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Research Article
Antimicrobial Activity of *Psidium guajava* and *Ocimum sanctum* Leaves Extracts Against Multi Drug Resistant Fish Pathogens

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Abstract

Background and Objective: Plant materials remain an important resource to combat diseases in the world. The development of antibiotic-resistant due to the indiscriminate use of antibiotics has led to the use of natural products that have antimicrobial effects. Antimicrobial evaluation of aqueous, ethanolic and methanolic extracts of guava and holy basil leaves were investigated using agar well diffusion method. **Materials and Methods:** The extracts were tested against four clinical strains of bacterial isolates from *Clarias gariepinus*. Minimum Inhibitory Concentration (MIC) of guava, holy basil leaves and phytochemical screening of these plants were determined using standard methods. Data were analyzed using descriptive statistics. **Results:** The guava and holy basil leaves of aqueous, ethanolic and methanolic extracts had inhibition zones of 20±0.01, 18±0.02, 24±0.02, 17±0.02, 22±0.03 and 18±0.02 mm diameter, respectively against *Bacillus subtilis*; 24±0.01, 10±0.01, 25±0.01, 20±0.02, 25±0.03 and 22±0.03 mm diameter against *Staphylococcus aureus*; 24±0.01, 10±0.02, 20±0.02, 10±0.03, 24±0.01 and 17±0.01 mm diameter against *Streptococcus iniae*; 15±0.01, 10±0.01, 24±0.02, 10±0.02, 19±0.01 and 0.8±0.03 mm diameter against *Aeromonas hydrophila*; 0.7±0.01, 0.4±0.01, 0.3±0.01, 0.4±0.01, 0.10±0.00 and 0.10±0.00 mm diameter against *Aspergillus niger*. The leaves extracts were active and it inhibited the growth of the micro-organisms. Minimum inhibitory concentration of these plants on the bacteria tested was 1000 μg mL^{-1}. The phytochemical screenings of these plants revealed the presence of saponins, tannins, flavonoids and phenol. **Conclusion:** The results indicated that these plants had antibacterial activity on the tested organisms and show their potentials for their use in the treatment of fish pathogens.

Key words: Antibacterial, holy basil leaves, guava leaves, fish pathogens, *Clarias gariepinus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish are susceptible to several bacterial infections mainly when reared in high density conditions. Disease outbreaks elevated the mortality rate and decrease the productivity efficiency, causing high economic loss of the fish farmers[1]. The continuous use of synthetic antimicrobial agent in aquaculture has resulted in more resistant bacterial strains in the aquatic environment[2]. The development of antibiotic resistant is multifactorial including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors[3].

This situation has forced scientist to search for a new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents but the cost of production of synthetic drug is high and they produce adverse effects compared to plant derived drugs[4]. Medicinal plants as the alternative agents are effective to treat the infectious diseases and mitigate many of side effects that are associated with synthetic antimicrobials[5].

Treatment of bacterial diseases with different medicinal plants have been safely used in organic agriculture, veterinary and human medicines and treatment with medicinal plants having antibacterial activity are a potentially beneficial alternative in aquaculture[6]. The medicinal plants may be used as potential and promising drugs against fish pathogens in the organic aquaculture[7]. Medicinal plants such as *Psidium guajava* and *Ocimum sanctum* can be used in fish health management but the mechanism of action of *P. guajava* and *O. sanctum* as antimicrobial agents are yet to be adequately researched.

The present study elucidates the antimicrobial activity of *P. guajava* and *O. sanctum* against different microbes.

MATERIALS AND METHODS

Plant collection: The *P. guajava* and *O. sanctum* leaves were used in the study and these plants were obtained in Igodan Lisa, Okitipupa, Nigeria on the 15th-17th January, 2015. The plants were identified by Mr. S.M. Erinoso in the Department of Biological Sciences (Botany Programme), Ondo State University of Science and Technology, Okitipupa, Nigeria.

Preparation and extraction of guava and holy basil leaves: Guava and holy basil leaves were air-dried in the Fisheries and Aquaculture Laboratory, Ondo State University of Science and Technology, Okitipupa, Nigeria, for 4 weeks (15th January-15th February, 2015). The extraction of guava and holy basil leaves were done as described by Ajaiyeoba and Fadare[8]. The air-dried extracts of guava and holy basil leaves were kept in a separate container and store at 25°C until required.

Culture media, chemicals and preparation: Media such as nutrient broth (Oxoid), nutrient agar (Biolife), Potato Dextrose Agar (PDA) (Oxoid) and Mueller-Hinton agar (HiMedia) were used for the study. Also, distilled water, ethanol and methanol were used for extraction process. These media and the solvent were purchased from a re-known company in Nigeria. All media used were prepared according to manufacturer's instruction. All these media are allowed to cool after sterilization to about 45°C before pouring into Petri dishes.

Source of test organisms: The micro-organisms isolated from *Clarias gariepinus* juveniles were *Aeromonas hydrophila*, *Streptococcus iniae* sp., *Bacillus substilis* and *Staphylococcus aureus*. The isolation characteristics of bacteria using bio-chemical test were carried out at Microbiology Laboratory, Faculty of Science, Ondo State University of Science and Technology, Okitipupa, Nigeria, on 2nd March-5th April, 2015. *Aspergillus flavus* was collected from the stock of the Department of Microbiology at Ondo State University of Science and Technology, Okitipupa, Nigeria. The pure cultures were labeled, sub-cultured on nutrient agar slants and nutrient broth(s) and potato dextrose agar (PDA), preserved in the refrigerator at 4°C until it is required for study.

Isolation of micro-organism/counts: The gills, skin, intestine and liver sample of *C. gariepinus* were separately macerated and put into sterile clapped test tube containing sterilized distilled water, homogenized and serial dilution was performed as described by Shalaby *et al.*[9]. Total viable count and Enterobacteriaceae counts were determined, the result were expressed in $\log_{10}$ CFU g^{-1}.

Antimicrobial assay: A well diffusion assay as described by Bello *et al.*[10] was used. Distilled water was used as negative control while antibiotics, chloramphenicol (10 and 20 mg mL^{-1}) were used as positive control. The plates were examined for zones of inhibition which was scored positive, if the width of the clear zone was 10 mm or longer.

The diameter of the inhibition zones was taken to be proportional to the logarithm of the antimicrobial compounds in guava and holy basil leaves[11].

Determination of minimum inhibitory concentration of plant extract by microdilution method: Double dilution of 2000 μg mL^{-1} of guava and holy basil leaves extract were made in 2 mL volume of broth to 15.63 μg mL^{-1}. One row of the test was inoculated with 0.02 mL of 1 in 100 dilution of the overnight broth culture of the organism[12]. The test was incubated at 37°C for 24 h aerobically. The minimum inhibitory concentration was the lowest concentration that prevented the growth of bacterial after 24 h incubation[13].

Determination of phytochemical screening: Phytochemical constituents such as saponins, phenols, tannins, flavonoids, glucosinolates, triterpenes and steroids, proteins and amino acids were done as described by Olusola *et al.*[14] and Adeoye and Oyedapo[15] methods.

RESULTS

Evaluation of phytochemical constituents in guava and holy basil leaves: The result of the phytochemical screening revealed the presence of saponins, tannins, flavonoids, phenols and protein. Glucosinolates and polysterols were absent in both plants as shown in Table 1.

Evaluation of microbial load in *Clarias gariepinus*: The results of the microbial load of fish tissue (skin, gills, intestine and liver) showed that skin had the highest Enterobacteriacea counts and total viable counts while the control recorded no Enterobacteriacea counts and total viable counts as shown in Table 2.

Evaluation of plant extracts bioactivity against fish pathogens: The results showed that aqueous, ethanolic and methanolic extracts of the guava leaves had better antimicrobial properties against the tested pathogens when compared with extracts of holy basil leaves and the control (Table 3).

Minimum inhibitory concentration (MIC) of plant extracts using microdilution method: The result showed 1000 and 2000 μg mL^{-1} of aqueous, ethanolic and methanolic extracts of guava and holy basil leaves, respectively against the tested pathogens except *S. iniae*, *B. subtilis* in aqueous and ethanolic extracts of guava leaves, respectively (Table 4).

Table 1: Determination of some important phytochemical of guava and holy basil leaves

Parameters	Guava leaves	Holy basil leaves
Saponins	+++	+
Tannins	+	+
Flavonoids	+	+
Glucosinolates	-	-
Phenol	+	+
Proteins	+	+
Polysterols	-	-

+++: Present and available in abundant quantity, +: Small quantity, -: Absent

Table 2: Microbial load of fish tissue

Fish site	Organism	Microbial load (log_{10} CFU g^{-1})
Liver	Enterobacteriacea counts	1.63±0.01
	Total viable counts	2.12±0.02
Intestine	Enterobacteriacea counts	2.17±0.02
	Total viable counts	2.48±0.03
Skin	Enterobacteriacea counts	2.47±0.01
	Total viable counts	2.66±0.06
Gills	Enterobacteriacea counts	2.27±0.04
	Total viable counts	2.33±0.01
Control	Enterobacteriacea counts	-
	Total viable counts	-

DISCUSSION

The result of the phytochemical screening showed the presence of saponins, tannins, flavonoids, phenol and protein. Glucosinolate and polysterol were not detected in both plants. The value of saponins obtained was abundant (+++) compared to the low quantity (+) observed in holy basil. The presence of these constituents in these plants was in agreement with the report of Kumar *et al.*[16], Shafqatullah *et al.*[17], Prasad *et al.*[18], Joshi *et al.*[19], Devendran and Balasubramanian[20] and Bihari *et al.*[21]. Also, this observation were similar with Arya *et al.*[22], who reported that the phytochemical screening of ethanol, aqueous and hydro alcoholic extracts revealed the presence of flavonoids, tannins, saponins, triterpenoids and alkaloids in *Psidium guajava*.

The epithelial surfaces of fish such as those of skin, gill or gastrointestinal tract are the first contact areas for potential pathogens[10]. The result of this work revealed that the microbial counts in the liver, intestine, skin and gill of *Clarias gariepinus* varies with the skin having the highest values of Enterobacteriacea and total viable counts. This agrees with Shalaby *et al.*[9] and Bello *et al.*[10] that bacterial load is greater on the skin and gills than any part of fish as these parts are ones constantly exposed to challenges.

Effects of different antibiotics on fish pathogenic bacteria under laboratory condition provided useful information on treatment of bacterial fish diseases[23]. The observation of the present study revealed that the both plants inhibited the

Table 3: Antimicrobial activities (diameter of inhibition zone, mm) of plant extracts on fish pathogens by well diffusion method

	Diameter of inhibition zone, mm								
	Aqueous		Methanol		Ethanol				
Pathogens	Guava leaves	Holy basil leaves	Guava leaves	Holy basil leaves	Guava leaves	Holy basil leaves	Chloramphenicol (10 mg mL^{-1})	Chloramphenicol (20 mg mL^{-1})	Control
Bacillus subtilis	20±0.01	18±0.02	22±0.03	18±0.02	24±0.03	17±0.02	20±0.02	25±0.02	-
Staphylococcus aureus	24±0.01	10±0.01	25±0.03	22±0.03	25±0.03	20±0.02	18±0.02	23±0.01	-
Streptococcus iniae	24±0.01	10±0.02	24±0.01	17±0.01	20±0.01	10±0.03	16±0.02	22±0.01	-
Aeromonas hydrophila	15±0.01	10±0.01	19±0.01	08±0.03	24±0.01	10±0.02	-	-	-
Aspergillus niger	07±0.00	04±0.00	01±0.00	01±0.00	03±0.00	04±0.02	ND	ND	-

ND: Not determined

Table 4: Minimum inhibitory concentration of aqueous, ethanolic and methanolic plant extracts against fish pathogens using microdilution method

		Minimum inhibitory concentration (μg mL^{-1})							
Parameters	Isolates	2000	1000	500	250	125	62.5	31.3	15.63
Guava leaves	*Bacillus subtilis*	-	-	-	+	+	+	+	+
		-	-	-	-	+	+	+	+
		-	-	+	+	+	+	+	+
	Staphylococcus aureus	-	-	-	+	+	+	+	+
		-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
	Streptococcus iniae	-	-	-	-	-	+	+	+
		-	-	+	+	+	+	+	+
		-	+	+	+	+	+	+	+
	Aeromonas hydrophila	-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
	Control (without isolates)	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
Holy basil leaves	*Bacillus subtilis*	-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
	Staphylococcus aureus	-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
		-	-	+	+	+	+	+	+
	Streptococcus iniae	-	-	+	+	+	+	+	+
		-	-	-	+	+	+	+	+
		-	-	+	+	+	+	+	+
	Aeromonas hydrophila	-	+	+	+	+	+	+	+
		-	+	+	+	+	+	+	+
		-	+	+	+	+	+	+	+
	Control (without isolates)	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-

+: Growth showed by turbidity of the broth, -: No growth

growth of pathogenic bacteria tested, *B. subtilis*, *S. iniae*, *S. aureus*, *A. hydrophila* and the fungi, *A. niger*. The negative control (distilled water) did not show any zone of inhibition while the positive control (Chloramphenicol) had inhibition of zone of diameter against the tested pathogens except *A. hydrophila.* Gram-positive and Gram-negative bacteria showed susceptibility toward all extracts, the zone of their inhibition range between 01-25 mm for aqueous, ethanolic and methanolic extracts of guava and holy basic leaves.

This result was similar to the report of Jeba and Rameshkumar[24], Singh *et al.*[25], Sanguri *et al.*[26], Rathod *et al.*[27], Mishra and Mishra[28]. Also, this result agrees with those obtained by Jaiarj *et al.*[29], who observed growth inhibition of *S. aureus* strains when these were diluted in water, ethanol and chloroform guava leaves extracts. Gnan and Demello[30] obtained similar results when testing the growth inhibition of *S. aureus* by guava leaves and fruit water extracts. Guava leaves has been shown to have significant effects on the Gram-negative and Gram-positive when compared to holy basil and chloramphenicol at 10 and 20 mg mL^{-1}.

The antibacterial activity of *Psidium guajava* is attributed to guajavenine and to psydiolic acid[31]. Also

the leaves contain large amount of tannin and triterpenoids (crategolics, gujavolic, oleanolics and ursolic acids) and essential oil containing β-sitosterol, β-bisabolene, β-caryophyllene, aromadendrene, B-selinene, guajevenine, nerolidiol[32] and se-ll-en-4α-01. The results of the present study observed that the guava leaves extracts were more active than chloramphenicol and control (distilled water). This report supported with the study of Thanangkol and Chaichangptipayut[33], who found that guava leaves were more efficient than synthetic drugs in the treatment of infections.

The minimum inhibitory concentration (MIC) of the guava and holy basil leaves extracts was determined using the micro dilution methods. It is found that aqueous, ethanol and methanol extracts of these plants were active against the pathogen tested and it was recorded that 1000 μg mL^{-1} was the least concentration that prevented the growth of bacteria after 24 h incubation except *A. hydrophila* and *S. iniae* for aqueous, methanol and ethanol extracts of holy basil and methanol extracts of guava leaves who recorded 2000 μg mL^{-1}. This result was aligned with the report of Geidam *et al*.[34] and Sanches *et al*.[35].

CONCLUSION

Guava and holy basil leaves is widely available, less toxic, suitable for boosting immune system and effectiveness of aqueous, ethanolic and methanolic extracts against fish pathogens thus make guava (*P. guajava*) leaves and holy basil (*O. sanctum*) leaves a very promising alternatives to commercial antibiotics that are losing efficacy in the treatment of fish diseases.

SIGNIFICANCE STATEMENT

This study discovered that the *P. guajava* and *O. sanctum* leaves extracts had significant potential for the development of new antimicrobial treatment and reduction of drug resistance, which will permit to find the treatment of several diseases caused by micro-organisms. This study will help the researchers to uncover the critical areas of plant extracts that many researchers were not able to explore. Thus a new theory on antimicrobial inhibition by the plant extracts may be arrived at.

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REFERENCES

1. Sharma, M., A.K. Mandloi, G. Pandey and Y.P. Sahni, 2012. Antimicrobial activity of some medicinal plants against fish pathogens. Int. Res. J. Pharm., 3: 28-30.
2. Muniruzzaman, M. and M.B.R. Chowdhury, 2004. Sensitivity of fish pathogenic bacteria to various medicinal herbs. Bangla. J. Vet. Med., 2: 75-82.
3. Jouda, M.M., 2013. The antibacterial effect of some medicinal plant extracts and their synergistic effect with antibiotic and non-antibiotic drugs. M.Sc. Thesis, Faculty of Science, Islamic University of Gaza, Gaza Strip, Palestine.
4. Abiramasundari, P., V. Priya, G.P. Jeyanthi and S.G. Devi, 2011. Evaluation of the antibacterial activity of *Cocculus hirsutus*. J. Drugs Med., 3: 26-31.
5. Punitha, S.M.J., M.M. Babu, V. Sivaram, V.S. Shankar and S.A. Dhas *et al*., 2008. Immunostimulating influence of herbal biomedicines on nonspecific immunity in grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection. Aquacult. Int., 16: 511-523.
6. Abutbul, S., A. Golan-Goldhirsh, O. Barazani, R. Ofir and D. Zilberg, 2005. Screening of desert plants for use against bacterial pathogens in fish. Israel J. Aquacult. Bamidgeh, 57: 71-80.
7. Turker, H., A.B. Yildirim and F.P. Karakas, 2009. Sensitivity of bacteria isolated from fish to some medicinal plants. Turk. J. Fish. Aquat. Sci., 9: 181-186.
8. Ajaiyeoba, E.O. and D.A. Fadare, 2006. Antimicrobial potential of extracts and fractions of the African walnut-*Tetracarpidium conophorum* Afr. J. Biotechnol., 5: 2322-2325.
9. Shalaby, A.M., Y.A. Khattab and A.M. Abdel Rahman, 2006. Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). J. Venomous Anim. Toxins Incl. Trop. Dis., 12: 172-201.
10. Bello, O.S., F.E. Olaifa, B.O. Emikpe and S.T. Ogunbanwo, 2013. Potentials of walnut (*Tetracarpidium conophorum* Mull. Arg) leaf and onion (*Allium cepa* Linn) bulb extracts as antimicrobial agents for fish. Afr. J. Microbiol. Res., 7: 2027-2033.
11. Farias, M.E., A.A. de Ruiz Holgado and F. Sesma, 1994. Bacteriocin production by lactic acid bacteria isolated from regional cheeses: Inhibition of foodborne pathogens. J. Food Protect., 57: 1013-1015.
12. Stokes, E.J and G.L. Ridgeway, 1980. Clinical Bacteriology. 5th Edn., Edward Arnold Ltd., London, UK., ISBN-13: 9780815182641, pp: 188.
13. Osoba, A.O., 1979. The control of gonococcal infections and other sexually transmitted diseases in developing countries-with particular reference to Nigeria. Niger. J. Med. Sci., 2: 127-133.

14. Olusola, S.E., S. Fakoya and I.B. Omage, 2017. The potential of different extraction methods of soursop (*Annona muricata* Linn) leaves as antimicrobial agents for aquatic animals. Int. J. Aquacult., 7: 122-127.
15. Adeoye, B.A. and O.O. Oyedapo, 2004. Toxicity of *Erythrophleum guineense* stem-bark: Role of Alkaloidal fraction. Afr. J. Tradit. Complement. Altern. Med., 1: 45-54.
16. Kumar, A., A. Rahal, S. Chakraborty, R. Tiwari, S.K. Latheef and K. Dhama, 2013. *Ocimum sanctum* (Tulsi): A miracle herb and boon to medical science-A review. Int. J. Agron. Plant Prod., 4: 1580-1589.
17. Shafqatullah, M. Khurram, Asadullah, Khaliqurrehman and F.A. Khan, 2013. Comparative analyses of *Ocimum santum* stem and leaves for phytochemicals and inorganic constituents. Middle East J. Scient. Res., 13: 236-240.
18. Prasad, M.P., K. Jayalakshmi and G.G. Rindhe, 2012. Antibacterial activity of *Ocimum* species and their phytochemical and antioxidant potential. Int. J. Microbiol. Res., 4: 302-307.
19. Joshi, B., G.P. Sah, B.B. Basnet, M.R. Bhatt and D. Sharma *et al*., 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J. Microbiol. Antimicrob., 3: 1-7.
20. Devendran, G and U. Balasubramanian, 2011. Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L. leaves. Asian J. Plant Sci. Resour., 1: 44-48.
21. Bihari, G.C., B. Manaswini, J. Prabhat and T.S. Kumar, 2011. Pharmacognostical and phytochemical investigation of various tulsi plants available in South Eastern Odisha. Int. J. Res. Pharmaceut. Biomed. Sci., 2: 605-610.
22. Arya, V., N. Thakur and C.P. Kashyap, 2012. Preliminary phytochemical analysis of the extracts of *Psidium* leaves. J. Pharmacogn. Phytochem., 1: 1-5.
23. Rahman, T., M.M.R. Akanda, M.M. Rahman and M.B.R. Chowdhury, 2009. Evaluation of the efficacies of selected antibiotics and medicinal plants on common bacterial fish pathogens. J. Bangladesh Agric. Univ., 7: 163-168.
24. Jeba, R.C. and G. Rameshkumar, 2013. Antimicrobial activity of *Ocimum* species. Int. J. Pharmaceut. Sci. Health Care, 3: 1-9.
25. Singh, A.R., V.K. Bajaj, P.S. Sekhawat and K. Singh, 2013. Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum sanctum* L. J. Nat. Prod. Plant Resour., 3: 51-58.
26. Sanguri, S., S. Kapil, P. Gopinathan, F.K. Pandey and T. Bhatnagar, 2012. Comparative screening of antibacterial and antifungal activities of some weeds and medicinal plants leaf extracts: An *in-vitro* study. Elixir Applied Bot., 47: 8903-8905.
27. Rathod, G.P., B.M. Kotecha, R. Sharma, H. Amin and P.K. Prajapati, 2012. *In vitro* antibacterial study of two commonly used medicinal plants in ayurveda: Neem (*Azadirachta indica* L.) and tulsi (*Ocimum sanctum* L.). Int. J. Pharmaceut. Biol. Arch., 3: 582-586.
28. Mishra, P. and S. Mishra, 2011. Study of antibacterial activity of *Ocimum sanctum* extract against gram positive and gram negative bacteria. Am. J. Food Technol., 6: 336-341.
29. Jaiarj, P., P. Khoohaswan, Y. Wongkrajang, P. Peungvicha, P. Suriyawong, M.L.S. Saraya and O. Ruangsomboom, 1999. Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. J. Ethopharmacol., 67: 203-212.
30. Gnan, S.O. and M.T. Demello, 1999. Inhibition of *Staphylococcus aureus* by aqueous Goiaba extracts. J. Ethnopharmacol., 68: 103-108.
31. Berdy, J., A. Aszalos, M. Bostian and K.L. Mcnitt, 1981. CRC Handbook of Antibiotic Compounds. Vol. 8, Part I & II, CRC Press, Boca Raton, FL., USA.
32. Morton, J.F., 1981. Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan. Vol. 1, Thomas Publisher, Springfield, IL., USA., ISBN-13: 9780398040369, pp: 745-750.
33. Thanangkol, P. and C. Chaichangptipayut, 1987. Double-blind study of *Psidium guajava* L. and tetracycline in acute diarrhoea. Siriraj Hosp. Gazette, 39: 253-267.
34. Geidam, Y.A., A.G. Ambali and P.A. Onyeyili, 2007. Preliminary phytochemical and antibacterial evaluation of crude aqueous extract of *Psidium guajava* leaf. J. Applied Sci., 7: 511-514.
35. Sanches, R.N., 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.). Braz. Arch. Biol. Technol., 48: 429-436.