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Research on Comparative Study of Antimicrobial Activity on Neem and Guava Leaves

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Abstract

This work aims to evaluate the antimicrobial potential of ethanolic extracts of Neem leaves and guava leaves. The antibacterial activity of guava (Psidium Guajava) and Neem (Azadirachta Indica) extracts against Staphylococcus Aureus, Escherichia coli,, Salmonella typhimurium, Vibrio cholerae and Bacillus cereus Guava and Neem extracts showed higher antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria except for V. Parahaemolyticus, P. Aeruginosa, and A. Hydrophila. Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations (MIC) of different plant extracts against Gram-positive bacteria (Bacillus cereus, Staphylococcus aureus), Gram-negative bacteria (Escherichia coli, Salmonella typhimurium, and Vibrio cholera. The extracts exhibited both antibacterial and antifungal activities against tested microorganisms. To enhance our understanding of antimicrobial activity mechanism of plant extracts, the changes in internal pH and membrane potential were measured in Staphylococcus aureus (SA) and Escherichia coli (EC) cells after exposure to the plant extracts. The results indicated that the plant extracts significantly affected the cell membrane of Gram-positive and Gram-negative bacteria, as demonstrated by the decline in pH as well as cell membrane hyperpolarization. In conclusion, plant extracts are of great value as natural antimicrobials and can be used safely as food preservatives.

Keywords: Neem; Guava; Antimicrobial activity; Gram positive; Gram negative

Introduction

Antimicrobial activity can be defined as a collective term for all active principles (agents) that inhibit the growth of bacteria; prevent the formation of microbial colonies; and may destroy microorganisms. Traditionally; the crude extracts of different parts of medical plants; including root; stem; flower; fruit; and twigs; were widely used for treatments of some human diseases. Medicinal plants contain several phytochemicals such as flavonoids; alkaloids; tannins; and terpenoids; which possess antimicrobial and antioxidant properties. The antimicrobial activities of some plant species have been widely researched. For example; the crude extracts of Neem; guava; cinnamon; garlic; basil; curry; ginger; sage; mustard; and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria.

Technically a microorganism or microbe is an organism that is microscopic. The study of microorganisms is called microbiology. Microorganisms can be bacteria; fungi; archaea or protists. The term microorganism does not include viruses and prions; which are generally classified as non-living. A microorganism or microbe is an organism of microscopic size; which may exist in its single-celled form or as a colony of cells. Microorganisms can have very different habitats; and live everywhere from the poles to the equator; deserts; geysers; rocks; and the deep sea. Some are adapted to extremes such as very hot or very cold conditions; others to high pressure; and a few; such as Deinococcus radiodurans; to high radiation environments. Microorganisms also make up the microbiota found in and on all multicellular organisms [1, 2].

Azadirachta Indica L. (Neem)

The plant product or natural products show an important role in disease prevention and treatment through the enhancement of antioxidant activity; inhibition of bacterial growth; antimicrobial activity and modulation of genetic pathways. The therapeutic role of the number of plants in disease management is still being enthusiastically researched due to their less side effects and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effects on normal tissues and on various biological activities. It largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants [3, 4].

Neem ingredients are applied in Ayurveda; Unani; Homeopathy; and modern medicine for the treatment of many infectious; metabolic; or cancer diseases [5, 6]. Different types of preparation based on plants or their constituents are very popular in many countries in diseases management. In this vista; Neem (Azadirachta Indica); a member of the Meliaceae family; commonly found in India; Pakistan; Bangladesh; and Nepal; has therapeutics implication in diseases cure and formulation based on the fact that Neem is also used to treat various diseases. Azadirachta Indica has a complex of various constituents including nimbin; nimbidin; nimbolide; and limonoids and such types of ingredients play a role in disease management through modulation of various genetic pathways and other activities. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves and were known to have antifungal and antibacterial activities. Numerous biological and pharmacological activities have been reported including antibacterial; antifungal and anti-inflammatory. Earlier investigators have confirmed their role as anti-inflammatory;

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anti-arthritic; antipyretic; hypoglycaemic; anti gastric ulcer; antifungal; antibacterial; and antitumor activities] and a review summarized the various therapeutics role of Neem This review summarizes the role of Neem and its active ingredients in the disease prevention and treatment through the modulation of various biological pathways [6-13] (Table 1).

Botanical Description of Neem

Neem tree belongs to the family Meliaceae which is found in abundance in tropical and semitropical regions like India; Bangladesh; Pakistan; and Nepal. It is a fast-growing tree with 20–23 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound; imparipinnate; with each comprising 5–15 leaflets. Its fruits are green drupes which turn golden yellow on ripening in the months of June–August.

Active Compounds of Azadirachta Indica L. (Neem)

Azadirachta Indica L. (Neem) shows therapeutics role in health management due to the rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbolinin; nimbin; nimbidin; nimbidol; sodium nimbinate; gedunin; salannin; and quercetin. Leaves contain ingredients such as nimbin; nimbanene; 6-desacetyl nimbinene; nimbandiol; nimbolide; ascorbic acid; amino acid; n-hexacosanol ;7-desacetyl-7-benzoyl azadiradione; 7-desacetyl-7-benzoyl gedunin; 17-hydroxy azadiradione; and nimbiol Quercetin and ß-sitosterol; polyphenolic flavonoids; were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin [14-16].

Mechanism of Action of Active Compounds

Neem (Azadirachta indica); a member of the Meliaceae family; has therapeutics implication in the diseases prevention and treatment. But the exact molecular mechanism in the prevention of pathogenesis is not understood entirely. It is considered that Azadirachta indica shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin; nimbolinin; nimbin; nimbidin; nimbidol; salannin; and quercetin.

Possible mechanism of action of Azadirachta Indica is presented as follows: Neem (Azadirachta Indica) plants parts shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown. Azadirachtin; a complex tetranortriterpenoid limonoid present in seeds; is the key constituent responsible for both anti-feedant and toxic effects in insects]. Results suggest that the ethanol extract of Neem leaves showed in vitro antibacterial activity against both Staphylococcus aureus and MRSA with greatest zones of inhibition noted at 100% concentration [17,18] (Figure 1).

Psidium Guajava (Guava)

Guava; Psidium Guajava (Linn.); a member of Myrtaceae family; is a common tropical plant with a long history of traditional usage. It is used not only as food but also as folk medicine; and various parts of this plant have a number of medicinal properties ranging from antimicrobial

Table 1: Taxonomic position of Azadirachta Indica (Neem).

Order	Rutales
Sub order	Rutinae
Family	Meliaceae
Sub family	Meliaceae
	Meliae
Genus	Azadirachta

Figure 1: Neem leaves.

activity to anticancer property. An added advantage is that cultivation of guava is relatively easy as it thrives in a variety of soils and adapts to different climatic conditions; the fruits are also borne fairly in a short period. Due to the various commercial applications; guava trees are found throughout India. Although they are planted in almost all states; Andhra Pradesh; Assam; Bihar; Maharashtra; Uttar Pradesh; and West Bengal are the important cultivators of this plant [19].

Guava benefits-Medicinal properties

The high presence of tannins give Guava antidiarrheal properties; also have demonstrated pharmacological activity as antibacterial; antioxidant; antispasmodic; anti-inflammatory; anti-anemic; hemostatic and sedative. It is indicated in cases of dyspepsia; edema; swelling; dizziness; diarrhea; nausea; nervousness; HIV; skin conditions (Table 2).

Table 2: Taxonomic position of Psidium guajava.

Kingdom	Plantae
Order	Myrtaceae
Family	Psidium
Species	P.guajava
Genus	Psidium
Binomial name	Psidium guajava L

Botanical Description of Guava

Guava (Psidium Guajava L.) is a small branched tree or shrub up to 7–10 m tall. The root system is superficial. The trunk is woody; hard; with a characteristic smooth; pale mottled bark that peels off in thin flakes; after the trunk has grown to about 20 cm in diameter.

Content and active ingredients

It is known for the presence of gallic acid; ellagic acid; catechin; epicatechin; rutin and quercetin.Pentacyclic triterpene; guajanoico acid and B-sitosterol; uvaol; olenólico acid and ursolic acid. Guava leaves contain an essential oil rich in caryophyllene; nerolidol; beta bisabolene; aromandreno; p-selinene. Also contain flavonoids; beta sitosterol; triterpenoids; leucocyanidin and about 10% of tannins Phytochemical analyses of guava leaf reveal alkaloids; anthocyanins; carotenoids; essential oils; fatty acids; flavonoids (especially quercetin); lectins; phenols; saponins; tannins; triterpenes; and vitamin C (80 mg per 100 g of guava). The essential oil contains alpha pinene; caryophyllene; cineol; D-limonene; eugenol; and myrcene. The major constituents of the volatile acids include (E)-cinnamic acid and (Z)-3-hexenoic acid [5,10]. The guava fruit has a high water content with lesser amounts of carbohydrates; proteins; and fats. The fruit also contains iron;

vitamins A and C; thiamine; riboflavin; niacin; and manganese. The characteristic fruit odor is attributed to carbonyl compounds. Unripe fruits are high in tannins. The major constituent of the fruit skin is ascorbic acid; largely destroyed by canning and processing. The bark of the plant contains tannins (12% to 30%) and calcium oxalate crystals; while the seeds contain glycine-rich proteins; starch; and phenolic and flavonoid compounds [20-23] (Figure 2).

Figure 2: Guava leaves.

Literature Review

- The antimicrobial activity of Azadirachta Indica leaf extract (Neem leaf) was carried out on Staphylococcus aureus; Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli using the agar well diffusion method. Ethanolic; aqueous and methanolic extracts of the plant were used at varying concentrations: Antimicrobial Activity of Azadirachta Indica (Neem) Leaf Extract on Some Bacteria.

- The aim of the study was to determine the efficacy of Neem leaf extract against multidrug resistant (MDR) pathogenic bacteria. Laboratory stock culture of Pasteurella multocida; Salmonella pullorum; Salmonella gallinarum and Escherichia coli was revived.

Edris Ali: Conceptualization; Investigation; Writing-original draft; Writing-review & editing. Md. Sadequl Islam: Conceptualization; Methodology; Writing-original draft; Writing-review & editing. Md. Ismail Hossen: Methodology; Writing-review & editing. Mst. Minara Khatun: Data curation; Investigation; Methodology; Writing-review & editing. Md. Ariful Islam: Conceptualization; Data curation; Investigation; Supervision; Writing-original draft; Writing-review & editing.

- The antibacterial activity of guava (Psidium Guajava) and Neem (Azadirachta Indica) extracts against 21 strains of foodborne pathogens were determined--Listeria monocytogenes (five strains); Staphylococcus aureus (four strains); Escherichia coli O157:H7 (six strains); Salmonella Enteritidis (four strains); Vibrio parahaemolyticus; and Bacillus cereus; and five food spoilage bacteria: Pseudomonas aeruginosa; P. putida; Alcaligenes faecalis; and Aeromonas hydrophila (two strains). Latiful BariUniversity of Dhaka

- Center for Advanced Research in Sciences PhD.

- To determine the antimicrobial potential of guava (Psidium Guajava) leaf extracts against two gram-negative bacteria (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) which are some of foodborne and spoilage bacteria.

S Kim and DYC Fung (2004) Antibacterial effect of crude water-soluble arrowroot (Puerariae radix) tea extracts on food-borne pathogens in liquid medium. Letters in Applied Microbiology 39: 319-325.

The antibacterial activity of guava (Psidium Guajava) and Neem (Azadirachta Indica) extracts against 21 strains of foodborne pathogens were determined-Listeria monocytogenes (five strains); Staphylococcus aureus (four strains); Escherichia coli O157:H7 (six strains); Salmonella Enteritidis (four strains); Vibrio Parahaemolyticus; and Bacillus cereus; and five food spoilage bacteria: Pseudomonas aeruginosa; P. putida; Alcaligenes faecalis; and Aero monas hydrophila (two strains).

Need of Work

- Give knowledge about various antimicrobial agents.
- To discover various antimicrobial agents.
- Study various effects of Neem leaves and Guava leaves.
- Study the importance of Neem and Guava.

Aims and Objective

Aims

- To check the antimicrobial activity of Neem leaves and Guava leaves.
- To give knowledge about the chemical constituents of Neem leaves and guava leaves.
- To study the effect of antimicrobial agents on microbes.
- To study the various methods of extraction of Neem leaves and Guava leaves.
- To study the various methods of antimicrobial activity.

Objectives

- To accumulate the plant material.
- To authenticate the plant material.
- To extract plant fabric with the aid of the usage of soxhlet apparatus.
- Phytochemical screening of chemical components existing in both extracts.
- To find out about antimicrobial exercise of each Neem leaves and guava leaves extracts.
- To find out the synergistic impact of both plant extracts in opposition to gram fine S.aureus and gram negative E. coli.
- To measure the minimum inhibitory concentration (MIC) of the chosen plant extracts in opposition to gram positive S. aureus and gram poor E. coli.

Plan of Work

In the starting of 8th semester in January 2022 we select the subject

and Domain under the guidance. Then from February to March we planned a Review of literature survey. After that in mid of April we collect the Neem and Guava leaves. Prepare their extract and study antimicrobial activity of both plants. At the end of April covered the typing of the project. In May the final project was printed and submitted.

Materials and Methods

Drying

Neem and Guava leaves were collected and washed in water and dried in shade for 7-8 day's to minimize the moisture content. After samples were dried; stored in a protected place until similar use. These leaves had been in addition used for qualitative; quantitative; antimicrobial and other methods (Figures 3 and 4).

Grinding

Once the leaves had been properly dried; then placed into a blender to be grounded into powder; this powder is used for making aqueous extract.

Preparation of extract: About 200 g of the powder were separately soaked in 400 ml of 95% ethanol in a 500ml reagent bottle and stoppered. This was allowed to stand for 7-8 days to permit full extraction of the active ingredients. After soaking in solvent; the mixtures were transferred to Centrifugation machine and centrifuged for 10 min at 4; 000 rpm. The fluids were then filtered using Whatman No. 1 filter paper. Flask wrapped in aluminium foil to avoid evaporation and exposure to light. The supernatant was collected and stored at 4°C until use. An 80 mg/ml solution of each extract was prepared and appropriately diluted to obtain 40 mg/ml; 20 mg/ml; and 10 mg/ml concentrations needed for the bioassay (Figures 5 and 6).

Qualitative Phytochemical Screening

Tests for Alkaloids: 5 g of evaporated extract was boiled with 5 ml of 2% HCL on a steam bath for 5minutes; the mixture was filtered after cooling; and the filtrate was shared into 3 test tubes A B and C. 1 ml portion of the filtrate was treated with 2 drops of Mayer's reagent. To Confirm this result; 1 ml portion of the filtrate was treated with Dragendoff's reagent (Oseni 2011).

Figure 3: Dried leaves of Neem.

Figure 4: Dried leaves of Guava.

Figure 5: Centrifugation.

Figure 6: Neem and Guava leaves extract.

Test for Flavonoids: 5 g of extract was introduced into a test tube containing 10 ml ethyl acetate solution and was heated in boiling water for a minute. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of 1% aluminium chloride solution and left to stand for 10 min. The formation of a yellow coloration in the presence of 1ml of dilutes ammonia solution; indicates the presence of flavonoids (Oseni 2011).

Test for Saponins: 1 g of extract was boiled with 5 ml of distilled water for 5 min; the mixture was filtered while hot. To 1 ml of filtrate; two (2) drops of olive oil was added; the mixture was shaken and observed for the formation of an emulsion. 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken and then observed for the formation of stable frothing on standing (Oseni 2011).

Test for Tannins: To 2 g of the sample; 5ml of 45% ethanol was added and boiled for 5 minutes. The mixture was cooled and filtered. To 1 ml of the filtrate; three (3) drops of lead acetate solution was added. The formation of gelatinous precipitate indicates the presence of tannins. Also as a confirmation test; 1 ml of filtrate was treated with

0.5 ml of bromine water and the formation of a pale brown precipitate indicates the presence of tannins (Mbaeyi-Nwaoha and Emejulu 2013).

Test for Glycosides: 2 g of sample was mixed with 30 ml of distilled water and boiled for 5 min in a water bath. The mixture was cooled and filtered. To 5 ml of the filtrate; 0.2 ml of Fehling's solution A and B were added and boiled further in a water bath for 2 min. A brick red coloration indicates the presence of glycosides (Mbaeyi-Nwaoha and Emejulu 2013).

Test for Carbohydrate

Boil separately 2ml of Fehling's solution A and B and add 2 ml of the plant extract for 3 min. Observation of a deep blue to green coloration is indicative of a positive result (Mbaeyi-Nwaoha and Emejulu 2013).

Test for Steroids and Sterols: Salkowski's test: 5 g of extract was dissolved in 2ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence; indicating the presence of the steroids and sterols compound; in the extract (Oseni 2011).

Test for reducing sugars: The plant extract was treated with Fehling's solution (A and B) in a test tube. The colour change from deep blue to brick red indicates the presence of reducing sugar.

Test for oils: About 0.2 g of the plant extract was pressed between filter papers and observed for transparency. A control was also prepared by placing 2 drops of olive oil on another filter paper and also observes for translucency. If the filter paper becomes transparent; it shows the presence of oils in the paste.

Test for terpenoids: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids (Figures 7 and 8).

Antimicrobial Sensitivity Bioassay

The antimicrobial assay was performed by using the agar well diffusion method (Habamu 2010, Perez 1990). Wells of 10 mm in diameter were made into previously seeded Nutrient agar plates. Each well was filled with (1.0 ml) of the extract. The same quantity of sterile distilled water and 50% ethanol both without plant extract served as controls. The plates were preincubated for 2 h to allow diffusion of extract before incubating overnight at 37°C. The diameter of the clear zone was measured in mm using a well-calibrated meter rule. Triplicate plates were prepared for each extract and controls (Tables 3 and 4).

Preparation of nutrients media: Nutrient Agar is a general purpose; nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi; and contains many nutrients needed for the bacterial growth.

Figure 7: Phytochemical test for Guava.

Figure 8: Phytochemical test for Neem.

Table 3: Qualitative phytochemical screening of Neem.

Chemical tests	Ethanol extract	Aqueous extract
Alkaloids	+	+
Steroids	+	-
Saponins	+	-
Tannins	+	-
Flavonoids	+	+
Carbohydrates	+	+
Glycosides	+	+

Table 4: Qualitative phytochemical screening of Guava.

Chemical tests	Ethanol extract	Aqueous extract
Alkaloids	+	+
Steroids	+	+
Saponins	-	-
Tannins	-	-
Flavonoids	+	+
Carbohydrates	+	+
Glycosides	+	+

Composition of Nutrient Agar

- 0.5% Peptone: It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria.
- 0.3% beef extract/yeast extract: It is the water-soluble substances which aid in bacterial growth; such as vitamins; carbohydrates; organic nitrogen compounds and salts.
- 1.5% agar: It is the solidifying agent.
- 0.5% NaCl: The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.
- Distilled water: Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.
- pH is adjusted to neutral (7.4) at 25 °C.

Preparation of Nutrient Agar

- Suspend 28 g of nutrient agar powder in 1 litre of distilled water.
- Heat this mixture while stirring to fully dissolve all components.

- Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
- Once the nutrient agar has been autoclaved; allow it to cool but not solidify.
- Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.
- Replace the lid of each Petri dish and store the plates in a refrigerator.

Determination of Minimum Inhibitory Concentration (The minimum inhibitory concentration): (MIC) of the extracts were determined using the method described by Vinothkumar (2010) by diluting the extracts double fold (beginning with 40mg/ml) with nutrient broth in a series of test tubes and to each of the tubes; equal volume of the test organism was added and incubated at 37 °C for 24 h. Controls were prepared by inoculating tubes without the extracts but with the cell suspensions. The tubes were then examined for the presence of turbidity after the incubation period. The least concentration with no observable bacterial growth; when compared with the control; was considered as the (MIC).

Result

Observation table: The comparative study of Neem leaves and guava leaves show that Neem is more effective than Guava (Tablea 5 and 6).

Table 5: Antimicrobial activity of Neem (zone and inhibition mm).

Sr.no	Name of the organism	Ethanol extract of neen
1	E coli	11mm
2	Vibrio cholerae	17mm
3	Salmonella typhimurium	10mm
4	Staphylococcus aureus	12mm
5	Bacillus pumilus	23mm

Table 6: Antimicrobial activity of Guava (zone and inhibition mm).

Sr.no	Name of the organism	Ethanol extract of Guava
1	E.coli	-
2	Vibrio cholerae	-
3	Salmonella typhimurium	-
4	Staphylococcus aureus	11mm
5	Bacillus pumilus	6mm

Conclusion

Natural antimicrobial agent have been extra famous due to their efficacy in opposition to antibiotic resistant microorganism and campaign for consumption of herbal product according to previous reports; extract of Neem and Guava established antimicrobial activity towards specific microorganisms. The result of this study also suggests that guava and Neem extracts possess compounds containing antimicrobial properties that can be useful to control growth of microorganisms.

References

1. Tyrell Kelly (2017) Oldest fossils ever found show life on Earth began 3.5 billion years ago. University of Wisconsin-Madison.
2. Schopf JW, Kitajima K, Spicuzza MJ, Kudryavtsev AB, Valley JW (2017) SIMS analyses of the oldest known assemblage of microfossils document their taxon-correlated carbon isotope compositions. Proc Natl Acad Sci U S A 115: 53-58.
3. Zong A, Cao H, Wang F (2012) Anticancer polysaccharides from natural resources: a review of recent research. Carbohydrate Polymers. Carbohydr Polym 90: 1395-1410.
4. Efferth T, Koch E (2011) Complex interactions between Phytochemicals. The Multi-Target Therapeutic concept of Phytotherapy. Curr Drug Targets 12: 122-132.
5. Brahmachari G (2004) Neem-an omnipotent plant: retrospection. ChemBioChem 5: 408-421.
6. Ketkar AY, Ketkar CM, Jacobson M, Ketkar MS, Schmutterer H (2004) Various uses of neem products. 518-525.
7. Govindachari TR, Suresh G, Gopalakrishnan G, Banumathy B, Masilamani S (1998) Identification of antifungal compounds from the seed oil of Azadirachta indica. Phytoparasitica 26: 109-116.
8. Singh N, Sastry MS (1997) Antimicrobial activity of Neem oil. Indian J Pharmacol 13: 102-106.
9. Kher A, Chaurasia SC (1997) Antifungal activity of essential oils of three medical plants. Indian Drugs 15: 41-42.
10. Bandyopadhyay U, Biswas K, Sengupta A, Moitra P, Dutta P, et al. (2004) Clinical studies on the effect of Neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer. Life Sciences 75: 2867-2878.
11. Sultana B, Anwar F, Przybylski R (2007) Antioxidant activity of phenolic components present in barks of Azadirachta indica; Terminalia arjuna; Acacia nilotica; and Eugenia jambolana Lam. trees. Food Chemistry 104: 1106-1114.
12. Ebony PE, Atang Who IJ, Eyong EU, Egbung GE (2008) The antidiabetic efficacy of combined extracts from two continental plants: Azadirachta indica (A. Juss) (Neem) and Vernonia amygdalina (Del.) (African Bitter Leaf). Am J Biochem Biotechnol 4: 239-244.
13. Paul R, Prasad M, Sah NK (2011) Anticancer biology of Azadirachta indica L (neem): a mini review. Cancer Biology and Therapy 12: 467-476.
14. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U (2002) Biological activities and medicinal properties of neem (Azadirachta Indica). Current Science 82: 1336-1345.
15. Ali A (1993) A Study on Antiurolithiatic Activity of Melia Azadirachta L. Aqueous Extract in Rats. Textbook of Pharmacognosy 2: 27-31.
16. Hossain MA, Shah MD, Sakari M (2011) Gas chromatography–mass spectrometry analysis of various organic extracts of Merremia borneensis from Sabah. Asian Pac J Trop Med 4: 637-641.
17. Kokate C, Purohit AP, Gokhale SB (2010) A Study on Antiurolithiatic Activity of Melia Azadirachta L. Aqueous Extract in Rats. Pharmacognosy. Maharashtra; India: Nirali Prakashan 2: 27-31.
18. Mordue (Luntz) AJ, Nisbet AJ (2000) Azadirachtin from the neem tree Azadirachta indica: its action against insects. Anais da Sociedade Entomológica do Brasil 29: 615-632.
19. Sarmiento WC, Maramba CC, Gonzales MLM (2011) An in vitro study on the antibacterial effect of neem (Azadirachta indica) leaf extracts on methicillin-sensitive and methicillin-resistant Staphylococcus aureus. PIDSP Journal 12: 40-45.
20. Gutiérrez RMP, Mitchell S, Solis RV (2008) Psidium guajava: A review of its traditional uses; phytochemistry and pharmacology. J Ethnopharmacol 117: 1-27.
21. Olajide OA, Awe SO, Makinde JM (1999) Pharmacological studies on the leaf of Psidium guajava. Fitoterapia 70: 25-31.
22. Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, et al. (2002) Triterpenoids from the leaves of Psidium guajava. Phytochemistry 61: 399-403.
23. Latza S, Ganber D, Berger RG (1996) Carbohydrate esters of cinnamic acid from fruits of Physalis peruviana; Psidium guajava and Vaccinium vitis-idaea. Phytochemistry 43: 481-485.