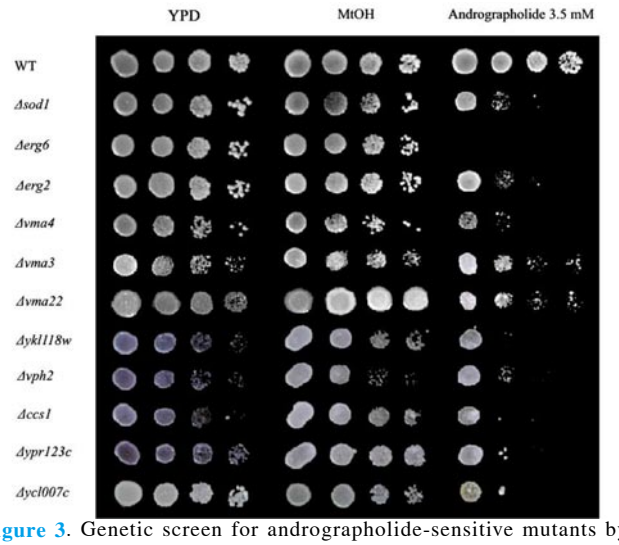

Figure 3. Genetic screen for andrographolide-sensitive mutants by spotting dilution assay on YPD, methanol-supplemented YPD, and 3.5 mM andrographolide-supplemented YPD.

Figure 2. Heat map obtained from hierarchical cluster analysis.

It demonstrates the variations in contents and types of compound in crude extracts of *A. paniculata* exposed to different treatments. The deep red tab represents possible compounds having antimicrobial activity against the human pathogenic bacteria, *S. aureus*.

4. Discussion

Methanol has high polarity that can extract more compounds than other solvent so that the methanol extract might contain other compounds apart from AD. In a previous study of soxhlet extraction, methanol could extract AD from *A. paniculata* 5.5 times over dichloromethane[12]. This is the directly opposite from results described here, which indicate that dichloromethane is the most suitable solvent. Sequential extraction by sonication was conducted in this study as an alternative to soxhlet extraction, and therefore different results might be expected here due to improved extraction efficiency[15]. Also, the compounds were sequentially extracted according to their solubility in each solvent. Thus, this might improve the efficiency of the extraction process since there would be less interference from the sample in the process. This study focused on AD and its derivatives, as well as terpenoid production in *A. paniculata*. Hence, to avoid the interference of other compounds, dichloromethane was used as extraction solvent.

The application of GA_3 could interfere α-tocopherol and ascorbic acid accumulation[16]. Another study on *A. annua* revealed that GA_3 application could induce the upregulation of genes encoding for proposed key enzymes in artemisinin biosynthesis, resulting in higher artemisinin content[17]. The disturbance of metabolic production in hairy root of *A. annua* can induce plant to produce new compounds that have anti-tumor activity against the human tumor cell line of 95-D[18]. Exogenous application of GA_3 may interfere the biosynthetic pathway of terpenoid compounds, leading to alter metabolic flux towards other compounds including AD and its derivatives. On the other hand, PBZ has inhibitory activity against cytochrome P-450 dependent enzymes that can interfere sterol biosynthesis such as sterol 14 -methyl-demethylase and *ent*-kaurene oxidase enzyme in gibberellin biosynthesis[19], but has no significant effect on the variations of AD and its derivative diterpenoids when compared to water treatment. The amount of AD, a diterpene compound, in *A. paniculata* showed enhancements after GA_3 treatment in comparison with the control.

PBZ can block *ent*-kaurenoic acid formation, which is known as the early step of gibberellin biosynthetic pathway via precursor competitive inhibition[20]. It is also reported that application of PBZ could reduce the sterol formation[21]. As presented in this experiment, the accumulations of triterpene and sterol compounds were decreased after treated with PBZ, while sesquiterpenes dramatically increased. It was reported that squalene, sesquiterpene and their intermediate compounds could inhibit 3-hydroxy-3-methylglutaryl-CoA reductase at the limiting step of the mevalonate pathway[21]. GA_3 showed low effect on increasing content of sesquiterpene, apart from the effect on diterpene levels, GA_3 treatment in *A. paniculata* showed controversy phenomena with the previous report of *A. annua*, that leads to increasing levels of a sesquiterpene compound named artemisinin when treated with GA_3[22].

Two sesquiterpenoid compounds, globulol and patchoulane were found only in PBZ treated plants, which have not been reported to be detected in *A. paniculata*. These two compounds have antimicrobial activities and exhibited significant anti-inflammatory activities[23] and have cytotoxic activity in human ovarian cancer cells[19].

The anti-*S. aureus* activity of extracts from GA_3 and PBZ treatments confirms that the external stimulants affected AD and DDA contents, as well as the antimicrobial property of the crude extracts. However, it is possible that other compounds present in the extracts might also contribute to the antibacterial activity.

In our present study, the effect of PBZ to AD and DDA content were not significantly different from control. The anti-*S. aureus* activity of PBZ treatment extracts was significantly decreased when compared to those with GA_3 and water, confirming that the external stimulants affected AD and DDA contents, as well as the antimicrobial property of the crude extracts. However, it is possible that other compounds present in the extracts might also contribute to the antibacterial activity. Various reports have reported the antibacterial activity of 22, 23-dihydro-stigmasterol or β-sitosterol[24] as well as caryophyllene oxide against *S. aureus*[25], although the activity of *trans*-phyt-2-ene, a neophytadiene isomer, has no literature precedence. DDA shows cholangiocarcinoma cell lines[26], but has not been reported to show anti-*S. aureus* activity prior to this study. While evidence suggests that the quantity of DDA in dichloromethane extract affects anti-*S. aureus* activity, the antimicrobial activity of the extracts from treatment with water, GA_3, and paclobutrazol may be a result of synergistic effects arising from various active constituents in these extracts.

Based on these results, the Δ*sod1* and Δ*ccs1* strains are the mutants lacking genes involved in superoxide detoxification. *SOD1* encodes the cytosolic Cu/Zn superoxide dismutase (SOD) while *CCS1* is SOD chaperone functioning in filling copper into Cu/Zn-SOD (*Saccharomyces* genome database, 2014). *ERG2* and *ERG6* are genes involved in ergosterol biosynthesis. *ERG6* encodes Δ(24)-sterol C-methyltransferase enzyme that converts zymosterol to fecosterol while *ERG2* encodes C-8 sterol isomerase that catalyzes the isomerization of fecosterol to episterol in ergosterol biosynthesis[13]. In addition, the Δ*vma4*, Δ*vma3*, Δ*vma22*, and Δ*vph2* strains are mutants lacking genes involved in the assembly and functions of vacuolar H^+-ATPase (V-ATPase), which plays an important role in the maintenance of intracellular pH homeostasis. It seems that AD might interact with the lipid bilayer and disrupt ergosterol biosynthesis. Consequently, the yeast mutants depletedthe ergosterol and accumulate the abnormal sterol, leading to the alteration of permeability and fluidity of yeast membrane. In addition, AD might also indirectly affect the mitochondrial electron transport, causing the generation of surplus ROS, which can further damage ergosterol and perturb the V-ATPase activity[27]. Since ergosterol is also required for V-ATPase activity in homeostasis and integrity, the malfunction of certain genes might lead to the lethality of yeast mutants.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

[1] Mansouri H, Asrar Z, Mehrabani M. Effects of gibberellic acid on primary terpenoids and 9-tetrahydrocannabinol in *Cannabis sativa* at flowering stage. *J Integr Plant Biol* 2009, **51**(6): 553-561.

[2] Pholphana N, Rangkadilok N, Thongnest S, Ruchirawat S, Ruchirawat M, Satayavivad J. Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm.f.) Nees. *Phytochem Anal* 2004; **15**(6): 365-371.

[3] Mishra K, Dash AP, Dey N. Andrographolide: A Novel antimalarial diterpene lactone compound from *Andrographis paniculata* and its interaction with curcumin and artesunate. *J Trop Med* 2011; **2011**: 6.

[4] Rajagopal S, Kumar RA, Deevi DS, Satyanarayana C, Rajagopalan R. Andrographolide, a potential cancer therapeutic agent isolated from *Andrographis paniculata*. *J Experim Ther & Oncol* 2003; **3**(3): 147-158.

[5] Lee KC, Chang HH, Chung YH, Lee TY. Andrographolide acts as an anti-inflammatory agent in LPS-stimulated RAW264.7 macrophages by inhibiting STAT3-mediated suppression of the NF-κB pathway. *J Ethnopharmacol* 2011; **135**(3): 678-684.

[6] Tan HK, Muhammad TST, Tan ML. 14-Deoxy-11,12-didehydroandrographolide induces DDIT3-dependent endoplasmic reticulum stress-mediated autophagy in T-47D breast carcinoma cells. *Toxicol Appl Pharmacol* 2016; **300**(Supplement C): 55-69.

[7] Sule A, Ahmed QU, Latip J, Samah OA, Omar MN, Umar A et al. Antifungal activity of *Andrographis paniculata* extracts and active principles against skin pathogenic fungal strains *in vitro*. *Pharm Biol* 2012; **50**(7): 850-856.

[8] Sule A, Ahmed QU, Samah OA, Omar MN, Hassan NM, Kamal LZM et al. Bioassay guided isolation of antibacterial compounds from *Andrographis paniculata*. *American J Appl Sci* 2011; **8**(6): 525-534.

[9] Taye B, Giday M, Animut A, Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pacific J Trop Biomed* 2011; **1**(5): 370-375.

[10] Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Complement Altern Med* 2006; **6**(1): 1-6.

[11] Worakan P, Karaket N, Maneejantra N, Supaibulwatana K. A phenylurea cytokinin, CPPU, elevated reducing sugar and correlated to andrographolide contents in leaves of *Andrographis paniculata* (Burm. F.) Wall. Ex Nees. *Appl Biochem Biotechnol* 2017; **181**(2): 638-649.

[12] Saeed AI, Bhagabati NK, Braisted JC, Liang W, Sharov V, Howe EA et al. TM4 microarray software suite. In: *Methods in enzymology*. Academic Press; 2006, p. 134-193.

[13] Li W, Mo W, Shen D, Sun L, Wang J, Lu S et al. Yeast model uncovers dual roles of mitochondria in the action of artemisinin. *PLoS Genet* 2005; **1**(3): e36.

[14] Shima J, Ando A, Takagi H. Possible roles of vacuolar H^+-ATPase and mitochondrial function in tolerance to air-drying stress revealed by genome-wide screening of *Saccharomyces cerevisiae* deletion strains. *Yeast* 2008; **25**(3): 179-190.

[15] Dupont S, Lemetais G, Ferreira T, Cayot P, Gervais P, Beney L. Ergosterol biosynthesis: a fungal pathway for life on land? *Evolution* 2012; **66**(9): 2961-2968.

[16] Jadhao D, Thorat B. Purification (crystallization) of biotactive ingredient andrographolide from *Andrographis paniculata*. *World J Pharm Pharm Sci* 2014; **3**(10): 747-763.

[17] Kuppusamy P, Yusoff MM, Parine NR, Govindan N. Evaluation of *in-vitro* antioxidant and antibacterial properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. *Saudi J Biol Sci* 2015; **22**(3):293-301.

[18] Anuradha VE, Jaleel CA, Salem MA, Gomathinayagam M, Panneerselvam R. Plant growth regulators induced changes in antioxidant potential and andrographolide content in *Andrographis paniculata* Wall.ex Nees. *Pest Biochem Physiol* 2010; **98**(2): 312-316.

[19] Banyai W, Mii M, Supaibulwatana K. Enhancement of artemisinin content and biomass in *Artemisia annua* by exogenous GA_3 treatment. *Plant Growth Regul* 2011; **63**(1): 45-54.

[20] Zhai DD, Supaibulwatana K, Zhong JJ. Inhibition of tumor cell proliferation and induction of apoptosis in human lung carcinoma 95-D cells by a new sesquiterpene from hairy root cultures of *Artemisia annua*. *Phytomedicine* 2010; **17**(11): 856-861.

[21] Rademacher W. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. *Ann Rev Plant Physiol Plant Mol Biol* 2000; **51**(1): 501-531.

[22] Ruffing AM. Metabolic engineering of hydrocarbon biosynthesis for biofuel production. In: *Liquid, gaseous and solid biofuels – conversion techniques*. InTech; 2013.

[23] Tricarico PM, Crovella S, Celsi F. Mevalonate pathway blockade, mitochondrial dysfunction and autophagy: A possible link. *Int J Mol Sci* 2015; **16**(7): 16067-16084.

[24] Harkat-Madouri L, Asma B, Madani K, Bey-Ould Si Said Z, Rigou P, Grenier D et al. Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. *Industr Crops Prod* 2015; **78**(Supplement C): 148-153.

[25] Ahn JH, Lee TW, Kim KH, Byun H, Ryu B, Lee KT et al. 6-acetoxy cyperene, a patchoulane-type sesquiterpene isolated from *Cyperus rotundus* rhizomes induces caspase-dependent apoptosis in human ovarian cancer cells. *Phytother Res* 2015; **29**(9): 1330-1338.

[26] Sen A, Dhavan P, Shukla KK, Singh S, Tejovath G. Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from *Momordica charantia*. *Sci Secure J Biotechnol* 2012; **1**(1): 9-13.

[27] Magiatis P, Skaltsounis AL, Chinou I, Haroutounian SA. Chemical composition and *in-vitro* antimicrobial activity of the essential oils of three greek *Achillea* species. *Zeitschrift für Naturforschung C* 2014; **57**(3-4): 287-290.