Banat's Journal of Biotechnology
Contact: web: http://www.bjbabe.ro, e-mail: contact@bjbabe.ro

Chemical composition and antibacterial activities of ethanol extract of *Arisaema langbiangense* rhizome (Araceae)

DOI: 10.7904/2068–4738–XI(21)–12

Hong Thien VAN[1], Ngo Trang VO[1], Ngoc Trang NGUYEN[1], Thao Nguyen LUU[1], Tan Viet PHAM[1], Pham Tan Quoc LE[1*]

[1]*Institute of Biotechnology and Food–technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, VIETNAM*
[*]*Corresponding Author: lephamtanquoc@iuh.edu.vn*

Abstract. *Arisaema langbiangense was* an endemic species recorded in Langbiang mountain, Bidoup–Nui Ba National Park, Lac Duong District, Lam Dong Province, Vietnam. The species was a rare member belonging to Araceae family and there has not been any research on its phytochemical composition and antimicrobial activity. In this study, the phytochemical composition and antibacterial activity of ethanol extract *from A. langbiangense* rhizome was analysed using liquid chromatography coupled with mass spectrometry (LC–MS) and disk diffusion method, respectively. Consequently, 12 compounds were found in the ethanol extract of *A. langbiangense* rhizome and the extract was proved to be able to inhibit the growth of *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*.

Keyword: Antibacterial activity, *Arisaema langbiangense,* phytochemical composition, ethanol extract, LC–MS.

Introduction

Arisaema Martius, including of 200 species in over the world and 21 species in Vietnam, is belonged to the Araceae family [PHAM, 2000; GUSMAN and GUSMAN, 2006; BOYCE *et al.*, 2012; NGUYEN, 2017].

Arisaema is a genus which contains the medicinal plants of the Araceae family and has been used extensively in traditional Vietnamense medicine and the other countries.

The phytochemical composition, antimicrobial and antioxidant activities of the compounds which are extracted from the leaves and the rhizomes of several plants of *Arisaema* genus have been shown by many recent studies [PHAM, 2000; IQBAL *et al.*, 2008; MUBASHIR *et al.*, 2016; AZAM *et al.*, 2016].

Arisaema langbiangense Luu, Nguyen–phi & H.T., was first described by Van [VAN *et al.*, 2016].

The type specimens were collected in Langbiang mountain, Bidoup–Nui Ba National Park, Lac Duong District, Lam Dong Province, Vietnam.

A. langbiangense is the herb evergreen and this species different from others in *Arisaema* genus in having green flesh of rhizome, long pseudostem, forwards bent spadix appendix and 5(6)–lobed obovoid ovaries.

To date, *A. langbiangense* is a rare species and has only been found from the type location.

As the consequence, the number of studies about this species is limited and the bioactivity of this species is still unknown.

In 2018, the specimens of *A. langbiangense* were collected again by Hong Thien Van in location where the type specimens were collected in 2016 [VAN *et al.*, 2017].

In this study, we first determined the phytochemical composition and antibacterial activity of ethanol extract of *A. langbiangense* rhizome, and provided the more information for further application of this species.

Material and methods

Plant material

Samples of *A. langbiangense* were collected from Langbiang mountain, Bidoup–Nui Ba National Park, Lac Duong District, Lam Dong Province, Vietnam, location of about $108^0$26'58''E, 12^0

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2020, XI(21)

0'29''N, December 4, 2018, 1702 m in elevation (Figure 1).

Bacterial strains

The antibacterial activity of the extract was conducted using five bacterial strains, including two Gram–positive bacteria (*Bacillus cereus* (ATCC 11774), *Staphylococcus aureus* (ATCC 25923), and four Gram–negative bacteria (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13976), *Salmonella typhimurium* (ATCC 13311).

Those five studied bacteria were obtained from the microbiology collection, Department of Biotechnology, Institute of Food and Biotechnology, Industrial University of Ho Chi Minh city, Viet Nam.

The strains were maintained in 20 % glycerol solution at 20 °C and activated by cultivation in Luria–Bertani broth at 37 °C for 24 h before the antibacterial activity assay.

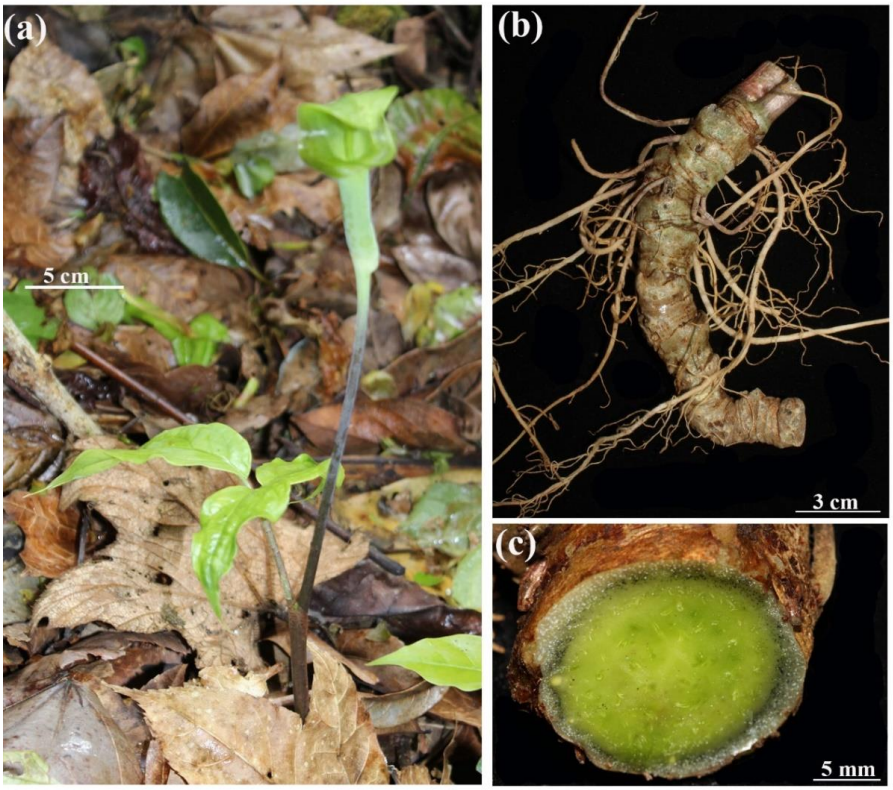

Figure 1. *Arisaema langbiangense*. Habitat (a), Rhizome (b) and Green flesh of rhizome (c)

Methods

The fresh sliced rhizomes were dried at a 50 °C until constant weight and grounded into medicinal powder.

The 100 g of the resulting powder was soaked in 1000 mL of 99 % ethanol for 7 days at the room temperature and filtered to collect the extract.

The extract was then condensed under vacuum pressure at 60 °C to obtain the brown medicinal paste.

To ensure the absolute absence of ethanol in the paste, sublimation dryer was utilized [ALTEMIMI *et al*. 2017].

Liquid chromatography mass spectrometry (LC–MS)

For identification of the components in the extract, LC–MS analysis was performed at the Central Laboratory for Analysis, University of Science, Vietnam National University of Ho Chi Minh City.

An aliquot of ethanol extract was injected into HPLC Agilent 1200 infinity liquid chromatography system (Agilent Technologies, CA, USA) coupled with MicroTOF–QII mass spectrometer (Bruker Daltonics, Germany).

The components were separated on ACE3–C_{18} analytical column (4.6 ×150mm, 3.5 µm) as stationary phase at 40 °C.

The mobile phase used consisted of deionized water with 0.1 % formic acid and acetonitrile with 0.1 % formic acid and the flow rate was set at 0.3 mL/min.

The gradient elution program was summarized in Table 1.

In mass spectrometer, the extract was then ionized using electrospray ionization source (ESI) at positive mode and the mass spectra data were recorded on mode for a mass range 50–2000 m/z.

Data Analysis software (Bruker, Germany) was used for data analysis.

Table 1.

Gradient elution program for the chromatographic separation

Time (min)	Solvent A*	Solvent B*
0	90	10
15	0	100
30	0	100
31	90	10
40	90	10

(*): presented as the percentage of volume of mobile phase, Solvent A: Deionized water with 0.1 % formic acid, Solvent B: Acetonitrile with 0.1 % formic acid

Antibacterial activities

The method by BAUER *et al.* was used to study the antibacterial activity of the ethanol extract of *A. langbiangense* rhizome.

The studied bacteria were inoculated into LB Broth for growing until the turbidity of 0.5 McFarland standards.

100 µL of culture was then spread on the surface of Mueller Hinton plate before a sterile 6mm diameter discs were placed on.

Each disc was added with 20 µL of ethanol extract and the plates were maintained at 4 °C for 2 hours for the extract diffusing into the medium.

The plate was incubated at 37 °C for 24 h before the diameter of the zone of inhibition of tested bacteria was measured.

Sterilize distilled water and Gentamycin antibiotic discs (Nam Khoa BioTek, Viet Nam) were used as positive control [BAUER *et al.*, 1996].

The one–way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) procedure (Stat graphics software (Centurion XV)) were used to analyse experimental results as well as significant differences among the means from triplicate analyses at (p<0.05).

Also, the obtained values were presented in the form of a mean ± standard deviation (SD).

Results and discussion

Phytochemical analysis

The molecular weight of components in the chromatogram of Figure 2 was compared with other studies on species of Araceae and other families as well.

As a result, there were 12 compounds present in the ethanol extract with similar molecular weights with compounds found in previous studies (Table 2).

As seen in table 2, all of 12 compounds reported were present in plants, of which Homalomenol F was a compound found in methanolic paste extract of *Homalomena oculta,* a species of Araceae [HU *et al.*, 2010].

(–)–(2R*,3S*,6S*)–N,2–Dimethyl–3–hydroxy–6–(9–phenyl nonyl) piperidine was discovered by Zhao and collab. as a new piperidine alkaloid from methanol paste extract of *Arisaema decipiens* rhizome.

Similarly, 6–Deacetylnimbin and Nimbin belonging to tetranortriterpenoids were also found in methanolic paste extract from rhizome of *A. decipiens* [ZHAO *et al.*, 2010].

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2020, XI(21)

Arisaema is a genus with large number of species of Araceae in which some species have been applied in medicine family [PHAM, 2000; GUSMAN and GUSMAN, 2006; IQBAL *et al.*, 2008; BOYCE *et al.*, 2012; MUBASHIR *et al.*, 2016; AZAM *et al.*, 2016; NGUYEN, 2017].

However, the investigations on phytochemical composition and biological activity of species belonging to this geneus are still limited.

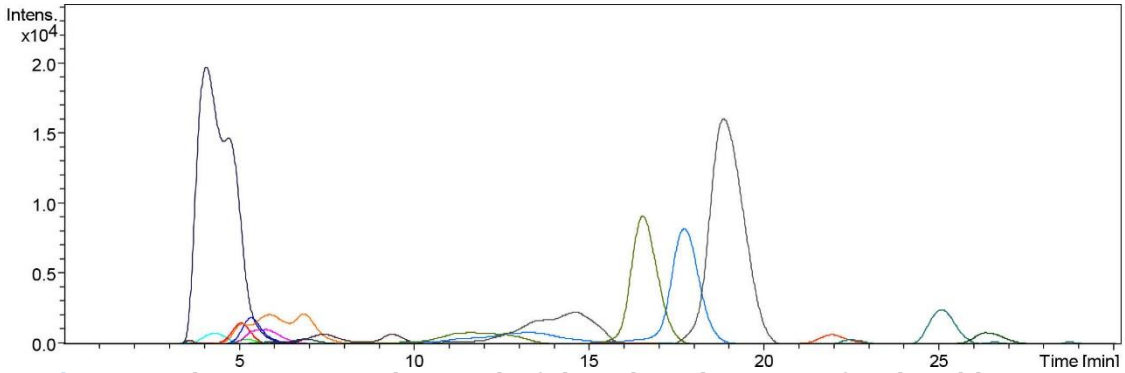

Figure 2. **Chromatogram (LC–MS) of the ethanol extract of *A. langbiangense* rhizomes obtained by maceration**

For example, Jung and collab., determined four new cerebrosides from methanolic paste extract from the rhizome of *Arisaema amurense* [JUNG *et al.*, 1996].

Zhao and collab., found a new piperidine alkaloid in rhizome of *Arisaema decipiens* [ZHAO *et al.*, 2010]. Recently, Chakraborty and collab., have proved analgesic activity of methanolic extract of tubers of *Arisaema tortuosum* [CHAKRABORTY *et al.*, 2018]. From the above research status, we do hope that this study will be a platform for further studies on the phytochemical composition in ethanolic extract from rhizome of *A. langbiangense,* a rare and endemic species of Vietnam with the aim to apply this species into practice in the future.

Table 2.

Phytochemical composition of ethanol extract of *A. langbiangense* rhizome.

No.	Compounds	Molecular weight	References
1	(–)–(2R*,3S*,6S*)–N,2–Dimethyl–3–hydroxy–6–(9–phenylnonyl) piperidine	323	[ZHAO *et al.*, 2010]
2	Homalomenol F	353	[HU *et al.*, 2010]
3	Methyl 2–hydroxyoctade–canoate	310	[JUNG *et al.*, 1996]
4	4–Epi–oplopananol	276	[YANG *et al.*, 2016]
5	n–tritriacontane	464	[TAKABA *et al.*, 1997]
6	Henicosyl acrylate	366	[HAEHNEL *et al.*, 2014]
7	1,13–Tridecanediol	216	[NAKAMURA *et al.*, 1997]
8	6–Deacetylnimbin	498	[ZHAO *et al.*, 2010]
9	Triisodecylamine	437	[OVCHINNIKOV, 2013]
10	1–Triacontanol	438	[YOON *et al.*, 2007]
11	Cerotic acid	497	[YIDIZ *et al.*, 2016]
12	Nimbin	542	[ZHAO *et al.*, 2010]

Antibacterial activity

Data stated in Table 3 and Figure 3 showed that the paste extract was able to resist against five bacteria studied.

In general, zones of inhibition were completely transparent in the six bacteria.

The best antibacterial activity of *A. langbiangense* was shown in undiluted paste extract with zones of inhibition for *Salmonella typhimurium* (14.3±0.6) (Figure 3E), *E. coli* (11.6±0.3 mm) (Figure 3B), *B. cereus* (10.8±0.8 mm) (Figure 3A), *P. aeruginosa aureus* (9.8±0.3 mm), *S. aureus* (9.5±0.5 mm) (Figure 3F) and *S. enterritidis* (9.5±0.5 mm) (Figure 3D).

Meanwhile, among different dilute samples, only the two–fold dilute sample showed the antibacterial activity against *B. cereus* and *P. aeruginosa* in which their zones of inhibition were 9.6±0.3 mm and 8.3±0.6 mm, respectively (Figure 3A and Figure C).

Table 3.

The inhibition zone of ethanol extract of *A. langbiangense* rhizome against six tested bacteria. (–): No inhibition

Tested bacteria	Growth inhibition zone (mm)			
	Original extract	2–fold diluted extract	4–fold diluted extract	6–fold diluted extract
B. cereus	10.8±0.8[a]	9.6±0.3[a]	–	–
E. coli	11.6±0.3	–	–	–
P. aeruginosa	9.8±0.3[a]	8.3 ±0.6[a]	–	–
S. enterritidis	9.5±0.5	–	–	–
S. typhimurium	14.3±0.6	–	–	–
S. aureus	9.5±0.5	–	–	–

As aforesaid in the introduction, *A. langbiangense* is a rare and endemic to Vietnam and was only first described in 2016 [VAN *et al.*, 2017].

This paper, therefore, was the first research showing the phytochemical composition and the antibacterial activity of paste extract from the rhizome of *A. langbiangense.*

However, certain previous studies showed the antibacterial activity of paste extract from species belonging to genus *Arisaema.*

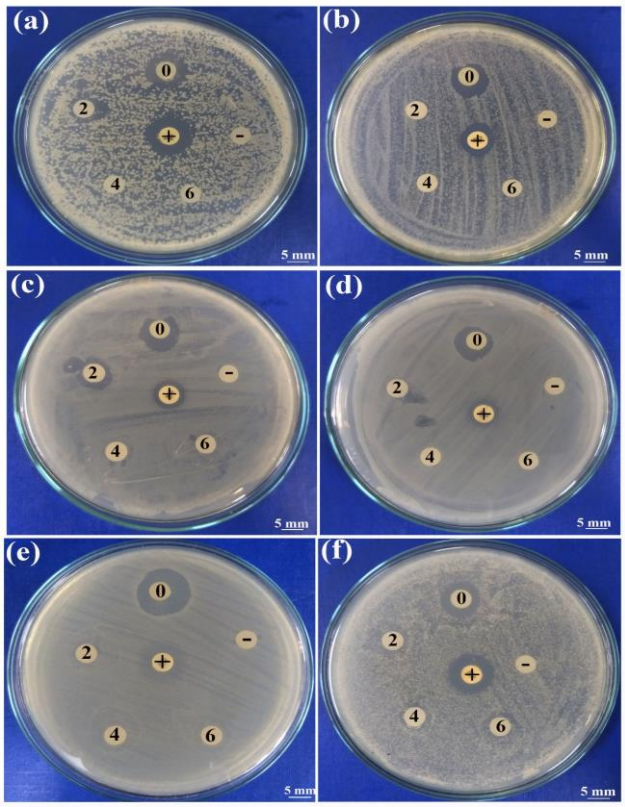

Figure 3. Antibacterial activity of ethanol extract of *A. langbiangense* rhizome against tested bacteria. *Bacillus cereus* (a), *E. coli* (b), *Pseudomonas aeruginosa* (c), *Salmonella enteritidis* (d), *Salmonella typhimurium* (e), and *Staphylococcus aureu* (f).

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2020, XI(21)

The paper discs numbered as 0, 2, 4, 6 were loaded with original extract, two–fold, four–fold, and six–fold diluted extracts, respectively.

The sample of ethanol extract was diluted by 5 % dimethyl sulfoxide (DMSO). (–) Negative control with sterilized distilled water, (+) Positive control with discs containing gentamicin.

For instance, Azam and collab., proved the methanolic paste extract from the rhizome of *A. tortuosum* was able to resist against 9 bacterial and 5 fungal strains studied [AZAM *et al.*, 2016].

In another study, Bibi and collab., demonstrated that methanolic paste extract from the rhizome of *A. flavum* was able resist against 5 bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus, Salmonella Setubal* and *Pseudomonas pickettii* [BIBI *et al.*, 2011].

Similarly, Mubashir and Shah showed that paste extracts (petroleum ether, chloroform and methanol) from rhizome of *A. propinquum* were resistant to 5 bacterial strains, such as *P. aeruginosa, P. vulgaris, S. aureus, B. subtilis* and *S. epidermidis* [MUBASHIR and SHAH, 2012].

Conclusions

In this study, 12 compounds were first identified in ethanol extract of *A. langbiangense* rhizome.

The antimicrobial activity of the ethanol extract against 6 bacterial strains, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enteritidis,* and *Salmonella typhimurium* was demonstrated.

The result of this study is a platform for further application of *A. langbiangense.*

Acknowledgements

We are grateful to staff of Tay Nguyen Institute for Scientific Research, especially Mr. Vu Kim Cong for his help in the field. We also thank Mr. Tran Ngoc Buu and Ms. Ho Nguyen Hoang Yen for their helpful cooperation.

References

1. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, **2017**. 6, 1–23.
2. Azam, S.; Saqib, M.S.; Zar, F.; Ahmad, B.; Khan, I.; Zeb, Z.; Khan, I. Antibacterial, antifungal, phytotoxic, antioxidant and hemagglutination activities of organic fractions of *Arisaema tortuosum*. *Pakistan Journal of Pharmaceutical Sciences*, **2016**. 29(3), 991–997.
3. Bauer, A.W.; Kirby; W.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* **1996**. 45, 493–496.
4. Bibi, Y.; Nisa, S.; Chaudhary, F.M. Antibacterial activity of some selected medicinal plants of Pakistan. *BMC Complementary and Alternative Medicine*, **2011**. 11, 52–62.
5. Boyce, P.C.; Sookchaloem, D.; Hetterscheid, W.L.A.; Gusman, G.; Jacobsen, N.; Idei, T.; Nguyen V.D. Araceae. *The Flora of Thailand,* **2012**. 11, 101–321.
6. Chakraborty, P.; Bala, N.N.; Das S. Analgesic activity of methanolic extract of tubers of *Arisaema tortuosum* (Wall.) Schott. in Swiss Albino Mice. *Dhaka University Journal of Pharmaceutical,* **2018**. 17, 37–41.
7. Gusman, G.; Gusman, L. *The genus Arisaema–A monograph for botanists and nature lovers*. 2nd ed. A.R.G, Gantner Verlag Kommanditgesellschaft, Ruggell, Lichtenstein, **2006**.
8. Haehnel, A.P.; Baumann, M.S.; Arens, L.; Misske, A.M.; Fleischhaker, F.; Kowollik, C.B. Global Trends for K_p? The Influence of ester side chain topography in Alkyl (Meth) acrylates−completing the data base. *Macromolecules*, **2014**. 47, 3483–3496.
9. Hu, Y.M.; Liu, C.; Cheng, K.W.; Sung, H.H.Y.; Williams, L.D.; Yang, Z.L.; Ye, W.C. Sesquiterpenoids from *Homalomena occulta* affect osteoblast proliferation, differentiation and mineralization in vitro.

Phytochemistry, **2008**. 69, 2367–2373.
10. Iqbal, M.; Bakht, J.; Shafi, M. Phytochemical screening and antibacterial activity of different solvent extracted samples of *Arisaema jacquemontii*. *Pakistan Journal of Pharmaceutical Sciences*, **2008**. 31(1), 75–81.
11. Jung, J.H.; Lee, H.; Kang S.S. Diacylglyceryl galactosides from *Arisaema amurense. Phytochemistry*, **1996**. 42(2), 447–452.
12. Mubashir, S.; Shah, W.A. *Arisaema propinquum*–a plant with potential as antibacterial, antioxidant and anticancer agent. *Drug Invention Today*, **2012**. 4(2), 345–347.
13. Nakamura, N.; Tanihara, Y.; Takayama, T. 1,13–Tridecanediol. Organic Compounds. *Acta Crystallographica*, **1997**. 53, 253–255.
14. Nguyen, V.D. *Flora of Vietnam–Araceae Juss*. Publishing House for Sciense and Technology, **2017**.
15. Ovchinnikov, V.V. Thermochemistry of heteroatomic compounds: analysis and calculation of thermodynamic functions of organic compounds of V–VII groups of mendeleev's periodic table. *American Journal of Physical Chemistry*, **2013**. 2, 60–71.
16. Pham, H.H. *Araceae. In: Pham–hoang H. (ed.), An Illustrated Flora of Vietnam,* Youth Publishing House, Ho Chi Minh City, Vietnam, **2000**.
17. Takaba, K.; Hirose, M.; Yoshida, Y.; Kimura, J. Effects of n–tritriacontane–16,18–dione, curcumin, chlorphyllin, dihydroguaiaretic acid, tannic acid and phytic acid on the initiation stage in a rat multi–organ carcinogenesis model. *Cancer Letters*, **1997**. 113, 39–46.
18. Van, H.T.; Nguyen P.N. Luu H.T. A new species of *Arisaema* (Araceae) from Vietnam. *Phytotaxa*, **2016**. 277(1), 90–94.
19. Yang, J.L.; Zhao, Y.M.; Shi, Y.P. Sesquiterpenoids from the Rhizomes of *Homalomena occulta. Natural Products and Bioprospecting*, **2016**. 6(4), 211–216.
20. Yildiz, Y.; Dasgupta, M. Unsaponifiable matter in Carnuba (*Cera carnuba*) wax, a modification of the USP/NF and FCC methods. *American Journal of Analytical Chemistry*, **2016**. 7, 611–616.
21. Yoon, S.J.; Ikeda, S.; Sadilek, M.; Hakomori, S.; Ishida, H.; Kiso, M. Self–recognition of N–linked glycans with multivalent GlcNAc, determined as ceramide mimetic conjugate. *Glycobiology,* **2007**. 17(9), 1007–1014.
22. Zhao, F. W.; Luo, M.; Wang, Y.H.; Li, M.L.; Tang, G.H.; Long, C.L. A piperidine alkaloid and limonoids from Arisaema decipiens, a traditional antitumor herb used by the Dong people. *Archives of Pharmacal Research*, **2010**. 33, 1735–1739.

Received: November 21, 2019
Article in Press: April 30, 2020
Accepted: Last modified on: May 20, 2020