CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1340635

Available online at: http://www.iajps.com Research Article

A COMPARATIVE INVESTIGATIONAL STUDIES ON PHARMACOGNOSY, PHYTOCHEMICAL ASPECTS AND ANTI-MICROBIAL ACTIVITY OF A *DELONIX REGIA* FLOWERS AND SEEDS ON METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS.*

G.V. Pavan Kumar[*1], Y.Malyadri, G.Pooja[2], B.Bhavani[4]

[*1,2, 3,4] Koringa College of Pharmacy, Korangi-533461, Kakinada, Andhra Pradesh, India,

Abstract:

An attempt was made to report the comparative pharmacognostic and phytochemical analysis of the flowers and seeds of Delonix regia by determination of ash values, extractive values, moisture content and fluorescence analysis.The aqueous extract of seeds and flowers reveal the chemical constituents by preliminary phytochemical screening and the extracts were subjected to evaluate the comparative anti-microbial activity of flowers and seeds of Delonix regia by agar bore well assay on gram stain microbes Methicillin resistant Staphylococcus aureus .The powdered flowers and seeds were subjected for the proximate analysis. Major Extraction was carried out by soxhlet extraction method using ethanol as solvent. The comparative anti-microbial activity of flowers and seeds of Delonix regia was evaluated in vitro. A greenish solid matter of 4.53 % w/w was obtained from the dried seeds and a pale yellow solid matter of 2.15 % w/w was obtained from the dried flower petals powder. Investigation of ethanolic extract of Delonix regia seeds and flowers produced a significant anti-microbial activity and the MIC values were found to be 1.75µg/ml and 2.0µg/ml respectively.The present work justifies the folklore claim of the medicinal importance of Delonix regia seeds and flowers for treatment of high resistant microbial strains, which was scientifically proven.

Keywords: *Delonix regia ,flowers,seeds, pharmacognsy, Phytochemical analysis anti-microbial activity.*

*** Corresponding author:**

Dr.G.V.Pavan Kumar,
Associate Professor,
Dept.of Pharmaceutical Chemistry,
Koringa College of Pharmacy,
e-mail:drgvpawankumar@gmail.com

QR code

Please cite this article in press G.V. Pavan Kumar et al., ***A Comparative Investigational Studies On Pharmacognosy, Phytochemical Aspects and Anti-Microbial Activity of a Delonix Regia Flowers and Seeds on Methicillin Resistant Staphylococcus Aureus.,*** *Indo Am. J. P. Sci, 2018; 05(07).*

INTRODUCTION:

The emergence and spread of multidrug resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy [1]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [2]. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare [3]. The most problematic bacteria include, but are not limited to, extended-spectrumβ-lactamase-producing Escherichia coli (ESBL-EC) and *Klebsiella pneumoniae* (ESBL-KP), carbapenem-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant Enterococcus (VRE) [4,5]. Thus, Infectious Diseases Society of America has recognized MRSA, VRE, ESBL-EP, ESBL-KP and *Pseudomonas aeruginosa* as notorious pathogens among the six major pathogens to which therapies with effective newer antimicrobials are urgently required [1,5]. Incidences of epidemics due to drug resistant microorganisms are now a common global problem posing enormous public health concerns [6]. The global emergence of multi drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections [7]. Antimicrobial drug resistance is also of economic concern with impact on doctors, patients, health-care administrators, pharmaceutical companies and the public [8]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [9]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs [10,11]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds [12]. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [6]. A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens [13-15]. Many plant species have been used by the indigenous people of India as traditional medicines, including as treatments for infectious diseases. Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds [16]. Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts [17]. The aim of this study is to investigate on pharmacognosy, Phytochemical aspects and the antibacterial properties of aqueous extract from *delonix regia* flowers and seeds by exploring the potency against a collection of clinical isolates of Methicillin resistant *Staphylococcus aureus.*

Delonix regia, the flame tree, is a type of flowering plant in the bean family "*Fabaceae",* subfamily"*Caesalpinioideae".* It is famous for its fern-like leaves and flamboyant display of flowers. In many tropical parts of the world it is grown-up as an ornamental tree and in English it is given the name royal *Poinciana* or *flamboyant* [18]. It is also one of several trees known as "flame tree".

The flowers of *Delonix regia* are large, with four spreading scarlet or orange-red petals up to 8 cm long, and a fifth upright petal called the standard, which is slightly larger and spotted with yellow and white [19,20]. The compound leaves have a feathery appearance and are a distinctive light, bright green and are doubly pinnate. Each leaf is 30–50 cm long with 20 to 40 pairs of primary leaflets or pinnae, each divided into 10–20 pairs of secondary leaflets or pinnules.

Fig.No. 1.*Delonix regia* seeds

Fig.No. 2. *Delonix regia* flowers

MATERIALS AND METHODS:

The test plant material flowers and seeds of *Delonix regia* were collected from East godavari district of Andhra Pradesh and the authenticity of the plant was confirmed by Dr. T.Satish Professor, Department of Pharmacognosy, Coringa college of Pharmacy, Kakinada. A voucher specimen of the collected sample (KCP/COG/02) was deposited in department of Pharmacognosy, for future reference.

The collected plant seeds and flowers were carefully observed for the foreign matter if any, shade dried for seven days, powdered mechanically and the powder Samples were passed through a Willy Mill to get 60-Mesh size and then stored in the good grade plastic containers which were maintained at room temperature until analysis.

a) Morphology

The collected fresh sample of flowers and seeds were subjected to morphological evaluation in which colour, odor, taste, shape and size were noted.

b) Proximate analysis

Proximate analysis contains determination of physical features like ash value, extractive values, moisture content, fluorescence analysis etc. The flowers and seeds powders of *Delonix regia* were evaluated in terms of ash values, extractive values, and moisture content[21].

I. Determination of Ash value

The total ash method is intended to measure the total amount of material lasting after explosion. This includes both "physiological ash", which is derived from the plant tissue itself and "non-physiological ash", which is residue of extraneous matter adhering to plant surface. Weigh & ignite flat, thin, porcelain dish.Weigh about 2g of *Delonix regia* dried flowers and seeds powder into the dish seperately. Support the dish on a pipe-clay triangle placed on a ring of retort stand. Heat with a burner using a flame about 2cm high & supporting the dish about 7cm above the flame, heat till vapors almost cease to be evolved; then lower the dish &heat more strongly until all the carbon is burnt off. Cool in a desiccator. Weigh the ash &calculate the % of total ash with reference to the air-dried sample. If a carbon free ash cannot be obtained in this way, then any one of the following method can be used. Exhaust the charred mass with hot water, collect the residue on a ash less filter paper incinerate the residue and filter paper, add the filtrate, evaporate to dryness & ignite at a temperature not exceeding 450ºC. Cool the crucible, add 15ml of alcohol; break up the ash with glass bar, burn off the alcohol & again heat the whole to a dull red heat. Cool, weigh the ash.

Determination of alcohol-soluble extractive

Weigh accurately about 5g of *Delonix regia* leaves and seed powder in a weighing bottle and transfer it into a dry 250ml conical flask. Fill a 100ml graduated flask to the delivery mark with the alcohol (90%) washout the weighing bottle and pour the washes, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24hrs, shaking frequently i.e., maceration. Filter through filter paper in a 50ml measuring cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed thin porcelain dish. Evaporate to dryness on water bath and complete dry in an oven at 100^0c. After that cool it in a desiccator and weigh. Calculate the % w/w of alcohol (90%) soluble extractive with reference to the air dried drug.

III. Moisture content: Weigh about 1.5g of powder drug into a weighed flat and thin porcelain dish. Dry in oven at 100ºC, Cool in desiccator and watch. Loss in weight is usually recorded as moisture.

IV. Fluorescence analysis

Both the powders were treated with altered reagents and observed under visible, UV light so as to analysis the fluorescence character of the powder sample under study.

Preparation of plant extracts - Successive solvent extraction

The plant extracts were prepared with some modifications in the guidelines of [22]. Plant material

was shade-dried and milled to a coarse powder to extract sequentially Powdered crude drug was successively extracted by maceration using different organic solvents increasing in their order of polarity like: Petroleum ether, Chloroform, Ethyl acetate, Methanol and water.Each solvent was replaced three times with fresh solvents and was allowed to remain in contact with the plant materials for 48 hrs. The concentrated extract was centrifuged for 20 min at 20°C. The supernatant was recovered, filter sterilized evaporated to dryness in vacuum and stored in refrigerator for further use.

Preliminary Phytochemical screening

All the extracts were subjected to preliminary phytochemical screening according to the following procedure [23].

Test for carbohydrates

Molisch's test: To the 5 ml of sugar solution in a test tube add 2 drops of molischs reagent mix thoroughly and add 3 ml of concentrated sulphuric acid along the sides of test tube by slightly inclincing the tube thus forming a layer of acid in the lower part.

Test for reducing sugars

Benedicts test: Take 5 ml of Benedict's reagent in a test tube and add 8 drops of the sample solution and boil for 2 minutes. Appearance of brick red precipitate indicates the presence of reducing sugars.

Test for pentose sugars

Mix equal amounts of test solution and HCL. Heat the mixture and add a crystal of fluoroglucinol. Appearance of red colour indicates the presence of pentose sugar.

Test for monosaccharides

Barfoed's test: It is a chemical test used for detecting the presence of monosaccharides. It is based on the reduction of copper acetate to copper oxide which forms a brick-red precipitate**.**

Test for hexose sugars

Seliwanoff's test: Take 2ml of sample solution into a test tube and add 2ml Seliwanoff's reagent and boil the solution for 30 seconds and cool. Appearance of red colour complex indicates the presence of ketoses**.**

Test for non-reducing sugars

Iodine test: Take 2ml of sample solution into a test tube and add 2 drops of iodine solution, heat the solution and then cool. Deep blue colour disappears and blue colour reappears, may be starch presence.

Tannic acid test: Take 3ml of sample solution into a test tube and add 3ml of 5% w/v solution of tannic acid. Appearance of yellow white precipitate indicates that the starch may be presence.

Test for proteins

Biuret test: To 3 ml of filtrate, two drops of 10% NaOH was added and heated to boil. To this a drop of 1% $CuSO_4$ solution was added and observed for colour. Formation of pink colour indicates the presence of proteins.

Test for Tannins

Lead acetate test: To the 2-3ml of alcoholic extract asdd lead acetate solution it gives white ppt it indicates presence of tannins.

Test for alkaloids

About 50 mg of the extract was stirred with 2 drops of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for alkaloids as follows:

Dragondroff's test (with solution of potassium bismuth iodide) To 2-3 ml of filtrate, added 2 drops of Dragondroff's reagent. Orange brown precipitate indicates presence of alkaloids.

Test for amino acids

Ninhydrin test (with tri keto-hydridine hydrate and 1% n-butanol) To 3 ml of filtrate, three drops of 0.5% Ninhydrin reagent was added and heated in boiling water bath for 10 minutes. Formation of violet / blue /purple colour indicates the presence of amino acids.

Test for Saponins: Evaporate extract to get 10ml oil. To oil add 25ml of 10% sodium hydroxide. Boil in boiling water bath for 30 minutes. Cool and add excess sodium sulfate solution. Foam forms and rises to the top. Filter, to filtrate add sulfuric acid. Evaporate, collect residue it contains glycerol and dissolve the residue in ethanol with ethanolic solution.

Test for steroids

Salkowski reaction: To 2ml of extract add 2ml of concentration sulfuric acid shake well. Chloroform layer appears red and acid layer appears shows greenish yellow fluorescence.

Test for saponin glycosides

Foam test: Shake the drug extract vigorously with water. Persistent foam observed indicates presence of glycosides.

Microbial strains

The MRSA strains used in this study were clinical isolates from patients of Bhavya superspeciality hospital Rajamundry, presenting with symptoms of *S.aureus*-associated diseases.The isolates were identified as *S.aureus* according to colonial and microscopic morphology, positive catalysis,

hemolysis and coagulase production. Standard strains of *Staphylococcus aureus* (SAS) were done in the Department of Microbiology, Koringa College of pharmacy, Kakinada, A.P

Assay for antibacterial activity

After identification on selective medium all bacteria were grown on Nutrient Agar (Hi-Media M001A) and in Nutrient Broth (Hi- Media M002) at 37°C. Antimicrobial activity of the Ethanolic plant extract of seed and flower was determined by the agar well diffusion method (Holder et al 1994), with modifications. 200 µL of overnight NB culture were added to 15 ml of molten MUELLER-HINTON Agar (Hi-Media M-173), mixed well, poured into a sterile PETRI dish and allowed to set. A sterile cork-borer (5 mm diameter) was used to make wells in the set agar. 25 µL of plant extracts, diluted 1:200 in sterile water, were added to triplicate wells and the plates were incubated overnight at 37°C. Antibacterial activity was recorded as a zone of growth inhibition of greater than 5 mm around the well. All *S. aureus* isolates were tested for methicillin resistance. The disk diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS) was used with a 1 µg PencillinG disk (Oxoid). Zone sizes were read after incubation at 35°C for 24 h. Isolates with zone sizes 10 mm were considered methicillin resistant[24].

RESULTS AND DISCUSSION:

The plant extracts were prepared in different concentrations ranging from 1.0 mg/ml to 2.0 mg/ml using seeds and flowers, the best results were obtained by enlisted Ethanolic concentration of plant parts- *delonix regia* 1.75 µg/ml for seeds and 2.0µg/ml of flowers for MRSA respectively. As the bioactivity of plant extracts depends on the ethanol concentration used in the extraction process. Since ethanol is safe to ingest thereby it is most commonly used organic solvent to extract plants extracts which is used as potential therapeutic agent by human society. These extracts were tested for antimicrobial activity against gram positive bacteria Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from burnt patients .These micro-organisms are responsible for causing serious infection in the human body thus need to be treated. The antimicrobial activity was studied using zone of inhibition and zone >18mm was considered as positive result as shown in Table 7,8 and Figure 3,4 for MRSA. It was observed that *delonix regia* seeds extract shows greater zone of inhibition against Methicillin resistant *Staphylococcus aureus* (MRSA), as compared to flower extract.

Table No: 1 Morphology of Flowers and seeds of *Delonix regia*

	Flowers	**Seeds**
Colour	Green	Brown
Odor	Odorless	Odorless
Taste	Bitter	Bitter
Shape	Ovate	Ovate
Size *length *width	Each leaf is 30–50 cm long with 20 to 40 pairs of primary leaflets	Each seed of 1-2 cm long and 1cm wide

Table No: 2 Ash values for the *Delonix regia* Flowers and seeds powder

S. No.	**Type of ash**	**Percentage of ash (flowers)**	**Percentage of ash (seeds)**
1.	Total ash value	11.5	13.5
2.	Water soluble ash value	6.2	7.4
3.	Acid insoluble ash value	5.3	6.1

Table No: 3 Florescence analysis of *Delonix regia* flowers

S. No.	Powdered drug	Day light	UV light
1	Powder as such	Green	Green
2	Powder + $Fecl_3$	Green	Light green
3	Powder + conc. HCl	Dark Green	Black
4	Powder + 1M NaOH	Green	White
5	Powder + $AgNO_3$	Green	Dark green
6	Powder + CCl_4	Green	Green
7	Powder + Methanol	Green	Green
8	Powder + Iodine solution	Brown	Brown

Table No: 4 Florescence analysis of *Delonix regia* seeds

S. No.	Powdered drug	Day light	UV light
01	Powder as such	Yellow	yellow
02	Powder + $Fecl_3$	Yellow	Light yellow
03	Powder + conc. HCl	Yellow	Dark yellow
04	Powder + 1M NaOH	Yellow	Yellow
05	Powder + $AgNO_3$	Yellowish	Orange
06	Powder + CCl_4	Yellowish green	Yellow
07	Powder + Methanol	Yellow	Yellow
08	Powder + Iodine solution	Yellow	Yellow

Moisture content:

Moisture content of *Delonix regia* flowers powder was found to be 8.4% v/w and moisture content of *Delonix regia* seeds powder was found to be 5.8% v/w.

Table: 5 Phytochemical analysis of Delonix regia plant seed and flower etracts

S.NO	TEST	NAME OF PHYTOCONSTITUENT	ANALYSIS OF REPORT Seeds	Flowers
1	**Salkowski Test**	Steroids	++	+
2	**Iscugajiu Test**	Tri Terpenoids	------------	------------
3	**Foam Test**	Saponins	++++++++	+++
4	**Mayers Test**	Alkaloids	++++	+++
5	**Molischs Test**	Carbohydrates	++++++	++++++
6	**Ferric Chloride Test**	Flavonoids	++++	++++
7	**Keller-Killiani Test**	Cardiac Glycosides	------------------	------------------
8	**Ferric Chloride Test**	Phenolic Compounds	++++++	++++++

Note: +++ indicates Miniscule Amount of phytochemical in the Extract

++++++ indicates Abundant Amount of phytochemical in the Extract

__________ indicates absence of phytochemical in the Extract

Table 6: Antimicrobial activity of Ethanolic extract of seeds against MRSA

Test organism	Inhibition Zone (mm)*	MIC of Seed extract µg/ml	Standard µg/ml Pencillin-G
MRSA	**26±0.3**	**2.0**	**2.33**
	24±0.6*	**1.75**	
	18±0.3	**1.5**	
	16±0.4	**1.25**	
	16±0.4	**1.00**	

***Values are mean±SD of three replications**

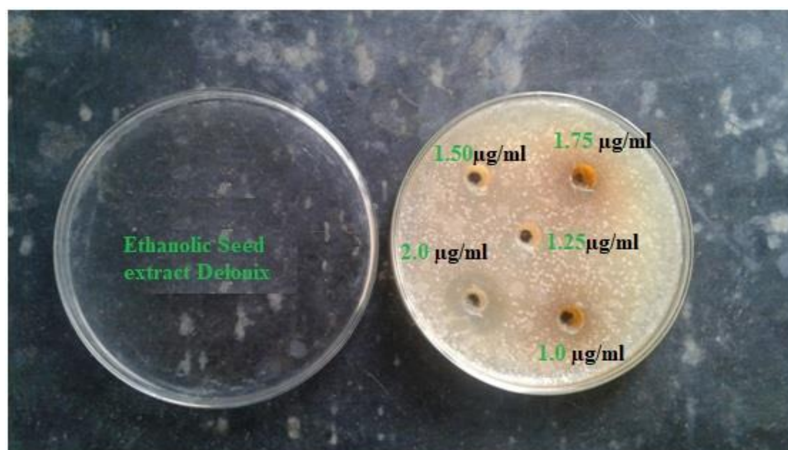

Figure: 3 Ethanolic seed extract zone of inhibition

Table 7: Antimicrobial activity of Ethanolic extract of flowers against MRSA

Test organism	Inhibition Zone (mm)*	MIC of Flowers extract µg/ml	Standard µg/ml Pencillin-G
MRSA	21±0.3	2.0	2.33
	18±0.6*	1.75	
	16±0.3	1.5	
	12±0.4	1.25	
	12±0.4	1.00	

***Values are mean±SD of three replications**

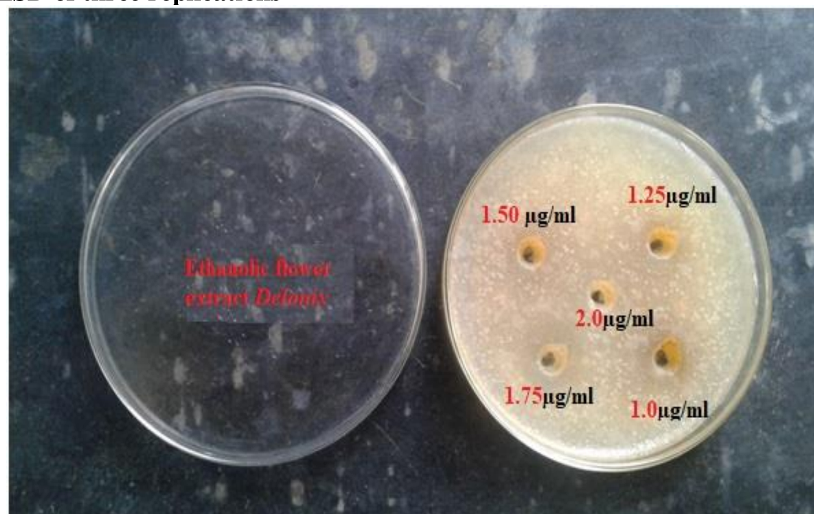

Figure: 4 Ethanolic Flower extract zone of inhibition

CONCLUSION:

Our studies have suggested that the plant *delonix regia* is found to be a potential antimicrobial agent against gram positive Methicillin resistant *Staphylococcus aureus* (MRSA) the seeds exhibits MIC value of 1.75 µg/ml for seeds and 2.0µg/ml of flowers of flowers respectively. The activity of the *delonix regia* extracts provides preliminary scientific validation for the traditional medicinal use of this plant. Thus it can be concluded that the plant can serve as potential therapeutic agent however, the application of any compounds to medicine will require safety and toxicity issues to be addressed.

ACKNOWLEDGEMENTS:

We would like to thank Faculty and management of Koringa College of pharmacy, for their support during the course of our research work.

REFERENCES:

1. Boucher HW, Talbot GH, Bradley JS (2009) Bad

bugs, no drugs: No ESKAPE!" An update from the Infectious Diseases Society of America, Clin Infect Dis 48: 1-12.
2. Cohen ML (1992) Epidemiology of drug resistance: Implications for a post antimicrobial era. Science 257: 1050-1055.
3. WHO (2002) Traditional Medicine Growing Needs and Potential - WHO Policy Perspectives on Medicines, No. 002, May, World Health Organization, Geneva, Switzerland.
4. Giamarellou H (2010) Multidrug-resistant Gram-negative bacteria: how to treat and for how long. Int J Antimicro Ag 36: 50-54.
5. Talbot GH, Bradley J, Edwards Jr JE, Gilbert D, Scheid M, et al (2006) Bad bugs need drugs: An update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis 42: 657-668.
6. Iwu MM, Duncan AR, Okunji CO (1999) New antimicrobials of plant origin. Perspectives on new crops and new uses. J. Janick (ed.), ASHS Press, Alexandria, VA.
7. Hancock REW, Kai Hilpert, Volkmer_enjert R, Walter T (2005) High-throughput generation of small anti-bacterial peptide with improved activity. Nat Biotechnol 23:1008-1012.
8. McGowan Jr JE (2001) Economic Impact of Antimicrobial Resistance. Emerging Infectious Diseases 7: 286-293.
9. Williams R (2000) Antimicrobial resistance a global threat.Essential Drug Monitor, 1: 28-29.
10. Pretorius JC, Magama S, Zietsman PC (2003) Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species. South African Journal of Botany 20: 188-192.
11. Moreillion P, Que YA, Glauser MP (2005) *Staphylococcus aureus* (Including Staphyloccal Toxic shock). In 'Principles and Practice of Infectious diseases (6th edn),' Published by Churchill Livingstone Pennyslyvania 2: 2333-2339.
12. Mahady GB (2005) Medicinal plants for the prevention and treatment of bacterial infections. Curr Pharm Des 11: 2405-2427.
13. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564-582.
14. Medina AL, Lucero ME, Holguin FO, Estell RE, Posakony JF, et al (2005) Composition and antimicrobial activity of *Anemopsis californica* leaf oil. J Agri Food Chem 53: 8694-8698.
15. Romero CD, Choph SE, Buck G, Martinez E, Garcia M, et al (2005) Antibacterial properties of common herbal remedies of the southwest. J Ethnopharmacol 99: 253-257.
16. Eloff JN (1998) Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol 60: 1-8.
17. Low Dog T (2009) Smart talk on supplements and botanicals. Alternative and Complementary Therapies 15: 101-102.
18. Modi A, Mishra V, Bhatt A, Jain A, Mansoori MH, Gurnany E, et al. Delonix regia: historic perspectives and modern phytochemical and pharmacological researches. Chin J Nat Med. 2016; 14(1):31–9.
19. Rahman M, Hasan N, Das AK, Hossain T, Jahan R, Khatun A, et al. Effect of Delonix regia leaf extract on glucose tolerance in glucose-induced hyperglycemic mice. Afr J Tradit Complement Altern Med. 2011; 8(1):34–6.
20. Shabir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan QM, et al. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [Delonix regia (Bojer ex Hook.) Raf]. Molecules. 2011; 16(9):7302–19.
21. Palombo EA, Semple SJ (2001) Antibacterial activity of traditional Australian medicinal plants. J Ethnopharmacol 77: 151-157.
22. Menut C, Sharma S, Luthra C (1993) Aromatic plants of tropical central Africa, Part X—Chemical composition of essential oils of *Ageratum houstonianum* Mill. and *Ageratum conyzoides* L. from Cameroon. Flavour Fragrance J 8: 1-4.
23. Medina AL, Lucero ME, Holguin FO, Estell RE, Posakony JF, et al (2005) Composition and antimicrobial activity of *Anemopsis californica* leaf oil. J Agri Food Chem 53: 8694-8698.
24. Agarwal P, Agarwal N, Gupta R, Gupta M, Sharma B (2016) Antibacterial Activity of Plants Extracts against Methicillin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant *Enterococcus faecalis*. J Microb Biochem Technol 8: 404- 407.