

SCREENING ANTIBACTERIAL ACTIVITY OF VIETNAMESE PLANT EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

LONG HOANG NGO¹, THI HAI YEN NGUYEN², VU KHAC TRAN³, VU VAN DOAN⁴, MINH VAN NGUYEN⁵, HIEU TRUNG BUI^{1*}

¹VK-Tech Center, Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City. ²Faculty of Science, Dong Nai University, Bien Hoa City. ³Department of Pharmaceutical Chemistry and Pesticides Technology, School of Chemical Engineering, Hanoi University of Science and Technology. ⁴Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam. ⁵Division of Biotechnology, College of Environmental and Bioresource Sciences, Chonbuk National University, Jeollabuk-do, Korea. Email: bthieu@ntt.edu.vn

Received: 18 September 2020, Revised and Accepted: 07 April 2021

ABSTRACT

Objectives: Infectious diseases caused by bacteria are a leading cause of death worldwide. Hence, the objectives of the study are aimed to evaluate the antibacterial activity against five human pathogenic bacteria of methanolic extracts from 66 plants collected from Vietnam.

Methods: The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of methanol extracts of 66 plant species against five bacterial strains.

Results: In this study, all the plant extracts were active against at least one strain with MIC values ranging from 24 to 2048 µg/mL. Twenty-five plant extracts were active against all three Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*). Of these, the extracts of *Macaranga trichocarpa* (Rchb. f. and Zoll.) Mull. Arg. (*Euphorbiaceae*), *Calophyllum inophyllum* L. (*Clusiaceae*) and *Caryodaphnopsis baviensis* (Lecomte) Airy Shaw (*Lauraceae*) exhibited the highest antibacterial activity (MIC =24–128 µg/mL), followed by extracts of *Betula alnoides* Buch-Ham. e x . D. Don (*Betulaceae*), *Acronychia pedunculata* (L.) Miq. (*Rutaceae*), *Croton alpinus* A. Chev. ex Gagnep. (*Euphorbiaceae*) (MIC =64–256 µg/mL). Furthermore, the extract of *Rhus chinensis* Mill. (*Anacardiaceae*) and *Annona reticulata* L. (*Annonaceae*) exhibited potent antibacterial activity against the two *Bacillus* species (MIC =32–64 µg/mL).

Conclusion: Results of this study reveal that plant extracts from Vietnam have highly antibacterial activity against Gram-positive bacteria. These results suggest that Vietnamese plant extracts may be a rich source of antibacterial drugs.

Keywords: Screening, Antibacterial, Human pathogenic bacteria, Vietnamese plant extracts.

© 2021 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2021v14i6.39790>. Journal homepage: <https://innovareacademics.in/journals/index.php/ajpcr>

INTRODUCTION

Infectious diseases remain a major health concern, being the second leading cause of death worldwide, and remain a dominant feature of domestic and international public health considerations in the 21st century [1,2]. Bacterial infections are prevalent in developing countries due to factors such as inadequate sanitation, poor hygiene, and overcrowded living conditions [3]. Antibiotics have proven to be powerful drugs for control of infectious diseases and remain one of the most important discoveries in modern medicine [4]. At present, the world is facing the widespread emergence of bacterial resistance to antibiotics [2]. Antibiotic resistance has been recognized by the World Health Organization as the greatest threat in the treatment of infectious diseases [4].

To combat antibiotic resistance, the development of new antibacterial agents that suppress bacterial resistance mechanisms is necessary. Plants have traditionally provided a source of new chemicals, and numerous clinical studies have demonstrated the therapeutic value of molecules of plant origin [4]. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants as traditional antibiotics [5]. Indeed, higher plant-derived products represent ~25% of drugs in current clinical use [4].

Considering the therapeutic potential of plants, the aim of the present study was to evaluate the *in vitro* antibacterial activity of Vietnamese plant extracts against five human pathogenic bacteria, *Escherichia*

coli, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*, which cause food poisoning and various infections in the community and in hospitals [6,7].

METHODS

Plant materials and extraction

A total of 68 plant leaves and branches were collected from different locations in Vietnam in 2012. Plant species were identified by Associate Prof. Xuan Phuong Vu, Dr. The Bach Tran and Dr. The Cuong Nguyen from the Institute of Ecology and Biological Resources, Vietnam. Voucher specimens have been preserved in the Herbarium of the Department of Phytochemistry and Research and Development Center of Bioactive Compounds, Vietnam Institute of Industrial Chemistry. Plant extracts were prepared as described in our previous study [8]. Briefly, air-dried and powdered aerial parts of the plant species (10 g) were extracted twice with 100 mL of methanol for 48 h at room temperature. Extracts were filtered and the filtrates were evaporated to dryness using a rotary evaporator, and then stored at -20°C until further use. Then, for the antibacterial activity assays, the extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and stored at 4°C as stock solutions.

Bacterial culture

Five bacterial species, comprising three Gram-positive (*B. cereus* American Type Culture Collection [ATCC] 21768, *B. subtilis* ATCC 6633, and *S. aureus* ATCC 6538) and two Gram-negative (*E. coli* ATCC

25922 and *P. aeruginosa* ATCC 9027) strains, were purchased from the (ATCC; Manassas, VA, USA). Bacterial strains were cultured on nutrient agar plates and incubated aerobically at 37°C for 24 h. For *in vitro* antibacterial assays, the bacteria were cultured in nutrient broth (NB) and incubated aerobically at 37°C for 24 h, and the bacterial suspension was adjusted to 10⁵ cfu/ml.

Antibacterial assay

The antibacterial activities of plant extracts were evaluated by broth microdilution assays and minimum inhibitory concentration (MIC) values were determined. Twofold serial dilutions of each plant extract were added to the wells of sterile 96-well plates containing 100 µl of inoculated NB medium. The final concentrations of the plant extracts were 15.6–2000 µg/ml and the final bacterial density was 10⁵ cfu/ml. DMSO (2%) was used as a negative control and did not affect bacterial growth; streptomycin sulfate was used as a positive control. The plates were incubated at 37°C for 24 h. The MIC values were determined as the lowest concentration that completely inhibited the growth of bacteria.

For weakly soluble plant extracts, the MIC was determined following addition of 20 µl of 0.2 mg/ml iodinitrotetrazolium chloride (Sigma-Aldrich, Korea) to the test wells and incubation at 37°C for 3 h. Viable bacteria reduce this yellow dye to pink [8]. Experiments were conducted twice in triplicate for each extract against individual bacterial species at all test concentrations.

RESULTS AND DISCUSSION

A total of 68 plant extracts were evaluated for antibacterial activity against five human pathogenic bacteria using the broth microdilution method. The plant extracts exhibited considerable antibacterial activity against the Gram-positive bacteria. *B. cereus* exhibited the greatest susceptibility to all of the plant extracts, followed by *B. subtilis* and *S. aureus*. Twenty-four plant extracts had the antibacterial activity against all three Gram-positive bacteria. Of these, the extracts of *Macaranga trichocarpa*, *Calophyllum inophyllum*, and *Caryodaphnopsis baviensis* had the highest antibacterial activity (MICs 31.3–125 µg/ml). Extracts of *Acronychia pedunculata*, *Betula alnoides*, *Croton alpinus*, *Garcinia cowa*, and *Annona reticulata* showed slightly weaker antibacterial activity (MICs 62.5–250 µg/ml). To the best of our knowledge, this is the first report of antibacterial activity of *C. alpinus*. In contrast, antibacterial activities and phytochemistry have been described for *A. pedunculata* [9], *B. alnoides* [10], *G. cowa* [11–13], and *A. reticulata* [14,15]. Furthermore, the extract of *Rhus chinensis* exhibited potent antibacterial activity against *Bacillus* species with MIC values of 31.3 µg/ml (Table 1). Conversely, most of the test plant extracts were inactive against the two Gram-negative bacteria. Only the extract of *C. inophyllum* at a concentration of 2000 µg/ml completely inhibited the growth of *E. coli* (Table 1).

In the following sections, the plants showing the greatest activities against Gram-positive bacteria are discussed in terms of their known phytochemical components, together with biological and pharmacological activities.

C. inophyllum

All parts of *C. inophyllum* have considerable therapeutic properties, such as antiseptic, expectorant, diuretic, astringent, and purgative effects [16]. The fruit oil is beneficial in treatment of rheumatism, gonorrhoea, and itching, the gum extracted from the stems is used to treat ulcers, and a decoction from the bark is used for hemorrhage and ulcers [17]. Oil of seeds and roots is used to treat wounds and scabies [16]. Phytochemical components of this plant have been reported previously [17]. Extracts from leaves and other parts exhibited antibacterial activities against various bacteria as described in the previous reports [16,18]. In our study, the methanol extract of leaves and branches of *C. inophyllum* had marked antibacterial activity against Gram-positive bacteria (MICs 31.3–62.5 µg/ml) and at 2000 µg/ml completely inhibited the growth of *E. coli* (Table 1).

C. baviensis

In this study, the methanol extract of *C. baviensis* exhibited significant antibacterial activity against Gram-positive bacteria (MICs 62.5–125 µg/ml) (Table 1). At present, there is lack of scientific documentation on the pharmacological and biological activities, as well as the phytochemical components, of *C. baviensis*. To the best of our knowledge, isolation of compounds from this plant has been reported only by Anh et al. [19], and this is the first report of antibacterial activity of an extract of *C. baviensis*.

M. trichocarpa

The methanol extract of *M. trichocarpa* showed the strongest antibacterial activity against three Gram-positive bacteria among the 68 plant extracts tested in this study (MICs 31.3–62.5 µg/ml) (Table 1). Leaves of some *Macaranga* species are used in folk medicine to treat swellings, cuts, sores, boils, and bruises. This genus is reported to be a rich source of isoprenylated, geranylated, and farnesylated flavonoids. Flavonoids and stilbenes are major constituents and most likely responsible for the activities of plants of this genus [20]. Flavanones and dihydrochalcones have been reported to show antibacterial activities against various bacterial species, including *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* [21]. However, the methanol extract of *M. trichocarpa* did not inhibit the growth of *E. coli* and *P. aeruginosa* at the maximum concentration tested (2000 µg/ml) (Table 1).

R. chinensis

This plant has long been used by practitioners of folk medicine in Asia. *R. chinensis* plant parts, particularly the galls on its leaves, *Galla chinensis*, have preventative and therapeutic effects on diverse ailments, including diarrhea, dysentery, rectal and intestinal cancer, diabetes mellitus, sepsis, oral diseases, and inflammation. Phytochemical studies on *R. chinensis* have demonstrated that it contains high levels of two phenolic compounds, gallic acid and methyl gallate. Recent studies revealed that *R. chinensis* compounds possess strong antiviral, anticancer, hepatoprotective, and antioxidant activities. Extracts from *G. chinensis* inhibited several bacteria, including *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, and others (MICs 0.5–8 mg/ml) [22]. In our study, the methanol extract of *R. chinensis* exhibited considerable antibacterial activity against the Gram-positive bacteria, especially against the two *Bacillus* species (MIC 31.3 µg/ml) (Table 1).

CONCLUSION

The Vietnamese plant extracts investigated in this study significantly suppressed the growth of Gram-positive bacteria. Discovery of biological activities in Vietnamese plants is a new venture. Although the antibacterial activities of some highly antibacterial plant extracts have been reported, *C. baviensis* and *C. alpinus* extracts have not previously been reported to show potent antibacterial activities.

Our results provide important information on the antibacterial activities of Vietnamese plant extracts to medical plant consumers, pharmacologists and researchers. Some Vietnamese plant extracts have potential for application as natural antibacterial agents and can be used for the development of new antibacterial drugs.

To develop new plant-derived antibacterial agents, further studies are necessary to isolate and characterize the active components from the antibacterial plants. Furthermore, additional research on combinations of the antibacterial components or plants with other antimicrobial agents would be useful to enhance their antibacterial potency.

ACKNOWLEDGMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 103.99-2020.67 and Nguyen Tat Thanh University (project No 2020.01.001). We thank Associate Prof. Phuong Xuan Vu and Dr. Bach The Tran at the Institute of Ecology and Biological Resources, Vietnam, for identifying plant materials.

Table 1: Antibacterial activities of plant extracts against human pathogenic bacteria^a

No.	Plant species	Family	MIC ($\mu\text{g/mL}$) ^b		
			<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
1.	<i>Barleria prionotis</i> L.	Acanthaceae	75±26	128±0	512±0
2.	<i>Ptyssiglottis kunthiana</i> (Wall. ex Nees) B. Hansen	Acanthaceae	597±209	- ^c	-
3.	<i>Alangium chinense</i> (Lour.) Harms	Alangiaceae	171±66	-	-
4.	<i>Rhus chinensis</i> Mill.	Anacardiaceae	32±0	32±0	427±132
5.	<i>Toxicodendron succedaneum</i> (L.) Kuntze	Anacardiaceae	1024±0	768±280	1024±0
6.	<i>Annona reticulata</i> L.	Annonaceae	64±0	64±0	384±140
7.	<i>Acorus gramineus</i> Soland. Ex Ait.	Araceae	1195±418	1877±418	2048±0
8.	<i>Amorphophallus paeoniifolius</i> (Dennst.) Nicolson	Araceae	128±0	256±0	-
9.	<i>Cryptolepis buchananii</i> Roem. and Schult.	Asclepiadaceae	341±132	-	-
10.	<i>Chromolaena odorata</i> (L.) King and Robinson	Asteraceae	-	-	768±280
11.	<i>Eupatorium fortunei</i> Turcz.	Asteraceae	384±140	-	-
12.	<i>Tithonia diversifolia</i> (Hemsl.) A. Gray	Asteraceae	-	512±0	768±280
13.	<i>Betula alnoides</i> Buch.-Ham. ex D. Don	Betulaceae	64±0	64±0	256±0
14.	<i>Markhamia stipulata</i> (Wall.) Seem. Ex K. Schum.	Bignoniaceae	384±140	-	-
15.	<i>Spathodea campanulata</i> P. Beauv.	Bignoniaceae	384±140	-	-
16.	<i>Plumbago zeylanica</i> L.	Plumbaginaceae	192±70	-	-
17.	<i>Canarium tramdenum</i> Dai and Yakovl.	Burseraceae	384±140	-	-
18.	<i>Calophyllum inophyllum</i> L.	Clusiaceae	32±0	53±17	64±0
19.	<i>Garcinia cowa</i> Roxb.	Clusiaceae	256±0	256±0	384±140
20.	<i>Quisqualis indicca</i> L.	Combretaceae	1024±0	512±0	1024±0
21.	<i>Rourea harmandiana</i> Pierre	Connaraceae	768±280	-	-
22.	<i>Hopea chinensis</i> (Merr.) Hand. Mazz	Dipterocarpaceae	48±18	512±0	128±0
23.	<i>Croton alpinus</i> A. Chev. ex Gagnep.	Euphorbiaceae	128±0	128±0	192±70
24.	<i>Endospermum chinense</i> Benth.	Euphorbiaceae	384±140	-	-
25.	<i>Euphorbia atoto</i> Forst. f.	Euphorbiaceae	384±140	-	-
26.	<i>Macaranga trichocarpa</i> (Rchb. f. and Zoll.) Mull. Arg.	Euphorbiaceae	64±0	24±9	24±9
27.	<i>Mallotus yunnanensis</i> Pax and K. Hoffm.	Euphorbiaceae	768±280	-	-
28.	<i>Albizia viableana</i> Pierre	Fabaceae	192±70	-	-
29.	<i>Cassia siamea</i> Lam.	Fabaceae	768±280	-	-
30.	<i>Castanopsis ceratocantha</i> Rehd. and Wils.	Fabaceae	256±0	512±0	768±280
31.	<i>Indigofera galeoides</i> DC.	Fabaceae	192±70	-	-
32.	<i>Lithocarpus ducampii</i> (Hickel and A. Camus) A. Camus	Fagaceae	384±140	-	-
33.	<i>Millettia setigera</i> Dunn	Fabaceae	384±140	-	-
34.	<i>Lysionotus chingii</i> Chun ex W.T. Wang	Gesneriaceae	149±52	512±0	683±264
35.	<i>Caryodaphnopsis baviensis</i> (Lecomte) Airy Shaw	Lauraceae	64±0	128±0	64±0
36.	<i>Litsea verticillata</i> Hance	Lauraceae	1195±418	1365±529	-
37.	<i>Barringtonia macrostachya</i> (Jack) Kurz	Lecythidaceae	1024±0	299±105	597±209
38.	<i>Magnolia coco</i> (Lour.) DC	Magnoliaceae	384±140	-	-
39.	<i>Manglietia insignis</i> (Wall.) Bl.	Magnoliaceae	768±280	-	-
40.	<i>Stachyphrynium placentarium</i> (Lour.) Clausager and Borchs.	Maranthaceae	768±280	-	-
41.	<i>Chisocheton cumingianus</i> subsp. <i>balansae</i> (C. DC.) Mabb.	Meliaceae	171±66	-	-
42.	<i>Melia azedarach</i> L.	Meliaceae	1365±529	2048±0	1195±418
43.	<i>Toona sureni</i> (Blume) Merr.	Meliaceae	341±132	-	-
44.	<i>Knema mixta</i> W. J. de Wilde	Myristicaceae	683±264	-	-
45.	<i>Myrsine linearis</i> (Lour.) Poir.	Myrsinaceae	597±209	853±264	683±264
46.	<i>Olax imbricata</i> Roxb.	Olacaceae	1195±418	597±209	1024±0
47.	<i>Piper hymenophyllum</i> Miq.	Piperaceae	1365±529	-	-
48.	<i>Rhamnus longipes</i> Merr. and Chun	Rhamnaceae	512±0	256±0	256±0
49.	<i>Ziziphus attopensis</i> Pierre	Rhamnaceae	192±70	-	-
50.	<i>Carallia brachiata</i> (Lour.) Merr.	Rhizophoraceae	341±132	1195±418	-
51.	<i>Coptosapelta flavescens</i> Korth.	Rubiaceae	128±0	256±0	-
52.	<i>Acronychia pedunculata</i> (L.) Miq.	Rutaceae	75±26	256±0	171±66
53.	<i>Clausena indica</i> (Dalzell) Oliv.	Rutaceae	384±140	-	-
54.	<i>Glycosmis petelotii</i> Guill.	Rutaceae	192±70	-	-
55.	<i>Luvunga sarmentosa</i> (Blume) Kurz	Rutaceae	192±70	-	-
56.	<i>Zanthoxylum nitidum</i> (Roxb.) DC.	Rutaceae	341±132	853±264	512±397
57.	<i>Scrophularia ningpoensis</i> Hemsl.	Scrophulariaceae	1024±0	1707±529	1365±529
58.	<i>Brucea javanica</i> (L.) Merr.	Simaroubaceae	683±264	2048±0	384±140
59.	<i>Brucea mollis</i> Wall. ex Kurz	Simaroubaceae	768±280	-	-
60.	<i>Sonneratia caseolaris</i> (L.) Engl.	Sonneratiaceae	683±264	683±264	-
61.	<i>Sterculia hyposticta</i> Miq.	Sterculiaceae	768±280	-	-
62.	<i>Schima surpeba</i> Gardner and Champ.	Theaceae	-	1024±0	384±140
63.	<i>Aphananthe aspera</i> (Thunb.) Planch.	Ulmaceae	768±280	-	-
64.	<i>Holoptelea integrifolia</i> Roxb. Planch.	Ulmaceae	384±140	-	-
65.	<i>Callicarpa dichotoma</i> (Lour.) Raeusch.	Verbenaceae	256±0	256±0	683±264
66.	<i>Tectona grandis</i> L.	Verbenaceae	128±0	384±140	-
	Streptomycin sulfate (positive control)		5±0	2.5±0	5±0

^aAll plant extracts were inactive against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) at maximum tested concentration (2048 $\mu\text{g/mL}$). ^bValues are mean±SD of two experiments with three replicates each, ^cInactive at maximum tested concentration (2048 $\mu\text{g/mL}$).

CONFLICT OF INTERESTS

All authors declare that they have no competing interests.

REFERENCES

1. Fauci AS. Infectious diseases: Considerations for the 21st century. *Clin Infect Dis* 2001;32:675-85.
2. Noumedem JA, Mihasan M, Lacmata ST, Stefan M, Kuate JR, Kuete V. Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria. *BMC Complement Altern Med* 2013;13:26.
3. Kannathasan K, Senthilkumar A, Venkatesalu V. *In vitro* antibacterial potential of some *Vitex* species against human pathogenic bacteria. *Asian Pac J Trop Med* 2011;4:645-8.
4. Abreu AC, McBain AJ, Simões M. Plants as sources of new antimicrobials and resistance-modifying agents. *Nat Prod Rep* 2012;29:1007-21.
5. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999;12:564-82.
6. de Barros Machado T, Leal IC, Kuster RM, Amaral AC, Kokis V, de Silva MG, et al. Brazilian phytopharmaceuticals--evaluation against hospital bacteria. *Phytother Res* 2005;19:519-25.
7. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. *Food Control* 2010;21:1199-218.
8. Vu TT, Kim H, Tran VK, Le Dang Q, Nguyen HT, Kim H, et al. *In vitro* antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria. *BMC Complement Altern Med* 2016;16:32.
9. Lesueur D, De Rocca Serra D, Bighelli A, Hoi TM, Thai TH, Casanova J. Composition and antimicrobial activity of the essential oil of *Acronychia pedunculata* (L.) Miq. from Vietnam. *Nat Prod Res* 2008;22:393-8.
10. Ghimire BK, Tamang JP, Yu CY, Jung SJ, Chung IM. Antioxidant, antimicrobial activity and inhibition of α -glucosidase activity by *Betula alnoides* Buch. bark extract and their relationship with polyphenolic compounds concentration. *Immunopharmacol Immunotoxicol* 2012;34:824-31.
11. Auranwiwat C, Trisuwan K, Saiai A, Pyne SG, Ritthiwigrom T. Antibacterial tetraoxygenated xanthenes from the immature fruits of *Garcinia cowa*. *Fitoterapia* 2014;98:179-83.
12. Siridechakorn I, Phakhodee W, Ritthiwigrom T, Promgool T, Deachathai S, Cheenpracha S, et al. Antibacterial dihydrobenzopyran and xanthone derivatives from *Garcinia cowa* stem barks. *Fitoterapia* 2012;83:1430-4.
13. Negi PS, Jayaprakasha GK, Jena BS. Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria. *LWT Food Sci Technol* 2008;41:1857-61.
14. Jamkhande PG, Wattamwar AS. *Annona reticulata* Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties. *J Tradit Complement Med* 2015;5:144-52.
15. Jamkhande PG, Wattamwar AS, Kankudte AD, Tidke PS, Kalaskar MG. Assessment of *Annona reticulata* Linn. leaves fractions for *in vitro* antioxidative effect and antimicrobial potential against standard human pathogenic strains. *Alex J Med* 2016;52:19-25.
16. Ali SM, Perveen S, Rizwani GH, Ahmad VU. Screening for the antimicrobial properties of the leaves of *Calophyllum inophyllum* Linn. (Guttiferae). *J Chem Soc Pak* 1999;21:174-8.
17. Cechinel Filho V, Meyre-Silva C, Niero R. Chemical and pharmacological aspects of the genus *Calophyllum*. *Chem Biodivers* 2009;6:313-27.
18. Saravanan R, Dhachinamoorthi D, Senthilkumar K, Thamizhvanan K. Antimicrobial activity of various extracts from various parts of *Calophyllum inophyllum* L. *J Appl Pharm Sci* 2011;1:102-6.
19. Nguyen Hoang A, Ripperger H, Porzel A, Tran Van S, Adam G. Neolignans from *Caryodaphnopsis baviensis*. *Phytochemistry* 1997;46:569-71.
20. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: A review. *J Med Plant Res* 2014;8:489-503.
21. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Z Naturforsch C Biosci* 2014;69:375-80.
22. Djakpo O, Yao W. *Rhus chinensis* and *Galla Chinensis*--folklore to modern evidence: Review. *Phytother Res* 2010;24:1739-47.