STUDIES ON THE ANTIBACTERIAL ACTIVITY OF *DENDROCALAMUS ASPER* BAMBOO EXTRACTS FROM DIFFERENT PARTS

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Abstract:

In this study, the bioassay of the antibacterial activity of *Dendrocalamus asper (D.asper)* extracts' phytochemical components was the main topic. Fixed oil, carotenoid, alkaloid, flavonoid, fatty acid, steroid, tannin, organic acid, saponin in all tested extracts. Steroids, fatty acids, and tannins were only found in tiny amounts in the leaves, and organic acids were only found in the roots. For the antibacterial activity, the least effective antibacterial properties were those of stem extracts. Extracts from bamboo roots, meanwhile, proved effective against *E. coli* bacteria. The most effective antibacterial substance overall was the ethanol leaf extract. While the hot water extract didn't demonstrate much anti-*E. coli* activity (the average inhibition zone diameters of the root extracts, stem extracts, and leaf extracts were 8.67±0.34 mm, 8.44±0.84 mm, and 8.44±0.84 mm, respectively), the ethanol extracts of the roots and leaves did (the average inhibition zone diameters were 10.00±0.00 mm and 11.33±0.58 mm, respectively). Hot water extracts of bamboo stems and roots both had the same level of antibacterial activity. For the leaf extracts in hot water that displayed less activity (9.11±0.38 mm was the average inhibition zone diameter). Steroids and tannins were thought to be important secondary metabolites that helped fight microbial infections when present in the ethanol leaf extract of *D. asper*.

Keywords: *Dendrocalamus asper*; *Escherichia coli,* anti-bacterial activity, inhibition zone, bamboo

Introduction

Bamboos are members of the grass family Poaceae's Bambusoideae subfamily. Although there are more than 1400 species of bamboo worldwide, Southeast Asia has the highest species diversity [1] [2]. One of the species on Earth with the quickest rate of growth is bamboo. Due to their versatility, they have a long history in human culture and currently help more than a billion people throughout the world with their subsistence needs [3]. Bamboo is widely used, as is well known. Its roots and leaves have also been utilized medicinally in addition to being employed in building construction. According to studies, bamboo leaves have anti-inflammatory, anti-cancer, and antibacterial effects [4] [5]. Bamboos are also well-known for being a very nutritious food source, and numerous studies have shown that they offer medical benefits. Over a thousand years ago, bamboo was first employed in traditional Chinese medicine [6] [7] [8]. In the past several years, bamboo garments have made their way into the textile market, and many commercial bamboo fabric goods are advertised as being hygienic and antibacterial. However, few pieces of scientific data are offered to support the majority of the claims made by industry parties [9]. In particular, not enough research has been done on the substances that give bamboo its antibacterial capabilities. Some Asian nations refer to the antibacterial molecule in bamboo plants as "kun," which in a straight translation denotes a hydroxyl functional group (-OH); however, this term is insufficient to characterize the chemical compound and where it is found in bamboo [10] [11].

Dendrocalamus asper (D.asper) is a tribe of roughly 35 species in the Bambuseae family, according to taxonomy. According to a study on *Dendrocalamus asper* done in 2017 [12], this genus shares more similarities than other bamboo species. *D. asper* grows to a height of 20–30 m, has internodes that are 8–20 cm long and in diameter, and its walls are comparatively thick

[13]. Bamboo's monocarpic flowering, which only happens around once every 25 to 60 years, causes both its flowering cycle and its seed-setting to be erratic [14]. Rhizome cuttings and, occasionally, air layering are used to propagate bamboo species since their seeds are few and short-lived. Although rhizome or offset cuttings are a tried-and-true technique of vegetative multiplication, their scarcity, bulkiness, and difficulty in extraction and transportation make them unsuitable for growing large-scale plantations [15]. There is no conclusive information regarding the origins of *D. asper*; however, reference [16] states that it is present throughout Southeast Asia, including Thailand, Vietnam, Malaysia, Indonesia, and the Philippines. *D. asper* thrives best in humid areas of tropical Asia with rich, heavy soils, from lowlands to an altitude of 1500 m, and an average annual rainfall of about 2400 mm. In semi-arid conditions, it can also thrive with the right management [17]. All portions of plants often accumulate bioactive chemicals, although the concentrations vary depending on the part of the plant. Neem (*Azadirachta indica* L.) and Tulsi (*Ocimum sanctum* L.) have antibacterial properties against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae*, and *Escherichia coli* [18]. The leaves of *D. asper* have long been used as a traditional medicine to treat diarrhea in animals like chickens, calves, piglets, and rabbits [19]. According to Beauchamp and Sofos [20], beef products were largely linked to foodborne outbreaks of diarrheagenic *E.coli.* As many as 50 avian pathogenic *E. coli* (APEC) from broiler chickens with colisepticemia showed high resistance (≥ 80%) against common antibiotics, such as nalidixic acid (100%), lincomycin (100%), erythromycin (97%), oxytetracycline (95%), chlortetracycline (95%), tetracycline (94%), flumequine (94%), tiamulin (91%), doxycycline (88%), difloxacin (83%), neomycin (81%), streptomycin (81%), trimethoprim-sulphamethoxazole (80%). Up to 56–100% of human fecal *E.coli* isolates exhibited tetracycline, ampicillin, chloramphenicol, and streptomycin resistance. In order to illustrate the biological activity of *D.asper*, several investigations have been carried out. The majority of these investigations have concentrated on extracts from particular *D.asper* body sections. However, thorough and comparative analyses of bamboo extracts from all parts using the same extraction solvent have not been carried out. In this study, *D.asper* was divided into three parts: the leaves, the stems, and the roots. These parts of bamboo were extracted with ethanol and hot water and examine the anti-bacterial activity extracts against *Escherichia coli*.

Materials and Methods

Sample collection

The adult bamboo was discovered growing in the Bay Nui region of Southern Vietnam's An Giang province. Fresh *D. asper* samples were well cleaned with tap water and then appropriately dried with the wind. After that, divide the mixture into pieces with a diameter of 0.5 to 1.0 cm.

Preliminary phytochemical screening

The following elements were subjected to phytochemical analysis using a slightly modified version of the standard protocol: Tannin, organic acid, carotenoid, alkaloid, flavonoid, steroid, fixed oil, and saponin, saponin triterpen, Saponin steroid [21].

Preparation bamboo Extracts

The bamboo samples (roots, stems and roots) were extracted using hot water and ethanol as solvents. In a round flask, 25 g of dried *D. asper* powder (1 g/ml) were dissolved in 100 ml of the solvent. For ethanol extracts, the powdered bamboos were treated with ethanol 70^0 for two hours at 60°C to 70°C, filtered, then put through a rotary evaporator with a 60 rpm setting at 45 °C [22]. For hot water extraction, the mixture was heated to between 80 and 90 °C in a hot water bath for two hours [22]. After filtering the mixture with Whatman No. 1 filter paper, the filtrate was put into a sterile amber bottle. The solvent (ethanol) was reagent grade (Merck). The media for antibacterial assay were Tryptone Soya Agar and Nutrient Broth, all were obtained from Oxoid.

E. coli used as tested was isolated from diarrheal livestock.

Evaluation of the antibacterial activity

The well and disc diffusion method were used to assess the antibacterial properties of the bamboo shoot extracts after 18 and 24 hours of incubation [23]. *Escherichia coli (E.coli).* Pure cultures of *E. coli* were cultivated for 24 hours at 37°C in Tryptone Soya Agar before being transferred to Nutrient Broth and standardizing to $1-2\times10^6$cells/ml. Prepare two Petri dishes for the prepared test bacteria, each filled with exactly 20 ml of media. The filtrate (10^6 CFU/ml) was loaded into a disc, placed on the agar with *E. coli* inoculations, create three equal-sized, 6mm-diameter agar wells on Petri dishes, each numbered from 1 to 3. 30 µl of an each extract from bamboo shoots was put into the wells at locations 1, 2, and 3, respectively and incubated for 24 hours at 37 °C for bacterial species. Ampicillin, a broad-spectrum antibiotic that prevents the formation of bacterial cell walls [24], was the positive control because it was utilized in the experiment. A ruler was used to measure the zone of inhibition. Measure the diameter of the zone of Inhibition if the perimeter is clearly defined; otherwise, measure the radius. On the underside of the Petri dish, the ruler or caliper was held while taking a direct millimeter reading. The measurement took into account the antimicrobial sample's size. Multiple zone readings were taken in order to determine the average size. Triplicates of each test were run. Standard deviation (SD) and means are used to report data. Using Microsoft Excel 2007, the results were statistically examined.

Results and Discussion

Characteristics of ethanol extracts and hot water extracts

The characteristics of the extracts of the *D.asper* bamboo can be seen in Table 1. The extracts from *D. asper* bamboo stems, roots, and leaves did not show significant differences in sensory factors such as color and flavor. All extracts are brown-yellow in color, mild in aroma, and have a thick consistency [25] [26]. The yields of ethanol extracts were as follows: leaves, 12.53±1.73; stems, 8.14±0.26; and roots, 9.81±1.51. The yields of hot water extracts were as follows: leaves, 10.50±2.39; stems, 7.52±0.28; and roots, 6.11±1.00.

Table 1. Characteristics of ethanol extracts and hot water extracts

Examination	Results					
	Ethanol extracts			Hot water extracts		
	Roots	Stems	Leaves	Roots	Stems	Leaves
Consistency	Thick	Thick	Thick	Thick	Thick	Thick
Color	Brown-yellow	Brown-yellow	Brown-yellow	Brown-yellow	Brown-yellow	Brown-yellow
Aroma	Mild, earthy odor	Mild, earthy odor	Mild, earthy odor	Mild, earthy odor	Mild, earthy odor	Mild, earthy odor
Extract yield	9.81±1.51	8.14±0.26	12.53±1.73	6.11±1.00	7.52±0.28	10.50±2.39
Water content	14.57±1.05	14.07±0.48	14.81±1.19	17.70±2.20	16.52±2.13	15.20±3.17

Phytochemical composition of D.asper bamboo extracts

To determine the presence or lack of specific phytochemicals, such as fixed oil, carotenoid, alkaloid, flavonoid, fatty acid, steroid, tannin, organic acid, saponin, saponin triterpen, saponin steroid, screening of the phytochemical components of ethanol and hot water shoot extracts of *D. asper was* done. Table 2 displays the results from a phytochemical analysis of the hot water and ethanol extract of *D. asper* bamboos. Fixed oil, carotenoid, and saponin were found in roots, stems, and leaves [22]. However, only the leaves contained traces of steroids, fatty acids, and tannins, while the roots were the only ones to contain organic acids. Previous investigations [27] [28] [29] also noted the presence of fixed oil, carotenoid, alkaloid, flavonoid, fatty acid, steroid, tannin, organic acid, saponin in *D.asper* species.

Table 2. Phytochemical composition of *D.asper* bamboo extracts

Phytochemicals	Roots	Stems	Leaves
Fixed oil	+	+	+++
Carotenoid	+	+	+++
Alkaloid	-	-	-
Flavonoid	-	-	-
Fatty acid	-	-	+
Steroid	-	-	+
Tannin	-	-	+
Organic acid	+	-	-
Saponin	++	++	+++
Saponin triterpen	-	-	-
Saponin steroid	-	-	-

Lengends: +++= very strong present of phytochemicals, ++= strong present of phytochemicals, + = Present; − = absent of phytochemicals

Antibacterial Activity of D. Asper extracts against Escherichia coli

Accurate data were obtained from the antibacterial activity assay using the well diffusion and disc diffusion techniques. The susceptibility patterns in Table 3 demonstrated that ampicillin, positive control resulted in an inhibition zone diameter of 22.00±0.00 mm for *Escherichia coli*. The positive control, had the highest mean, indicating that it is extremely effective against *E. coli*. Table 3 showed the antibacterial activity of the ethanol extracts and the hot water extracts of *E. coli*. The ethanol extracts of the roots and leaves showed activity against *E. coli* (average inhibition zone diameter were 10.00±0.00, and 11.33±0.58, respectively), while the hot water extract didn't show good activity average inhibition zone diameters of the root extracts, the stem extracts and the leaf extracts were 8.67±0.34, 8.44±0.84, respectively). There was no difference in the antibacterial ability of hot water extracts of bamboo stems and roots. For the hot water leaf

extracts that showed weaker activity (average inhibition zone diameters of the leaf extracts was 9.11±0.38) [30]. It seemed that *E. coli* isolated from diarrheal livestock was the most sensitive to ampicillin.

Table 3. Result of inhibition zone diameter measurement

Part	Average inhibition zone diameter (mm)	
	Ethanol extracts	Hot water extracts
Roots	10.00±0.00	8.67±0.34
Stems	7.67±0.58	8.44±0.84
Leaves	11.33±0.58	9.11±0.38
Ampicillin (Positive control)	22.00±0.00	

Interpretation:

No inhibition zone: inactive

<9 mm: partially active

10-14 mm: active

>15 mm: very active

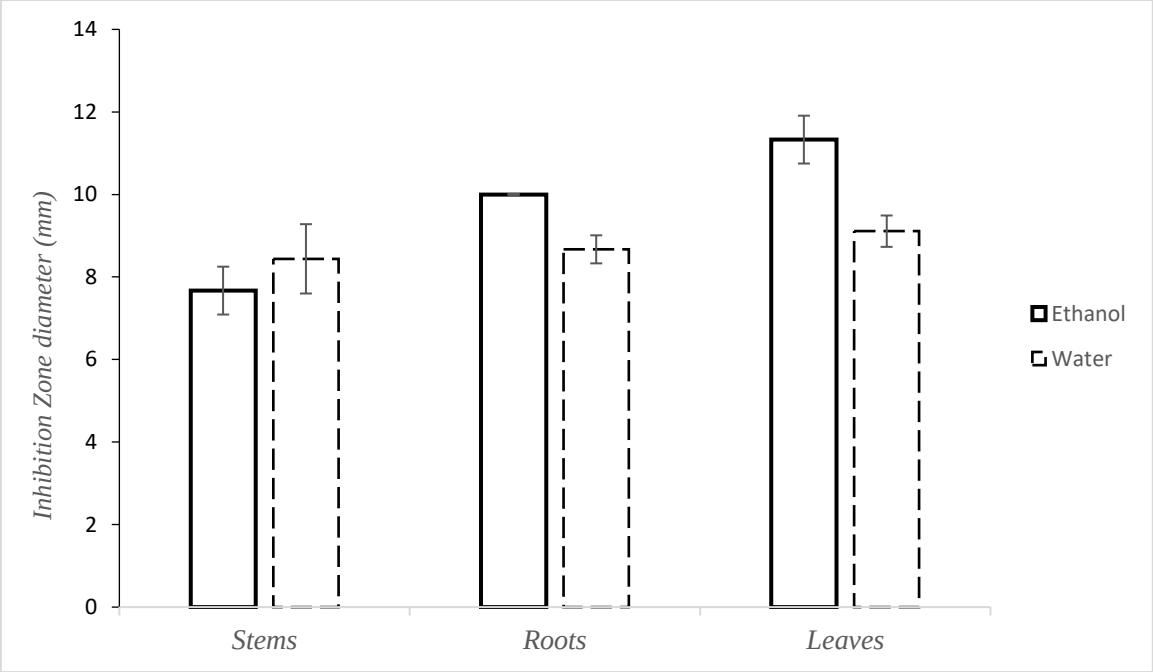

Figure 1. Correlation of the antibacterial ability of *D. asper* bamboo stems, roots, and leaf extracts against *E. coli* strains

The Figure 1. showed the correlation of the antibacterial ability of *D. asper* bamboo stems, roots, and leaf extracts with ethanol and water solvents against *E. coli* strains. All samples extracted

from bamboo showed antibacterial ability against *E. coli* bacteria. However, the antibacterial ability of stem extracts were the lowest. Meanwhile, bamboo root extracts were active against *E. coli* bacteria. In general, the ethanol leaf extract had the greatest antibacterial efficacy [19]. Thus, the antimicrobial potential of *D.asper* leaf extracts. All of the examined bamboo leaf extracts contain a variety of phytochemicals, which contribute to their antimicrobial properties. The presence of steroids and tannins in the ethanol leaf extract of *D. asper* was thought to be crucial secondary metabolites responsible for battling microbial infections. Furthermore, ethanol was the optimum extraction method for the compounds that have antibacterial activity [31] [32].

The results of the study demonstrated that *D. asper* has antibacterial activity that can guard against and eradicate *E. coli.* While *D. asper* ethanol and hot water extracts both have anti-bacterial properties that are also attributable to the presence of phytochemicals. Compared to commercially produced ampicillin, bamboo leaf ethanol extract has less antibacterial activity.

Acknowledgment

This research is funded by Vietnam National University Hochiminh City (VNU-HCM) under grant number C2021-16-04.

References

[1] Das M, Bhattacharya S, Singh P, Filgueiras TS, PalA, "Bamboo Taxonomy and Diversity in the Era of Molecular Markers Advances in Botanical Research," *Incorporating Advances in Plant Pathology, Elsevier Ltd,* vol. 47, 2008.

[2] Bystriakova N, Kapos V, Lysenko I, Stapleton C, "Distribution and conservation status of forest bamboo biodiversity in the Asia-Pacific region," *Biodiversity and Conservation,* vol. 12, p. 1833–1841, 2003.

[3] K, Salam, "Bamboo for economic prosperity and ecological security with special reference to north-east India, CBTC, Guwahati," *Indian folklore Journals,* vol. 409, p. 353, 2008.

[4] Lee, H.-O.; Baek, S.-H.; Han, D.-Mn, "Antimicrobial effects of Chamaecyparis obtusa essential oil," *Korean J. Appl. Microbiol. Biotechnol,* vol. 29, p. 253–257, 2001.

[5] Zhang, Y.; Jiao, J.; Liu, C.; Wu, X.; Zhang, Y, "Isolation and purification of four flavone C-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative high-performance liquid chromatography," *Food Chem.,* vol. 107, p. 1326–1336, 2008.

[6] YC, Tripathi, "Food and nutrition potential of bamboo," *MFP News,* vol. 8, no. 1, pp. 10-11, 1998.

[7] Chongtham N, Bisht MS, Haorongbam S, "Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food," *Comprehensive Reviews in Food Science and Food Safety,* vol. 10, pp. 153-169, 2011.

[8] Zhang Y, Ding XL, "Studies on anti-oxidative fractions in Bamboo leaves and its capacity to scavenge active oxygen radicals," *J. Bamboo Res,* vol. 15, pp. 17-24, 1996.

[9] Afrin, T., Tsuzuki, T., & Wang, X., "Bamboo fibres and their unique properties. In C.M. Wilson & R.M. Laing (Eds.)," *Combined (NZ and Aus) Conference of the Textile Institute, Dunedin, NZ,* p. 77–82, 2009.

[10] T. Afrin, T. Tsuzuki, R.K. Kanwar and X. Wang, "The origin of the antibacterial property of bamboo," *The Journal of The Textile Institute,* vol. 103, no. 8, p. 844–849, 2012.

[11] Koshijima, T., & Watanabe, T., "Association between lignin and carbohydrates in wood and other plant tissues," *Verlag/Berlin/Heidelberg: Springer,* 2003.

[12] Konzen, E.R.; Peron, R.; Ito, M.A.; Brondani, G.E.; Tsai, S.M, "Molecular identification of bamboo genera and species based on RAPD-RFLP markers," *Silva Fenn,* vol. 51, p. 1–16, 2017.

[13] Hossain MA, Kumar SM, Seca G, Maheran AA & Nor-Aini AS, "Mass propagation of *Dendrocalamus asper* by branch cutting," *Journal of Tropical Forest Science,* vol. 30, no. 1, p. 82–88, 2018.

[14] Nadgir AL, Phadke CH, Gupta PK, Parshrami VA, Nair S & Mascarenhas AF, "Rapid multiplication of bamboo by tissue culture," *Silvae Genetica,* vol. 33, p. 219–223, 1984.

[15] Pattanaik S, Das P, Borah E, Kaur H & Borah K., "Vegetative multiplication of Bambusa balcooa Roxb. using branch cuttings," *Journal of Bamboo and Rattan ,* vol. 3, p. 301–412, 2004.

[16] Benton, A, "Priority Species of Bamboo," *In Bamboo (Tropical Forestry); Liese, M., Kohl, M., Eds.; Springer: Cham, Switzerland,* vol. 10, p. 31–41, 2015.

[17] Singh, S.R.; Dalal, S.; Singh, R.; Dhawan, A.K.; Kalia, R.K., "Micropropagation of *Dendrocalamus asper* {Schult. & Schult. F.} Backer x k. Heyne): An exotic edible bamboo," *J. Plant Biochem. Biotechnol,* vol. 21, p. 220–228, 2012.

[18] Rathod GP, Kotecha BM, Sharma R, Amin H, Prajapati PK, "In vitro Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica L.*) and Tulsi (*Ocimum sanctum L.*)," *Int J Pharm Biol Arch,* vol. 3, pp. 582-586, 2012.

[19] Noryawati Mulyono, Bibiana W. Lay, Sri Rahayu, ndri Yaprianti, "Antibacterial Activity of Petung Bamboo (*Dendrocalamus Asper*) Leaf Extract Against Pathogenic *Escherichia coli* and Their Chemical Identification," *International Journal of Pharmaceutical & Biological Archives,* vol. 3, no. 4, pp. 770-778, 2012.

[20] Beauchamp CS, Sofos JN, "Diarrheagenic Escherichia coli. In: Juneja VK, Sofos JN, editors. Pathogens and toxins in foods: challenges and interventions," *1st ed Washington: ASM Pr,* pp. 71-94, 2010.

[21] Monique Brigitte Ouattara, Jean Hubert Bationo, Martin Kiendrebeogo, Odile Germaine Nacoulma, "Phytochemical Screening and Acute Toxicity Evaluation of Leaves Extract of Two Fabaceae's Species: *Sesbania pachycarpa DC.* and *Indigofera berhautiana Gillett*," *Journal of Biosciences and Medicines,* vol. 8, no. 7, 2020.

[22] Baguistan, Bon Jovi R., Waing, Kristine Grace D. and Valentino, Mary Jhaneg, "Phytochemical screening and determination of the biological activities of Bambusa *vulgaris var. striata* and *Dendrocalamus asper* shoot extracts," *International Journal of Biolog, Pharmacy and Allied Sciences (IJBPAS),* vol. 6, no. 11, pp. 2109-2119, 2017.

[23] Muanza, D., Kim, B., Euler, K., và Williams, L., "Antibacterial and antifungal activities of nine medicinal plants from Zaire," *International Journal of Pharmacognosy,* vol. 32, pp. 337-345, 1994.

[24] Gudisa Bereda, Department of Pharmacy, Negelle Health Science College, Guji, Ethiopia, "Clinical Pharmacology of Ampicillin," *Journal of Pharmaceutical Research & Reports,* vol. 3, no. 3, pp. 1-3, 2022.

[25] Mujahidah, nif Nur Artanti and Farikatul Sufi, "Antibacterial Activity Test of Ethanol Extract and SAP of Betung Bamboo Shoot (*Dendrocalamus asper*) Against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Bacteria," *Journal of Biodiversity and Biotechnology,* vol. 1, no. 1, p. 11–16, 2021.

[26] Justine Dave C. Abarquez, John Deux L. Balubal, Carlo Maverick Paul M. Pulido, Ivan Dave T. Salva, "Anti-bacterial activity of bamboo (Bambusa *vulgaris*) shoot ethanolic skin extract against *Staphylococcus aureus* and *Escherichia coli*," *HRB,* vol. 6, p. 3, 2019.

[27] Yakubu, M.T. and B.B. Bukoye, "Abortificent potentials of the aqueous extracts of Bambusa *vulgaris* leaves in pregnant Dutch rabbits," *Journal of Contraception,* vol. 80, no. 3, pp. 308-313, 2009.

[28] Anghore, D., Kulkarni, G.T. and A.R. Subramanian, "Preliminary phytochemical investigation of Bambusa *vulgaris var. striataHoltum*," *Journal of Chronotherapy and Drug Delivery,* vol. 5, no. 1, p. 27–31, 2014.

[29] Guerere, M., Mondon, J.M. and A. Pujanniaye, "Physiochemical composition of some seeds from plants of the isle reunion," *Anfalsi expert xhim toxicology,* vol. 78, pp. 281-286, 1985.

[30] Akinobu Tanaka, Qinchang Zhu , Hui Tan , Hiroki Horiba, Koichiro Ohnuki, Yasuhiro Mori, Ryoko Yamauchi, Hiroya Ishikawa, Akira Iwamoto, Hiroharu Kawahara and Kuniyoshi Shimizu, "Biological Activities and Phytochemical Profiles of Extracts from Different Parts of Bamboo (*Phyllostachys pubescens*)," *molecules,* vol. 19, pp. 8238-8260, 2014.

[31] Alo, M.N., Anyim, C., Igwe, J.C., Elom, M. and D.S.Uchenna, "Antibacterial activity of water, ethanol, and methanol extracts of *Ocicumgratissimum, Vernoniaamydalina,* and *Aframonummelegueta*," *Advances in Applied Science Research,* vol. 3, no. 2, pp. 844-842, 2012.

[32] Onyeagba, RA., Ugbogu, O.C., Okeke, C.U. and O.Iroakasi, "Studies on the antimicrobial effects of garlic (*Allium sativum Linn*), ginger (*Zingiberofficinale Roscoe*) and lime (*Citrus aurantifolia Linn*)," *African Journal of Biotechnology,* vol. 3, no. 10, pp. 552-554, 2004.