JOURNAL OF ADVANCEMENT IN
MEDICAL AND LIFE SCIENCES
Journal homepage: http://scienceq.org/Journals/JALS.php

ISSN:
2348-294X
V8I1.03

Research Article Open Access

Development of alternatives to synthetic antibiotics against important veterinary pathogens using native herbal plant of Bangladesh

K. B. M. Saiful Islam[1*], Kamrul Islam[2], Syeeda Shiraj-Um-Mahmuda[3], Sujan Kumar Sarkar[4]

1.Department of Medicine & Public Health, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh
2.Institute of Epidemiology, Disease control and Research (IEDCR), Dhaka-1212, Bangladesh
3.Department of Pathology, Faculty of Basic Science, Bangabandhu Sheikh Mujib Medical University, Dhaka-1000, Bangladesh
4.Department of Anatomy, Histology & Physiology, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

*Corresponding author: K. B. M. Saiful Islam, Email: vetkbm@yahoo.com,

Received: June 06, 2020, Accepted: July 25, 2020, Published: July 25, 2020.

ABSTRACT:

Antibiotic resistance has been recognized globally as one of the major health challenges for this modern era. Accordingly, the search for safe and effective antimicrobial agents has become a top priority for the biomedical field in recent years. Therefore, present study was undertaken to investigate the antimicrobial properties of herbal extract (guava) against bacterial pathogens of veterinary importance in *in vitro* conditions and thus to propose an alternative herbal candidate to synthetic antibiotics.Qualitative phytochemical analysis of guava leaves was performed to identify the bioactive chemical constituents like tannins, alkaloids, saponins, flavonoids, etc. Plant extracts were prepared using two readily available solvents namely aqua and ethanol. Guava leaves extracts were evaluated against both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli)* bacteria of veterinary importance that commonly cause diseases in animals and birds. The efficacy of these extracts was tested against the study organisms through a well-diffusion method employing 50mg leaf-extract solution per well. Bacterial growth inhibitory characteristics of the extracts were also investigated by determining the Minimum Inhibitory Concentration (MIC) of the extracts in Nutrient broth. Guava leaves are found to contain different phytochemicals like tannins, phenols, flavonoids, terpenoids, glycosides and saponins which have known antibacterial activity. Antibacterial assay of both the aqueous and ethanol extracts of guava leaves showed inhibitory activity against both gram-positive and gram-negative bacteria. The ethanol extract had better antibacterial activity than aqueous extracts with mean zones of inhibition of 11.8 ± 0.60 mm and 9.5 ± 0.95 mm against *Staphylococcus aureus* and *Escherichia coli*, respectively. MIC values revealed that a very low concentration of 6.25 -12.5mg/ml of the extracts inhibit the growth of the tested isolates. The finding revealed that guava leave extract has great potential as an antimicrobial compound against pathogenic microorganisms of veterinary importance. Guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties of such valuable herbal plant.

Keyword: *Guava leave extract, antimicrobial activity, pathogenic bacterial strains*

INTRODUCTION:

Antibiotics are regarded as one of the greatest medical discoveries of the 20th century, which are vigorously being used to treat deadly bacterial diseases in both humans and animals. But such vigorous use of antibiotics is contributing to a surge in drug-resistant germs or superbugs that render antibiotics powerless against deadly infections. Now-a-days antibiotic resistance has been recognized by the major world health organizations as one of the top health challenges for this modern era. Some of its causes are widely accepted, for example, the use of antibiotics in veterinary industry as in livestock or poultry [1]. There has been an increasing incidence of multiple resistances in veterinary pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial synthetic antimicrobial drugs commonly employed in the treatment of infectious diseases [2]. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. Recent work revealed the potential of several herbs as sources of drugs. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [3]. Numerous studies have identified compounds within herbal plants that are effective antibiotics [4]. At the same time, consumers are increasingly being interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents [5]. Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities [3]. Herbal medicine has long been recognized as one of the oldest forms of remedies used by humans [6]. The therapeutic properties of various medicinal plants have been used to treat human and animal diseases for centuries [7-8]). Many people in developing countries still rely on traditional healing practices and medicinal plants for their daily healthcare needs, in spite of the advancement in modern medicine [9]. Several studies carried out in Africa, Asia, Europe, Latin America and North America show that plants are routinely used as remedy for animal diseases [9- 13]. Historically, it is documented that humans utilize the same herbal preparations that they use to treat their sick animals [6]. The search continues for safe and effective antimicrobial agents for treating, therapeutically and

prophylactically, a wide variety of bacterial infections. This need has been urged recently by the emergence of many antimicrobial-resistant organisms like methicillin resistant staphylococci, multidrug, extended drug and pan drug resistant microorganisms. The best therapeutic antimicrobial agents cause virtually no adverse reactions, have a wide spectrum of activity, and are not likely to select resistance. Medicinal herbs are being increasingly studied by pharmacological researches, and many such herbs have a long history of medicinal use in Asia [5]. Herbs have many potential clinical and therapeutic applications in the modern medical setting, as numerous studies have revealed that they contain bioactive components, and have resulted in a better understanding of their physiological, therapeutic and clinical actions. Antimicrobial agents can also be derived from herbs, and over 1000 plants exhibit antimicrobial effects [14]. Traditionally, these herbs are said to provide safe and effective treatments against many diseases. Although studies on the antimicrobial properties of available herbal medicine are being extensively conducted in many countries based on their native herbs, surprisingly, there is no or little documented information about the local available herbs and their antimicrobial potentials in Bangladesh. In fact, no comprehensive study has yet been documented that reported the antibiotic potential of Bangladeshi herbs though the country is a blessed kid of nature harboring plenty of herbs in her land. Recently, considering the huge potential of herbal medicine, higher priority has been given to the *'Development of herbal drugs and treatment'* in *'Agricultural Research Priority: Vision- 2030 and beyond'* under Livestock Sub-sector of Bangladesh [15]. Therefore, in order to fulfill priority based present research need in Bangladesh, this study was undertaken to investigate the antimicrobial properties of a native herbal plant against bacterial pathogens of veterinary importance using *in vitro* conditions and to propose an herbal antibiotic as the alternatives to synthetic antibiotic against pathogenic microorganism of veterinary importance.

METHODOLOGY:

1. Collection and preservation of plant material:

Extensive literature review has been conducted on the ethnoveterinary medicinal use of herbal plants in Indo-Bangla Subcontinent. Based on the available literature review and availability in Bangladesh context Guava was selected to study under this experiment. The leaf samples were collected from the guava trees growing at the different areas of Bangladesh. Random leaf samples were collected into plastic zip lock bags with appropriate labeling and stored in an ice cooler until being transported to the laboratory for extraction. Collected fresh leaves were authenticated from an expert in the Department of Crop Botany, Sher-e-Bangla Agricultural University, Dhaka.

2. Preparation of plan extracts:

Both the aqueous and solvent extracts of the plant were evaluated for antimicrobial property. For aqueous extraction, 10 g of air-dried powder was added to 250 ml distilled water and boiled for 10 minutes. The solution is then filtered and centrifuged at low speed centrifugation to obtain supernatant free of debris and coarse particles. Aqueous extract was then prepared following the methods described by Parekh *et al*., 2005 [16]. For solvent extraction, 10 g of air-dried powder was taken in 100 ml of organic solvent (ethanol) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume [16] and stored at 4^{o}C in airtight bottles.

3. Preparation of stock solution:

Stock solutions of the different organic solvents soluble fractions were prepared by dissolving 100 mg of the extract in 1 ml of distilled water. Standard antibacterial agent (oxytetracycline, Cipla Ltd., Mumbai, India) at a concentration of 10 mg/ml was also used on all the bacteria organisms and their zones of inhibition were compared with those of the extract.

4. Preliminary phytochemical analysis:

Chemical tests for the screening and identification of bioactive chemical constituents in the guava were carried out with the extracts using the standard procedure as described earlier [17-19]). Plants were screened for commonly known phytochemicals like tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenoids, flavanoids etc. For each test, 1 ml of each solvent extract was used for analysis, in exception for the saponin test in which 3 ml solvent extract was used.

Test for Saponins: Extract was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Phenols and Tannins: Extract was mixed with 2 ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Terpenoids (Salkowski's Test): Extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration of the interphase was formed to show positive results for the presence of terpenoids.

Test for Flavonoids (Shinoda Test): Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop by drop. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

Test for Glycoside: Extract was mixed with 2 ml of glacial acetic acid containing 2 drops of 2% FeCl3 . The mixture was poured into another tube containing 2 mL of concentrated sulfuric acid. A brown ring at the interphase indicates the presence of glycosides.

5. In vitro determination of antibacterial activity of herbal plants:

Microbial strains: Based on the veterinary importance and common prevalence, clinical isolates of both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli)* bacteria were selected to test the guava extracts ability to inhibit the growth. The organisms were previously isolated from clinical cases and identified following standard protocol. Prior to sensitivity testing, the bacterial strains were cultured onto nutrient agar plates and incubated for 18 to 24 h at 37°C to obtain colonies. After overnight incubation, colonies were selected with a sterile disposable inoculating loop and transferred to a glass tube of sterile physiological saline and vortex thoroughly. Each bacterial suspension turbidity is then compared to that of the 0.5 McFarland standard solution (containing about 1.5×10^8 CFU/ml).

Antibacterial Activity: Antimicrobial susceptibility testing was performed using the well-diffusion method according to the standard of the National Committee for Clinical Laboratory Standards [20]. The plant extracts were tested on Mueller Hinton II plates to detect the presence of antibacterial activity. Prior to streaking the plates with bacteria, 5 mm diameter wells were punched into the medium using a sterile borer. All plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 McFarland standard solution; a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculum. The surface of

the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. The plates are allowed 3 to 5 min to dry the excess moisture. Fifty ul aliquots of each test extract was dispensed into each well after the inoculation of the plates with bacteria. The wells were also arranged in a triangle formation 2 inches apart. The same extract was used on each plate, with a total of three plates used for each extract for selecting bacterium. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with parafilm, labeled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts: The MIC was determined using the method described by Greenwood [21]. For each extract six sterile test tubes were arranged in a test tube rack in a row for each organisms and 0.5 ml of sterile nutrient broth was pipetted into each tube. Half a millimeter of the crude extract containing 100 mg per ml was pipetted into tube one to obtain a concentration of 50 mg per ml. There after there was a serial dilution of the extract to obtain concentrations of 25, 12.5, 6.25 and 3.13 mg per ml, respectively. 0.5 ml of the test organism was pipetted into each test tube and incubated at 37°C for 24 h. The MIC was recorded as the least concentration of plant extract that completely inhibit the growth of the test organism.

RESULTS AND DISCUSSION

Phytochemical Analysis:

Table 1 shows the summarized phytochemical screening of chemical constituents of guava extracts under study on qualitative basis.

The results revealed the presence of active compounds in the two different extracts. As the table shows, the ethanol extract indicates the presence of tannins, phenols, flavonoids, terpenoids, and glycosides, but absence of saponins. Distilled water is the only that showed the presence of all the phytochemicals. The analysis of the plant extracts revealed the presence of phytochemicals which are known to exhibit medical and physiological activities.

Table 1: Phytochemical constituents of *Psidium guajava* extracts.

Extracts	Phenols and tannins	Saponins	Trepenoids	Flavonoids	Glycoside
Aqueous	+	+	+	+	+
Ethanol	+	-	+	+	+

+: presence of constituent; -: absence of constituents

For example, tannins are polyphenolic compounds that bind to proline rich protein that interferes with protein synthesis [22-23] and has shown to have antibacterial activity [24-25]. Flavonoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections to which this aspect has been extensively studied and found to have antimicrobial activity against an array of microorganisms *in vitro* [3]. Their ability has been attributed to their ability to form complexes with extracellular and soluble proteins and bacterial cell walls [26]. Terpenoids although mainly used for their aromatic qualities have also been found to be potential agents against inhibiting bacteria. Saponins which are glycosides have been found to have inhibitory effects on gram-positive organism, *S. aureus* [22]. Therefore, the phytochemical analysis revealed that the ethanol and distilled water extract have chemical compounds that have been found to possess antibacterial activities, which could contribute to the results obtained from antibacterial analysis.

Antibacterial Activity The results of the study indicated that both the aqueous and ethanol extracts prepared from the leaves of *Psidium guajava*, showed inhibitory activity against test bacteria (Table 2). At 10 mg/100 μL, the ethanol extract had a slightly higher antibacterial activity with mean zones of inhibition 11.8 ± 0.60 mm and 9.5 ± 0.95 mm than aqueous extract with mean zone of inhibition 10.6 ± 1.2 mm and 7.9 ± 0.82 mm against *S. aureus* and *E. coli*, respectively. Seemingly, the Gram-positive bacteria, *Staphylococcus aureus*, were more susceptible to the extract, while the Gram-negative bacterium *E. coli* showed comparatively lower inhibition [Figure-1].

Table 2: Antibacterial activity of *Psidium guajava* leaves of the screened solvents extracts.

Plant extracts	Zone of inhibition∗ (mm)	
	S. aureus	*E. coli*
Water	10.6 ± 1.2	7.9 ± 0.82
Ethanol	11.8 ± 0.60	9.5 ± 0.95

∗Value of inhibition zone is expressed as mean ± standard deviation (n = 3)

—: no inhibitory activity.

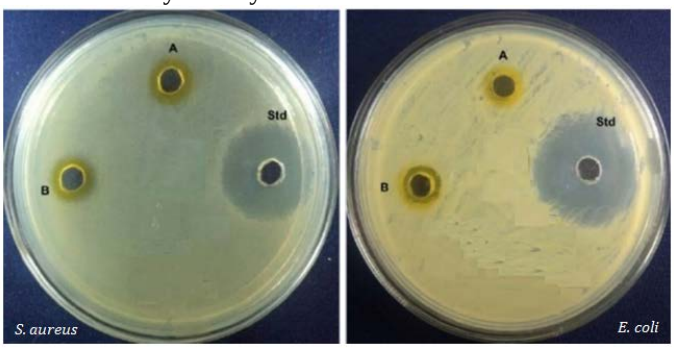

Figure-1: Antibacterial activity of aqueous and ethanol extracts of *Psidium guajava* leaves as determined by well-diffusion method. A) Aqueous Extracts of *Psidium guajava* leaves; B) Ethanol Extracts of *Psidium guajava* leaves; Std: Standard Antibiotics: oxytetracycline 10mg/ml

The resistance of the Gram-negative bacteria could be attributed to its cell wall structure. Gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding the plant extract. It has been reported earlier that Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria [27-29]. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts [27-28, 30-31]. Results found in this study were supported and/or opposed in the data reported in literature. Nascimento *et al.* (2000) conducted a study that supports the finding of the present study in which the guava extract was able to have inhibitory effects against *Staphylococcus* and *Bacillus* and no effect on the *Escherichia* and *Salmonella*, whereas Chanda and Kaneria (2011) oppose the findings concerning the Gram-negative bacteria [32-33]. However, Vieira *et al.* (2001) found guava sprout extracts were effective against inhibiting *E. coli* [34]. Sanches *et al.* (2005) found that the aqueous extract of guava was effective against *Staphylococcus* and *Bacillus* [35]. The methanolic extracts of guava reported by Lin *et al.* (2002) showed significant inhibitory activity against the growth of 2

isolates of *Salmonella* and enteropathogenic *E. coli* [36]. The Minimum Inhibitory Concentrations (MIC) of aqueous and ethanol extract of *P. guajava* are represented in Table 3 & 4.

Table 3: The minimum inhibition concentration of *Psidium guajava* aqueous leaf extract against the test organisms

	Minimum Inhibitory Concentration (mg/ml)				
	50.0	25.0	12.5	6.25	3.13
S. aureus	-ve	-ve	-ve*	+ve	+ve
E. coli	-ve	-ve*	+ve	+ve	+ve

+: ve = With bacterial growth; -: ve = Without bacterial growth; *: = MIC value

Table 4: The minimum inhibition concentration of *Psidium guajava* ethanol leaf extract against the test organisms

	Minimum Inhibition Concentration (mg/ml)				
	50.0	25.0	12.5	6.25	3.13
S. aureus	-ve	-ve	-ve	-ve*	+ve
E. coli	-ve	-ve	-ve*	+ve	+ve

+: ve = With bacterial growth; -: ve = Without bacterial growth; *: = MIC value

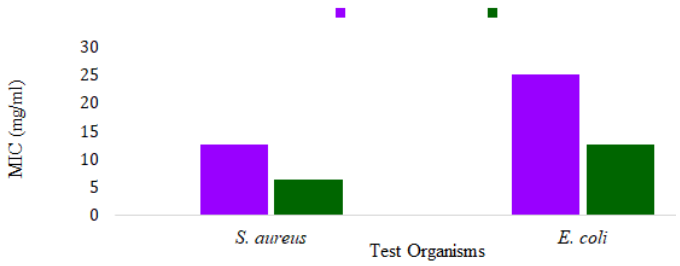

Figure-2: Minimum Inhibitory Concentration (MIC) of *Psidium guajava* aqueous and ethanol leaf extract against the test organisms

The result showed that dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill both the Gram-positive and Gram-Negative organisms. Lower MIC was shown by ethanol extract than aqueous extract [Figure-2].

From the results of MIC determination, the minimum inhibitory concentration showed that a very low concentration of 6.25 -12.5mg/ml the extracts inhibit the growth of the tested isolates. From the findings this is obvious that *P. guajava* extract can be a potential antibacterial agent if the active compound responsible is isolated.

CONCLUSION:

The present work demonstrates the antimicrobial potential of *Psidium guajava* leaves extract by using two readily available solvents. The results indicate that both the aqueous and ethanol extracts of the commonly available herbs Guava can be used as an alternative to antibiotics as they have antibacterial properties. Ethanol seems to be better than water for the extraction of the antibacterial properties of guava. The findings suggest that guava possesses compounds containing antibacterial properties that can effectively suppress the growth of pathogenic organisms causing diseases in animals and bird. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, *P. guajava* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *S. aureus* and *E. coli.*

ACKNOWLEDGMENTS:

We gratefully acknowledge the financial grant under Special Allocation for Science and Technology from the Ministry of Science and Technology, Government of Bangladesh. We are also thankful to Sher-e-Bangla Agricultural University Research System (SAURES) for their kind supervision and monitoring over the implementation of the research activities.

REFERENCES

1. Donoghue D.J. (2003) Antibiotic Residues in poultry tissues and Eggs: Human Health Concern. Poultry Science 82: 618-621.
2. Collignon P. (2003) A review – the use of antibiotics in food production animals – does this cause problem in human health. Manipulating pig production IX. Proceedings of the ninth biennial conference of the Australasian Pig Science Association (Inc.) (APSA), Femantle, Western Australia, 23-26 November: 73-80.
3. Cowan M. M. (1999) Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), pp. 564–582.
4. Nychas G.J.E. (1995) Natural antimicrobials from plants. In: Gould, G.W. (ed.), New Methods of Food Preservation. Blackie Academic and Professional, London; pp. 58-89.
5. Sinclair S. (1998) Chinese herbs: a clinical review of Astragalus, Ligusticum, and schizandrae. Alternative Medicine Review, 3: 338–344.
6. Eisenberg D., R. Davis, S. Ettner (1998) Trends in alternative medicine use in the United States 1990-1997; results of a follow up survey. Journal of American Medical Association, 280: 1569-1575.
7. McCorkle C.M. (1986) An introduction to ethnoveterinary research and development. Journal of Ethnobiology, 129-140.
8. Mathias M.E. (1994) Magic, myth and medicine. Economic Botany. 48 (1): 3-7.
9. Mathias E., and C.M. McCorkle (2004) Traditional livestock healers. Rev Sci Tech. 23 (1): 277-284.
10. Passalacqua N.G., G. De Fine, P.M. Guarrera (2006) Contribution to the knowledge of the veterinary science and of the ethnobotany in Calabria region (Southern Italy). Journal of Ethnobiology and Ethnomedicine. 2: 52
11. Yinegar H. E. Kelbessa, T. Bekele, E. Lulekal (2007) Ethnoveterinary medicinal plants in Bale Mountains National Park, Ethiopia. Journal of Ethnopharmacology, 112: 55-70.
12. Lans C. N., Turner, T. Khan, G. Brauer, W. Boepple (2007) Ethnoveterinary medicines used for ruminants in British Columbia Canada. Journal of Ethnobiology and Ethnomedicine, 3: 11-10.
13. Alves R.R.N., Lima H.N., Tavares Souto W.M.S. (2010) Plants used in animal healthcare in South and Latin America an overview. In Ethnoveterinary Botanical Medicine: Herbal medicines for animal Health.. 1 edition.

Edited by: Katerere RD, Luseba D. CRC Press New York USA:231-256.
14. Nychas G.J.E. (1995) Natural antimicrobials from plants. In: Gould, G.W. (ed.), New Methods of Food reservation. Blackie Academic and Professional, London, pp. 58-89.
15. Tareque A.M.M. and S.M.Z.H. Chowdhury (2010). Agricultural Research Priority : Vision- 2030 and beyond. Sub-sector: Livestock. Bangladesh Agricultural Research Council. Farmgate, Dhaka
16. Parekh J., R. Nair, S. Chanda, (2005) Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. Indian J. Pharmacol., 37: 408-409.
17. Harborne J. B. (1973). Phytochemical Methods, Chapman & Hall, London, UK, 1973.
18. Scalbert, A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30 (12): 3875–3883
19. Tanaka, T., N. Ishida, M. Ishimatsu, G. Nonaka, and I. Nishioka (1992) Tannins and related compounds. CXVI. Six new complex tannins, guajavins, psidinins and psiguavin from the bark of *Psidium guajava* L.. Chemical and Pharmaceutical Bulletin, 40 (8): 2092–2098
20. Anonymous (2009) Clinical and Laboratory Standards Institute, "Performance standards for antimicrobial susceptibility testing," Nineteenth informational supplement M100-S19, Clinical and Laboratory Standards Institute, Wayne, Pa, USA,.
21. Greenwood, D., (1989). Antimicrobial Sensitivity Testing. In: Antimicrobial Chemotherapy, Greenwood, D. (Ed.). Oxford University Press, New York, USA., pp: 91-100.
22. Tsuchiya, H., M. T. Sato, Miyazaki et al. (1996) "Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*, Journal of Ethnopharmacology, 50(1): 27–34.
23. Ulubelen, A. (2003). Cardioactive and antibacterial terpenoids from some Salvia species. Phytochemistry, 64(2):395–399
24. Akiyama, H., K. Fujii, O. Yamasaki, T. Oono, and K. Iwatsuki (2001) Antibacterial action of several tannins against *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy, 48(4):487–491
25. Min, B. R., W. E. Pinchak, R. Merkel, S. Walker, G. Tomita, and R. C. Anderson (2008) Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Scientific Research and Essays, 3(2): 66–73
26. G. E. Trease and W. C. Evans (1989) Pharmacognosy, Macmillan, London, UK, 11th edition,
27. Rameshkumar, K. B. V. George, and S. Shiburaj (2007) Chemical constituents and antibacterial activity of the leaf oil of *cinnamomum chemungianum*. Journal of Essential Oil Research, 19(1): 98–100.
28. Stefanello M.´E.A., A. C. Cervi, I. Y. Ito, M. J. Salvador, A.Wisniewski Jr., and E. L. Simionatto, (2008) Chemical composition and antimicrobial activity of essential oils of Eugenia chlorophylla (Myrtaceae). Journal of Essential Oil Research, 20(1):75–78
29. Tajkarimi, M. M. S.A. Ibrahim, and D.O. Cliver (2010) Antimicrobial herb and spice compounds in food. Food Control, 21(9): 1199–1218
30. Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology, 94 (3): 223–253
31. Qa'dan, F. A. D. A. Thewaini, R. Ali, Afifi, A. Elkhawad, and K. Z.Matalka (2005) The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. The American Journal of Chinese Medicine, 33(2): 197–204.
32. Nascimento G. G. F, J. Locatelli, P. C. Freitas, and G. L. Silva (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology, 31 (4):247–256,
33. Chanda S. and M. Kaneria (2011) Indian nutraceutical plant leaves as a potential source of natural antimicrobial agents," in Science against Microbial Pathogens: Communicating Current Research and Technological Advances, A. Mendez-Vilas, Ed., vol. 2, pp. 1251–1259, Formatex Research Center
34. Vieira, R. H. S. D. F. D. D. P. Rodrigues, F. A. Gonc¸alves, F. G. R. De Menezes, J. S. Arag˜ao, and O. V. Sousa (2001) Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. Revista do Instituto de Medicina Tropical de Sao Paulo, 43(3): 145–148
35. Sanches, N.R. D.A.G.Cortez, M. S. Schiavini, C. V. Nakamura, and B. P. D. Filho (2005) An evaluation of antibacterial activities of *Psidium guajava* (L.). Brazilian Archives of Biology and Technology, 48(3): 429–436
36. Lin, J. T. Puckree, and T. P.Mvelase (2002) Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. Journal of Ethnopharmacology, 79(1): 53–56.

Citation: K. B. M. Saiful Islam et al. (2020). MPB 3.1: A Useful Medicinal Plants Database of Bangladesh, J. of Advancement in Medical and Life Sciences. V8I1.03. DOI: 10.5281/zenodo.3960600.