ANTI-MICROBIAL ACTIVITY AND PELLICLE INHIBITION OF AERVA JAVANICA (BURM. F.) JUSS. EX SCHULT. FROM DISTRICT MARDAN, KP. PAKISTAN

NOREEN BIBI
MPhil Scholar, Department of Botany Hazara University, Mansehra, 21300, Khyber Pakhtunkhwa, Pakistan.

UZMA KHAN
Assistant Professor, Department of Botany, Hazara University, Mansehra, 21300, Khyber Pakhtunkhwa, Pakistan.

GHULAM MUJTABA SHAH
Chairman, Department of Botany, Hazara University, Mansehra. 21300, Khyber Puhtunkhwa, Pakistan.

MUHAMMAD FIAZ
Assistant Professor, Department of Botany, Hazara University, Mansehra, 21300, Khyber Pakhtunkhwa, Pakistan.

MUHAMMAD IKRAM
Assistant Professor, Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, 22060, Khyber Pakhtunkhwa, Pakistan.

KHURSHEED UR RAHMAN*
Department of Botany, Hazara University, Mansehra, 21300, Khyber Puhtunkhwa, Pakistan.

BUSHRA FIDA
MPhil Scholar, Department of Botany Hazara University, Mansehra, 21300, Khyber Puhtunkhwa, Pakistan.

FAZLI HAMID
MPhil Scholar, Department of Biochemistry Hazara University, Mansehra, 21300, Khyber Puhtunkhwa, Pakistan.

ABSTRACT

One crucial stage in the hunt for new compounds with potential applications in medicine is the screening of medicinal plants for antimicrobial activity. The present study was carried out at the Botany Department's Phytochemistry Lab at Hazara University Mansehra. The leaves of the species *Aerva javanica* were gathered in District Mardan. The Agar Well-Diffusion method was used to assess the antibacterial activity, and the zones of inhibition were expressed in millimetres. The following four microbial strains were employed: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*. The positive control in this experiment was vancomycine. The maximal inhibition zone of *Aerva javanica* methanolic extract against *Staphylococcus aureus* was 21 mm, whereas the minimum inhibition zone against

Escherichia coli was 15 mm. In order to identify the active bands, the plant crude extract was subsequently put via bioautography. It was evident that both plant bands exhibited strong antibacterial activity due to their high Rf values. To investigate the biofilm production of crude plant extract, the Pellicle Assay was carried out in test tubes. The same four bacterial strains were combated with a crude plant extract. While plants and broth media are found in the negative control test tube, bacteria and broth media are present in the positive control test tube.
Key word: Anti-microbial activity of leave extract and inhibition of pellicle formation of *Aerva javanica.*

INTRODUCTION

Traditional medicines are used as a source of first aid treatment in many countries around the world. The uses of plants to cure human diseases are as old as humans (Abdul *et al.*, 2012). Ecologically and physiologically active phytochemicals are widely distributed as plant constituents. Woody plants can synthesize and accumulate wide range of phytochemicals in their cells, comprising alkaloids, flavonoids, tannins, cyanosides, phenolic compounds, saponins and lignin. More than 60% of all the recent clinical drugs come from natural products. Substances that can inhibit the development of pathogens or kill pathogens and have least toxicity or no toxicity to host cells are considered applicants for the development of new anti-bacterials (Ahmad and Beg, 2001). Herbs are local heritage of global importance. Herbs have therapeutic properties due to the existence of complex chemicals of various compositions and are found in secondary plant metabolites in many parts of these plants (Patil *et al*., 2009). Bacterial resistance to numerous antimicrobial agents is one of the biggest challenges in the treatment of infections, necessitate of searching new sources of substances with antimicrobial properties and used against various pathogens (Namdeo, 2007). Natural products play an important role in drug development programs in the pharmaceutical industry. There are reports of traditional treatments using plants by tribal people or indigenous communities. Since antibacterial activity has great medicinal relevance in recent years, antibacterial activity has been screened, infections are greatly increased and resistant to antibiotics, and it has become an increasing therapeutic problem. Screening of medicinally important plants for antibacterial activity and phytochemicals is necessary for searching new compounds for medicinal use. Natural products of some plants can give a new source of antibacterial agents. There are several research groups currently involved in medicinal plant research (Joshua *et al*., 2006). Natural antibacterial agents can be derived from the bark, stems, leaves, flowers and fruits of plants (Nair and Chanda, 2007). Plants contain

antimicrobial constituents with vast therapeutic potential because they can aid therapeutically without any side-effects commonly linked with synthetic antimicrobial agents. In nature, in addition to physical stress, plants are exposed to too many infective microorganisms; naturally they have developed arsenals to counter the invasion of microbes (Prabhakar, *et al.*, 2013). In addition to the pre-existing general resistant components, a diversity of specific new compounds were synthesized in the post-infection phase. Obviously, they expect to have multiple antimicrobial compounds (Ausubel, 2006). Some of these antimicrobial compounds in plants can be used to combat bacterial diseases in humans. Nowadays, researchers have tried to develop new techniques to discover the antibacterial drugs needed for plants with high therapeutic activity and little or no side-effects. Most microbes have become resistant to today's antibiotics, so pharmaceutical companies are using different plants to get effective antibacterial agents and encounter resistance of microbial strains (Rafie *et al*., 2010; Angeh *et al.*, 2007; Ullah *et al*., 2009). MRSA is commonly referred to as supergerm and is often multi-drug resistant (Dettenkofer *et al*., 2008). This virulence factor causes infection of the ears and soft tissues, skin, blood, respiratory and urinary tract (Furukawa *et al*., 2008; Pereira *et al*., 2009; Gould, 2009). The development and spread of microbial resistance to existing antibiotics is due to the excessive use of commercial antibacterial drugs commonly used to treat various diseases. Although many plants with antimicrobial activity have been identified, a large number of plants have not been identified (Sharma *et al*., 2011). In this report, the antimicrobial activity of selected plant extracts against different bacterial strains has been analyzed.

Aerva javanica (Burm. f.) juss. Ex schult. *is* belong to amaranthraceae family and locally known is khar Buta. It has an upright, pale, rigid branch with height about 1.6 meters. Light green 20 to 40mm long alternate leaves, lanceolate, oblong-ovate, subsessile or short petiolate stalks covered with matte hair, upper surface light gray. The inflorescence generally a white nacked raceme of woody and sessile dense spikes. Flowers are small, milky white and the females are larger as compared to males. The fruit is soaked in a smooth white fleece, globular, single small black seed. It blooms from January to May (Soliman, 2006).

MATERIALS AND METHODS

The current work was performed in the Phytochemistry lab Department of Botany, Hazara University Mansehra. The plants *Aerva javanica* were collected from District Mardan, these plants are used as folk medicines in Pakistan. The identification of plants specimen was

determined by Dr. Abdul Majid, Assistant Professor at Botany department of Hazara University Mansehra, by checking the specimen characteristics with the Flora of Pakistan.

Material used for collection:

Tags, digger, hand bag, polyethene bags, pencil, camera and newspapers were used to collect plants from Mardan district, Pakistan.

Preparation of crude plant extracts

Fine powders of plants (dried) material were made by means of a grinder. This powder of each plant was kept in bottles or polyethene bags by labeling each with the plant name.

Culturing and Lawning of Bacteria

Four bacterial strains, (Table 1) of them were gram-positive, 2 are gram negative shown in Table 1. Nutrient media, Petri plates, cotton swabs, test tubes, spirit lamps, matchboxes, autoclave, iron loops and nutrient broth media were use in this research study.

Table 1. Bacterial Strains used

S.No	Name of bacterial strains	Gram +ive and Gram- ive
1	Pseudomonas aeruginosa (G)	Gram- ive
2	Bacillus subtilis (W)	Gram +ive
3	Escherichia coli (P)	Gram- ive
4	Staphylococcus aureus (Y)	Gram +ive

Selected Medicinal Plants

The studied medicinal plants, their local name, botanical name and families of these plants are given in (Table 2).

Table 2.s Plants used for Antibacterial Activity

S. No	Local Name	Botanical name	Family	Part Used
1	Khar Buta	Aerva javanica	Amaranthaceae	Leaves

Media Preparations and Sterilization

Agar Well Diffusion method was used to check antimicrobial activity of selected plants against different bacterial strains. Almost eight Petri plates were autoclaved for about 20 minutes

and then 8.4g of nutrients dissolved in 300ml of distilled water (28g/L) after complete dissolving it was then allowed to autoclaved at 121°C for 20 minutes. Media allowed to cool after autoclaving, transferred approximately 25ml in every petri plate in a hygienic environment and then Petri plates were allowed to cool for 1 hour at room temperature for agar solidification. Distilled water about 50ml and 0.4g (8g/liter) mixed to make broth media for four test tubes. Test tubes were autoclaved and broth media was poured into test tubes under hygienic condition. After pouring of media, the bacteria taken prepared slants by means of a sterilized coil and carefully cultured into a test tube. This process was done for every strain and every test tube. Test tubes were kept at 37°C for 24 hours after this process.

Antibacterial Assessment of Selected plants

The antimicrobial assay of selected plants was evaluated using Wells Diffusion method described by (Walters *et al.,* 2011; Carron *et al.,* 1987; Kiveck *et al.,* 2001; Stepanovic *et al.,* 2003) with minor changes. The antimicrobial potential of the crude extracts of the two plants was screened using four bacterial strains. The Petri plates labelled two plants and four bacterial strains. Cotton swabs were dipped in 24 hours old nutrient inoculums and then bacterial lawning were made on every plate four times, circling the Petri plates at angle 60°. Wells with a diameter of 6mm were made by a cork borer. The plants extract was put into the wells by using a micropipette. The agar plate was kept in an incubator at 37°C for 24 hours. After incubation, the zone of inhibition was prepared from each plants extracts and measured in millimeters (mm) with the help of a ruler. Antimicrobial assay of plant extracts was set according to following parameters; zone of inhibition less than 0mm, in-active: 1.1-1.2 mm; moderate active: 1.4-1.9mm, good assay: greater than 1.9mm; very active (Alves *et al.,* 2000).

Antibiotic Sensitivity Test by Disc Diffusion method

The antibiotic sensitivity test was performed by Kirbey-Bauer Disc Diffusion method. The antibiotic tested along with specified codes and concentrations was given. Antibiotic then poured in petri plates having well containing plant extract. These Petri plates were then kept in incubator at 37°C (24 hours). This technique of zone interpretation of each antibiotic were used according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2013).

Standard Control

An antibiotic was used as a standard control, vancomycin (VA 30) was used against all the microbial strains.

Thin Layer Chromatography (TLC)

Preparation of TLC Plate

Two pieces of filter paper was cut (What Man filter paper no; 4) for two plants in equal length and width 70mm and 20mm. These filter papers were used for bioautographic. A line was marked on base of the plate and also on top of the plate with pencil. Base of filter paper was baseline and top was solvent front. On baseline we load the plant sample.

Solvent System Preparation

By mixing ethanol and ethyl acetate of ratio 3:1 solvent system was made. Then solvent system was transferred in beakers protected with plate.

Preparation of Chromatogram

Take a micro capillary of 10µl micro caps. Dip it into plant extract and carefully contact its ends onto the baseline of the paper to load the sample. This process was repeated 3 times. Filter paper is then carefully place in the beaker of solvent system. After a while when the plants extract was near the upper ends, the paper was takes out from beaker and the TLC plates was observed with a 240nm and 365nm UV lamp. The spot of the marker visualization was used to calculate the Rf value.

Rf value

R.f values also called retention factor and it is defined as

"The distance traveled by compounds divided by distance traveled by solvent"

R.f value = distance traveled by compound/ distance traveled by solvent.

RESULTS

Antibacterial assay of selected plants

Antimicrobial assay of crude extract (methanol) of plants was used against four bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis*) in order to assess antimicrobial potential of these plants and inhibition of microbial growth. Results were taken after 24-hours of incubation period and inhibition zone was measured in millimeter (mm).

Antibacterial activity of *Aerva javanica* by Well Diffusion method

The *Aerva javanica* extract were used for antibacterial assay against 4 selected strains of bacteria, *P. aeruginosa, E. coli, B. subtilis* and *S. aureus.* The crude extract of *Aerva javanica* showed highly significant results against selected microbial strains.

Staphylococcus aureus

The crude extract of *Aerva javanica* was tested against *Staphylococcus aureus*. The extract showed highly significant inhibition. 21±1.73 mm zone of inhibition was recorded against *Staphylococcus aureus* as compared to standard antibiotic Vancomycin, which showed 13± 1.15 mm inhibition zone (Table 1).

Pseudomonas aeruginosa

The antibacterial activity of *Aerva javanica* extract give significant result which showed 19±1.15mm inhibition zone against *Pseudomonas aeruginosa*, while standard control also showed 19±1.15mm zone of inhibition against *Pseudomonas aeruginosa* (Table 1).

Bacillus subtilis

The methanolic extract of *Aerva javanica* showed good result against *Bacillus subtilis*. Methanolic extract of *Aerva javanica* which showed 17±0.57mm zone of inhibition as compared to antibiotic showed 23± 2.30 mm zone of inhibition against *Bacillus subtilis* (Table 1).

Escherichia coli

In case of *Escherichia coli* extract of *Aerva javanica* gave highly significant value in which 15±1.15mm zone of inhibition was recorded as compared to control which also showed 15±1.15mm of inhibition against *Escherichia coli* (Table 1).

Table 3. Inhibition zone by Agar Well Diffusion method of methanol extract of *Aerva javanica.*

S.No	Plant Name	Bacterial strains	Zone of inhibition (mm)	Vancomycine (as control)
1.	*Aerva javanica*	*E. coli*	15±1.15 mm	15±1.15 mm
2.		*P. aeruginosa*	19±1.15 mm	19±1.15 mm
3.		*B. subtilis*	17±0.57mm	23± 2.30 mm
4.		*S. aureus*	21±1.73 mm	13± 1.15 mm

Key: E.C; *Escherichia coli*, B.S; *Bacillus subtilis*, P.A; *Pseudomonas aeruginosa*, S.A; *Staphylococcus aureus*,

Table 4. Descriptive statistics for antimicrobial activity of *Aerva javanica*

Bacteria	Mean	SD	SE Mean	C.V.	Minimum	Maximum
B. subtilis	17mm	1	0.5774	5.8824	16mm	18mm
P. aeruginosa	19mm	2	1.1547	10.526	17mm	21mm
E. coli	15mm	2	1.1547	13.333	13mm	17mm
S. aureus	21mm	3	1.7321	14.286	18mm	24mm

Key: S.D (standard deviation) CV (coefficient of variance) SE (standard error) Mean

Table 5. Comparison of plant and Vancomycine against bacterial strains

Bacterial strains	*A. javanica* Extracts	Vanco-mycine
S. aureus	21± 1.73 mm	13± 1.15 mm
P. aeruginosa	19± 1.15mm	19± 1.15 mm
B. subtilis	17± 0.57mm	23± 1.15mm
E. coli	15± 1.15mm	15± 1.73 mm

Key: E.C; *Escherichia coli*, B.S; *Bacillus subtilis*, P.A; *Pseudomonas aeruginosa*, S.A; *Staphylococcus aureus*, A.J; *Aerva javanica*

Table 6. Pellicle Inhibition of plant extracts against different bacterial strains.

Plant sample	Incubation period	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
Aerva javanica	7 days	++	++	+	++

Rf-values of bioactive bands for selected plants

The developing of TLC plate for selected plant extracts in solvent Ethanol: Ethyl acetate having ratio of 3:1, following RF values for separated bands were recorded for *Aerva javanica* that is 0.83, 0.16, and 0.98 were 0.83, 0.66, 0.58 and 0.16.

Table 7. Shows Rf value of P1 and P2 in solvent system Ethylacetate: Ethanol (1:3)

Plant	Solvent system	Rf values under UV	Active bands colours
Aerva javanica	Ethyl acetate: Ethanol (1:3)	0.83, 0.16, 0.98	Light Blue
Butea monosperma	Ethyl acetate: Ethanol (1:3)	0.83, 0.66, 0.58, 0.16	Light Purple

DISCUSSION

Antibiotic resistance in many infectious agents makes finding new sources of antibiotics a global challenge and a priority for research institutes, pharmaceutical companies, and academies (Sharma *et al*., 2009). In the present report two plant species of District Mardan *Aerva javanica* and *Butea monosperma* were evaluated for antimicrobial-activity and antibiofilm activity against different pathogens with Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa).* *Aerva javanica* is locally known as "Sparai", Khar Buta etc whereas its English name is desert cotton. The plant contains many important bio molecules such as alkaloids, tannins, saponins, sulphates, flavonoids, lipids and carbohydrates. The plant has many uses; it is an effective helmintis fighter and an anti-inflammatory (Vertichelvan *et al.,* 2000) helpful in significant activity against diabetes, cough and infected lesions (Vertichelvan and Jegadeesan, 2002). It is also used to treat urinary disorders, respiratory complications, nasal hemorrhage and cracks (Waikar and Bonventre, 2007). The powder of the plant Aerva javanica is used to treat livestock ulcers and headache seeds, but its decoction is helpful in reducing swelling and alleviating toothaches. Chest pain, diarrhoea, and ascariasis can all be treated with the entire plant (Samejo *et al*., 2012). Methanolic extract of *Aerva javanica* leaves showed different

inhibition zone at all extract concentration against all bacterial strains. The methanol extract of *Aerva javanica* has been checked against four bacterial strains and the concentration revealed marked antimicrobial potential counter to Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis.* 21±1.73mm zone of inhibition was measured against *Staphylococcus aureus* as compared to standard antibiotic Vancomycin, which showed 13±1.15mm inhibition zone, 17±0.57mm zone of inhibition was recorded against *Bacillus subtilis* as compared to antibiotic which showed 23±2.30mm zone of inhibition against *Bacillus subtilis*. The plant also showed a significant result against Gram negative bacteria *Pseudomonas aeruginosae* and *Escherichia coli*. 19±1.15mm inhibition zone was recorded against *Pseudomonas aeruginosa* while standard control also showed 19±1.15mm zone of inhibiition against *P. aeruginosa.* In case of *Escherichia coli* extract of *Aerva javanica* gave significant value in which 15±1.15mm zone of inhibition was recorded as compared to control which also showed 15±1.15mm of inhibition against *E. coli.* The result of current study also showed the significant antibiofilm activity of *Aerva javanica*. Significant anti-biofilm effect (+) was showed against *Staphylococcus aereus*, good anti-biofilm (++) effect was observed for *Aerva javanica* against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacterial strain which result in partial removal of layer. Our work also agrees with (Srinivas and Reddy, 2012) conducted antibacterial activity of *Aerva javanivca*. Methanol, chloroform and hexane extract showed marked antibacterial activity against different strains of bacteria. Their result indicated that methanolic extract of the plant showed more antibacterial activity and a wide range of phytochemicals. But no report available on their anti-biofilm assay.

Our investigation also agrees with the studies of (Reddy and Reddy. 2009) examined antibacterial assay of *Butea monosperma* against different pathogens *Bacillus megatarium, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* and flower extracts revealed highest antibacterial activity against *Escherichia coli* and root extracts were effective against *Bacillus subtilis*. There is no report available on anti-biofilm assay. Bacterial strain which result in partial removal of layer. It means that the plant *Aerva javanica* showed maximum inhibitory effect against Gram-positive pathogenic strain *Staphylococcus aureus* by Agar Well Diffusion method and Pellicle Inhibition assay.

CONCLUSSION

The experimental results and investigations conclude that plant species *Aerva javanica* and *Butea monosperma* selected for the current study showed marked antibacterial activity and Anti-biofilm assay against all the tested microbes. These plants can be used in cure of infections caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. It is evident from the result that the plant *Aerva javanica* showed significant to good antibacterial activity against all the pathogenic strains. In Pellicle Inhibition *Butea monosperma* showed significant inhibition against *Bacillus subtilis*, good inhibition against *Staphylococcus aeureus* and *Pseudomonas aeruginosa* and moderate inhibition against *Escherichia coli*. *Aerva javanica* also showed a pattern of significant inhibition against *Staphylococcus aureus*, good anti-biofilm effect was observed for *Aerva javanica* against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Pellicle inhibition of *Aerva javanica* were not studied previously. We have conducted for the first time the effect of crude extract (methanolic extract) of these plants polysaccharides inhibition of bio-film against different bacterial strains. For both anti-bacterial and anti-biofilm activity these studied plants extract showed good results hence should be used against the tested bacteria. The current report verified that laboratory experiments are very applicable to check the capacity of traditional uses of plants against bacterial growth. However, present report of in vitro antimicrobial evaluation of plants has led to further phytochemical and pharmacological studies to discover the main platforms for new antibiotic drugs.

ACKNOWLEDGMENTS:

The authors are thankful to the Department of Botany Hazara University, KP, Pakistan, for providing an opportunity to complete this project.

CONFLICTS OF INTEREST:

There is no conflict of interest.

DATA AVAILABILITY STATEMENT:

All required data is provided in the manuscript; no external dataset is required or utilized.

REFERENCES

Abdul, W. K., J. Saleem, P. Shaista, A. K. Rahmat, S. Asma, J. Abdul, Tanveerand A. S. Anwar. 2012. Phytochemical analysis and Enzyme Inhibition Assay of Aerva javanica for ulcer. Chem. Central J., 6: 76.

Ahmad, I. and A.Z. Beg. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol., 74: 113-23.

Alves, T. M. D. A., A.F Silva, M. Brandao, T.S.M. Grandi, E.D.F.A. Smania, A. Smania Junior and Zani. 2000. Biological screening of Brazilian medicinal plants, Memórias do Instituto Oswaldo Cruz., 95(3): 367-373.

Angeh, J.E., X. Huang, L. Sattler, G.E. Swan, H. Dahse, A. Hartl and J.N. Eloff. 2007. Antimicrobial and antinflammatory activity of four known and one new triterpenoid from Combretum imberbe (Combretaceae). J. Ethnopharmacol., 110: 56-60.

Ausubel, F.M, Lewis, K. 2006. Prospects of plant derived antibacterials. Nat. Biotechnol., 24: 1504-1507.

Carron, E. A., J.M. Maran, A. Montero, A. Fernandozalgo and A. Dominguez. 1987. Antimicrobial properties of some extracts obtained from some Mediterranean plants of medicinal value. Plantes Medicinales et Phytotherapie. 21: 195-202.

Dettenkofer, M., A.F. Widmer, and W.V. Kern. 2008. MRSA and other multi resistant pathogens: An ever increasing problem even in ambulant medicine. Dtsch. Med. Wochenschr., 133: 370-371.

Furukawa, M., Minekawa, A. Haruyama, T. Narui, Y. Sugita, G. Sugita, R. Kusunoki and T.K. Ikeda. 2008. Clinical effectiveness of ototopical application of mupirocin ointment in methicillin-resistant Staphylococcus aureus otorrhea. Otol. Neurotol., 29: 676-678.

Gould, I.M. 2009. Antibiotics, skin and soft tissue infection and meticillin resistant Staphylococcus aureus: Cause and effect. Int. J. Antimicrob. Agents., 34(1): 8-11.

Joshua, G. W. P., C. Guthrie-Irons, A.V. Karlyshev and B.W. Wren. 2006. Biofilm formation in Campylobacter jejuni. Microbio., 152: 387-396.

Kivack, B. T. Mert and H. Tansel. 2001. Antimicrobial and cytotoxic activity of Ceratoniasilinqua extracts. Turkish J. Biol., 26: 197-200.

Nair, R. and S.V. Chanda. 2007. Antibacterial activities of some medicinal plants of western region of India. Turk. J. Biol., 31: 231-6.

Namdeo, A. 2007. Plant cell elicitation for production of secondary metabolites: A review. Pharmacog. Rev., 1: 69-79.

Patil, S.B., M.N.S. Naikwade and C.S. Magdum. 2009. Review on phytochemistry and pharmacological aspects of Euphorbia hirta Linn. J.P.R.H.C., 1: 113-33.

Pereira, E.M., R.P. Schuenck, K.L. Malvar, N.L. Iorio, P.D. Matos, A.N. Olendzki, W.M. Oelemann and K.R. Dos Santos. 2009. Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus haemolyticus: methicillin- resistant isolates are detected directly in blood cultures by multiplex PCR. Microbiol. Res., 165: 243-249.

Prabhakar, S., P. Prakash, G. Ramchandra, R. Sunil, J.P. Alok, S. Apoorva, G. Ashish and S. Ajay. 2013. Physicochemical & antibacterial evaluation of Hydro-alcoholic flower extract of Butea monosperma. Inventi Rapid: Planta Activa., 2: 1-2.

Rafie, S., C. MacDougall and C.L. Jame. 2010. Cethromycin: A promising new ketolide antibiotic for respiratory infections. Pharmacotherapy. 30: 290-303.

Reddy, K.S. and V.M. Reddy. 2009. Anti-hyperglycaemic Activity of ethanol extract of Aerva javanica leaves in Alloxan-induced diabetic mice. J. Pharm. Res., 2: 1259-1261.

Samejo, M.Q., S. Memon, M.I. Bhanger and K.M. Khan. 2012. Chemical compositions of the essential oil of Aerva javanica leaves and stems. Pak. J. of Analytical and Environ. Chem., 13: 48-52.

Sharma, N., V. Garg. 2011. Antihyperglycemic and antioxidative attribute of hydroethanolic extract of Buteamonosperma (Lam.) seeds and its active constituents. Ind. J. Exp. Biol., 49: 756-766.

Soliman, M. A. 2006. Cytogenetical studies on *Aerva javanica* (Amaranthaceae). *Fl. Medit*., 16: 333-339.

Srinivas, P. and S.R. Reddy. 2012. Screening for antibacterial principle and activity ofAerva javanica. Asian Pacific J. of Tropical Biomedicine., 8: 838-8.

Stepanovic, S., N. Antic, I. Dakic, and M. Svabic-Vlahovic. 2003. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbio. Res., 158: 353-357.

Ullah, F., S.A. Malik and J. Ahmed. 2009. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial Escherichia coli from urinary tract infections in Pakistan. Afr. J. Biotechnol., 8: 3921-3926.

Vetrichelvan, T and M. Jegadeesan. 2002. Anti-diabetic activity of alcoholic extract of Aerva lanata in rats. J. *Ethnopharmacol*., 80: 103-107.

Vetrichelvan, T., M. Jegadeesan, M.P. Senthil, N.P. Murali and K. Sasikumar. 2000. Diuretic and anti-inflammatory activities of Aerval lanata in rats. Indian J. Pharma. Sci., 62: 300-302.

Waikar, S.S and J.V. Bonventre. 2007. Biomarkers for the diagnosis of acute kidney injury. Curr. Opin. Nephrol. Hypertens., 16: 557-564.

Walter, C. Y. N. T. H. I. A., Z. K. Shinwari, I. M. R. A. N. Afzal, and R. N. Malik. 2011. Antibacterial activity in herbal products used in Pakistan. Pak. J. Bot., 43: 155-62.