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# *In vitro* antibacterial activity of leaf extract of *Leucas aspera* against human pathogenic bacteria

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## Abstract

To study the phytoconstituents and antibacterial activity of *Leucas aspera,* leaf extracts of petroleum ether, ethyl acetate and aqueous were tested against human pathogenic bacteria. About forty gram of powered leaf was extracted successively with 200 ml of aqueous (97-103$^0$C), ethyl acetate (77-85$^0$C) and petroleum ether (62-66$^0$C). The preliminary phytochemical analysis of the leaf extract revealed the presence of glycosides, tannins and terpenoids in petroleum ether and aqueous extract, alkaloids, coumarins, glycosides, flavonoids, quinines, saponins, tannins, terpenoids and almost all the tested compounds in the ethyl acetate extract. The crude extract of petroleum ether, ethyl acetate and aqueous were screened for antibacterial activity. Among the three extractions, the extract of ethyl acetate showed potent antibacterial activity by inhibiting the growth of all the tested human pathogenic bacteria, Maximum inhibition zone of 23mm, 23mm, 21mm and 20mm against the growth of *Streptococcus faecalis*, *Pseudomonas aeruginosa, Shigella dysenteriae*, and *Corynebacterium diphtheria* respectively. Minimum Inhibitory Concentrations (MIC) of crude extract of *Leucas aspera* was determined against gram positive and gram negative bacterial pathogens ranged from 600µg/ml to 2400µg/ml. Further, Minimum Bacterial Concentration (MBC) of 1200µg/ml was observed against the growth of *Staphylococcus faecalis* and *Pseudomonas aeruginosa*, 2400µg/ml against *Bacillus subtilis* and *Shigella dysenteriae*, 4800µg/ml against *Corynebacterium diphtheria* and

*Salmonella typhi* respectively. The findings of this study revealed that the metabolites of leaf of *Leucas aspera* are the potential source for the development of new antibacterial compounds.
**Keywords**: *Leucas aspera*, Phytochemical constituents, Antibacterial activity, MIC, MBC

## Introduction

Nature has provided a complete store-house of remedies to cure all aliments of mankind. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Numerous of the existing chemotherapeutic drugs like vinblastine, methotrexate, taxol, and so forth, were first recognized in vegetation. In earlier stage drugs are available in the form of tinctures, teas, poultices, powders, and other herbal formulations, at the present this is serve as the foundation of novel medicine discovery (Madan and Sunil, 2011; Lakshmi *et al*., 2011).

According to World Health Organization (WHO), from 119 plant-derived medicines, about 74% are used in modern medicine in ways that correlate directly with their traditional uses. WHO also estimates that 4 billion people, 80% of the world's population, presently use herbal medicine for primary healthcare. Herbal medicine is a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional oriental, Native American and Indian medicine. Even among prescription drugs, at least 25% contain at least one compound derived from higher plants. The percentage might be higher if we include over-the counter (OTC) drugs.

In developing countries, the practice of medicine still relies heavily on plant and herbal extracts for the treatment of human ailments. Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, and many of them have been used as traditional medicines. Cancer preventive properties are photochemical is obtained from plants (Barrett, 1991).

There is a growing focus on the importance of medicinal plants and traditional health systems in solving the health care problems of the world. Botanical, phytochemical, biological, and molecular techniques are used to find drugs from plat material. This method is used to find medicines for several existing disease including cancer, Alzheimer's etc (Snuet *et al*., 2011).

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. As such herbal remedies have been used to cure a variety of disorders or conditions such as diabetes, cardiovascular problems, weight control, dermal infirmities, sexual malfunction, and of course cancer. Therefore, the antibacterial studies should be concentrated on the unexplored medicinal plants for their medicinal values. Hence, the present study is aimed to investigate the efficacy of the solvent extracts of leaf of *Leucas aspera* against human pathogenic bacteria.

## Materials and Methods

### Collection of plant material

Fresh plant leaf of *Leucas aspera* was collected from the natural habitat of Government Arts College (Men) campus, Krishnagiri during the month of August – September 2015. Taxonomic identification of the plant was carried out with the help of the flora of presidency of madras (Gamble, 1935). The leaves of the *Leucas aspera* were washed thoroughly three times with water and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were sealed in separate polythene bags, until the time of extraction.

### Preparation of crude extract

The dried leaf materials were pulverized into fine powder using a grinder (mixer). About forty gram of powdered fruits was extracted successively with 200 ml of aqueous (97-103$^0$C), ethyl acetate (77-85$^{0C}$) and petroleum ether (62-66$^0$C) in Soxlet extractor until the extract was clear. The solvent extracts were evaporated by using rotary vacuum evaporator (Model: PBV – 7D) and the resulting pasty form extracts were stored in a refrigerator at 4$^0$C for further use (Chessbrough, 2000).

### Qualitative analysis

*Leucasaspera leaf* is subjected to phytochemical screening and qualitative phytochemical screening tests. All the extracts such as petroleum ether, ethyl acetate, and aqueous were analyzed with standard methods of Trease and Evans (1989) and Harborne (1998). The presence of constituents tested was recorded as present (+) or absent (-).

### Test for alkaloids

Formation of precipitate or turbidity while adding 5 mL of 2N HCl was added to 0.25 g of plant extracts followed by few drops of Mayer's reagent indicates the existence of alkaloids.

### Test for glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

### Test for flavonoids

The 2 ml of test solution was added with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

### Test for coumarins

In the test solution development of yellow colour while adding 10% of NaOH indicates the existence of coumarins in test sample.

### Test for quinines

When adding sulphuric acid with plant extract, the formation of red colour point out the occurrence of quinines.

### Test for saponins

The development of lather occurs while adding the 2 ml of each test solution with water and shook indicates the presence of saponins.

### Test for terpenoids

The 2 ml of test solution was added with 2ml of chloroform and a 3ml of Conc. $H_2SO_4$ mixed well and the formation of reddish brown indicates the presence of terpenoids.

### Test for tannins

A 2ml of each test solution was added with distilled $H_2O$ and a pinch of lead acetate, formation of white precipitate indicates the presence of tannins.

### Test microorganisms

The human pathogenic bacteria such as *Bacillus subtilis, Corynebacterium diphtheria, Staphylococcus faecalis, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteriae* were used during the present study and were obtained from Department of microbiology, Periyar University, Salem. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4$^{o}$C.

### In vitro antibacterial assay

The agar plate diffusion assay method was used to evaluate the antibacterial activity against the tested microorganisms. A 100µL of bacterial liquid culture in an exponential growth phase was spread onto the surface of Nutrient agar plate by using sterile cotton swabs. All the culture plates were allowed to dry for about five minutes. Agar well was prepared by using sterile cork borer (6 mm in diameter) and filled with 100µL of the crude extract. Petroleum ether, ethyl acetate and water (100%) were used as negative controls. The plates were incubated at 30$^{o}$C for 24 h. The diameter of inhibition zone around each of the well was measured at the end of the incubation time. Experiments were performed in triplicate and the antibacterial activity was expressed as the average of diameters of the inhibition zone produced by the test extracts.

### Determination of MIC

The minimum inhibitory concentrations (MIC) were performed by a serial dilution technique using 96-well microplates. The different plant extracts *viz.* petroleum ether, ethyl acetate, aqueous was taken (1 mg/ml) and serial dilution of the extract with nutrient broth and respective inoculums were used. The microplates were incubated for 72 hours at 28$^{o}$C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

### Determination of MBC

The minimum bacterial concentrations (MBC) were determined by serial sub-cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm

and compared with the standards Ampicillin for Bacteria (Hi-media lab, India) as the positive control. All experiments were performed in duplicate and repeated three times.

## Results and discussion

Currently, a number of antibiotics have lost their effectiveness due to the development of resistant strains of bacteria, which has primarily occurred through the expression of resistance genes (Davies, 1994; Moorthy *et al*., 2012). In addition to including resistance, antibiotics are sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions (Ahmad, 1998). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases (Berahou *et al*., 2007; Salmao *et al*., 2008).

In the present study, the preliminary phytochemical investigation of various solvent extract of leaf of *Leucas aspera* revealed the presence of of glycosides, tannins and terpenoids in petroleum ether and aqueous extract, alkaloids, coumarins, glycosides, flavonoids, quinines, saponins, tannins, terpenoids and almost all the tested compounds in the ethyl acetate extract. As per our preliminary phytochemical studies on the successive solvent extracts of petroleum ether, ethyl acetate and aqueous extracts, the ethyl acetate extracts revealed the presence of maximum number of phytoconstituents in it (Table 1). These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antibacterial, antiulcer, anticancer, larvicidal and chemo protective activities.

**Plate 1. Habit of *Leucas aspera* (willd.) Linn.,**

**Table 1. Preliminary phytochemical screening of various solvent extract of *Leucas aspera***

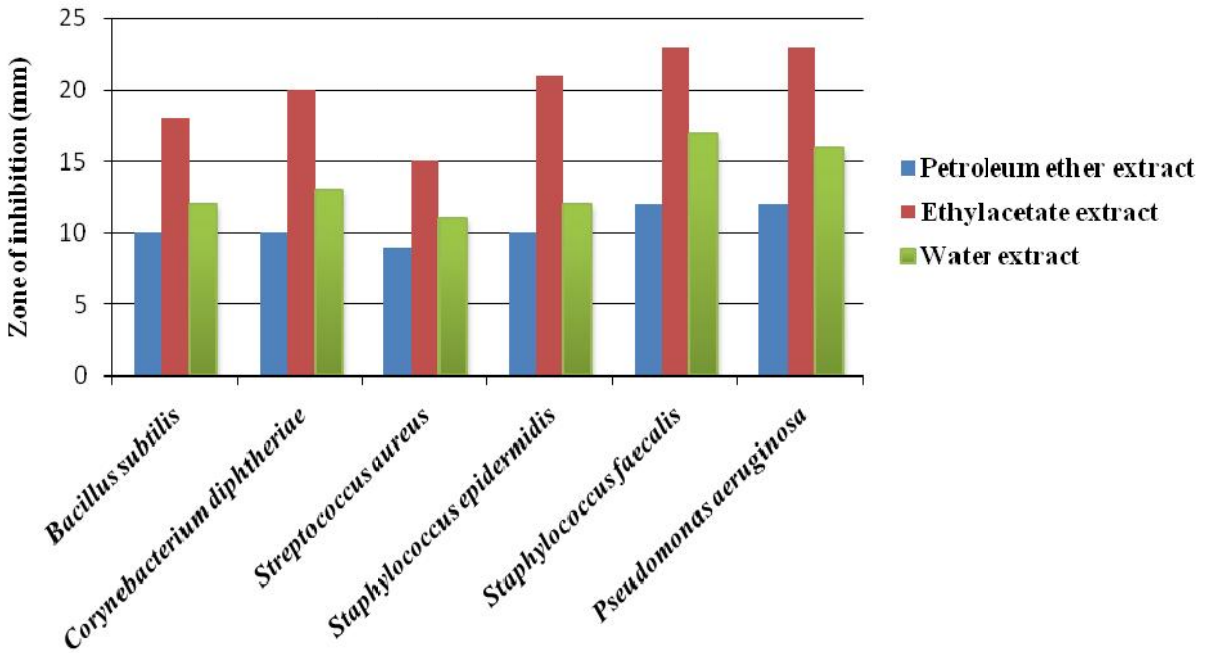
| S.No | Test | Petroleum ether extract | Ethyl acetate extract | Aqueous extract |
|---|---|---|---|---|
| 1 | Alkaloids | _ | + | _ |
| 2 | Coumarins | _ | + | _ |
| 3 | Glycosides | + | + | + |
| 4 | Flavonoids | _ | + | _ |
| 5 | Quinines | _ | + | _ |
| 6 | Saponins | _ | + | _ |
| 7 | Tannins | + | + | + |
| 8 | Terpenoids | + | + | + |

*+ Sign indicates presence and – sign indicates absence.*

The antibacterial activity of crude petroleum ether, ethyl acetate and aqueous extracts of *Leucas aspera* leaf were tested against six human pathogenic bacteria. Among the three extractions, the extract of ethyl acetate showed potent antibacterial activity by inhibiting the growth of all the tested human pathogenic bacteria. Highest inhibition zone of 23 mm was observed against *Streptococcus faecalis* and *Pseudomonas aeruginosa, Shigella dysenteriae* 21 mm and *Corynebacterium diphtheriae* 20 mm. Next to this, the significant zone of inhibition 18 mm was noted against *Bacillus subtilis.* Crude extract showed moderate activity against *Salmonella typhi* with the zone of inhibition was 15 mm. At the same time, the aqueous extract had significant activity and the zone of inhibition ranging from 11 to 17 mm. But, the petroleum ether extract exhibited lowest antimicrobial activity and the zone of inhibition ranging from 9 to 12 mm against all the tested microorganisms whereas, negative control petroleum ether, ethyl acetate and aqueous had negative antibacterial activity against all the tested human pathogenic bacteria (Table. 2 & Plate 2-3). (Figure.1). Similarly, Zakaria *et al*., (2009) reported antibacterial antimicrobial activity of petroleum ether, ethyl acetate and water extracts of leaf of *Muntingia calabura* were tested against bacterial and fungal pathogens. Among them, the ethyl acetate extract showed potent antimicrobial activity by inhibiting the growth of all tested gram positive, gram negative bacterial and fungal pathogens, followed by the petroleum ether and aqueous extracts.

Doughari and Okafor (2006) analyzed the antibacterial activity by using the aqueous and organic leaf extracts of *Senna siamae* against clinical isolates of *Salmonella typhi* using the disc diffusion method. The ethyl acetate extracts showed the highest activity, followed by acetone extracts, while the aqueous extracts showed the lowest activity. Similar result observed in our studies. The crude extract of petroleum ether, ethyl acetate and aqueous were screened for antibacterial activity. Among the three extractions, the extract of ethyl acetate showed potent antibacterial activity by inhibiting the growth of all the tested plant and human pathogenic bacteria. The result revealed that the ethyl acetate extract of *Leucas aspera* leaf are the potential source for the development of new antibacterial compounds. Similar results were obtained by different workers in various systems (Ilango *et al.,* 2008; Alam Morshed *et al*. 2011).

**Figure 1. Antibacterial activity of petroleum ether, ethyl acetate and water extract of leaf of *Leucas aspera* against human pathogenic bacteria.**



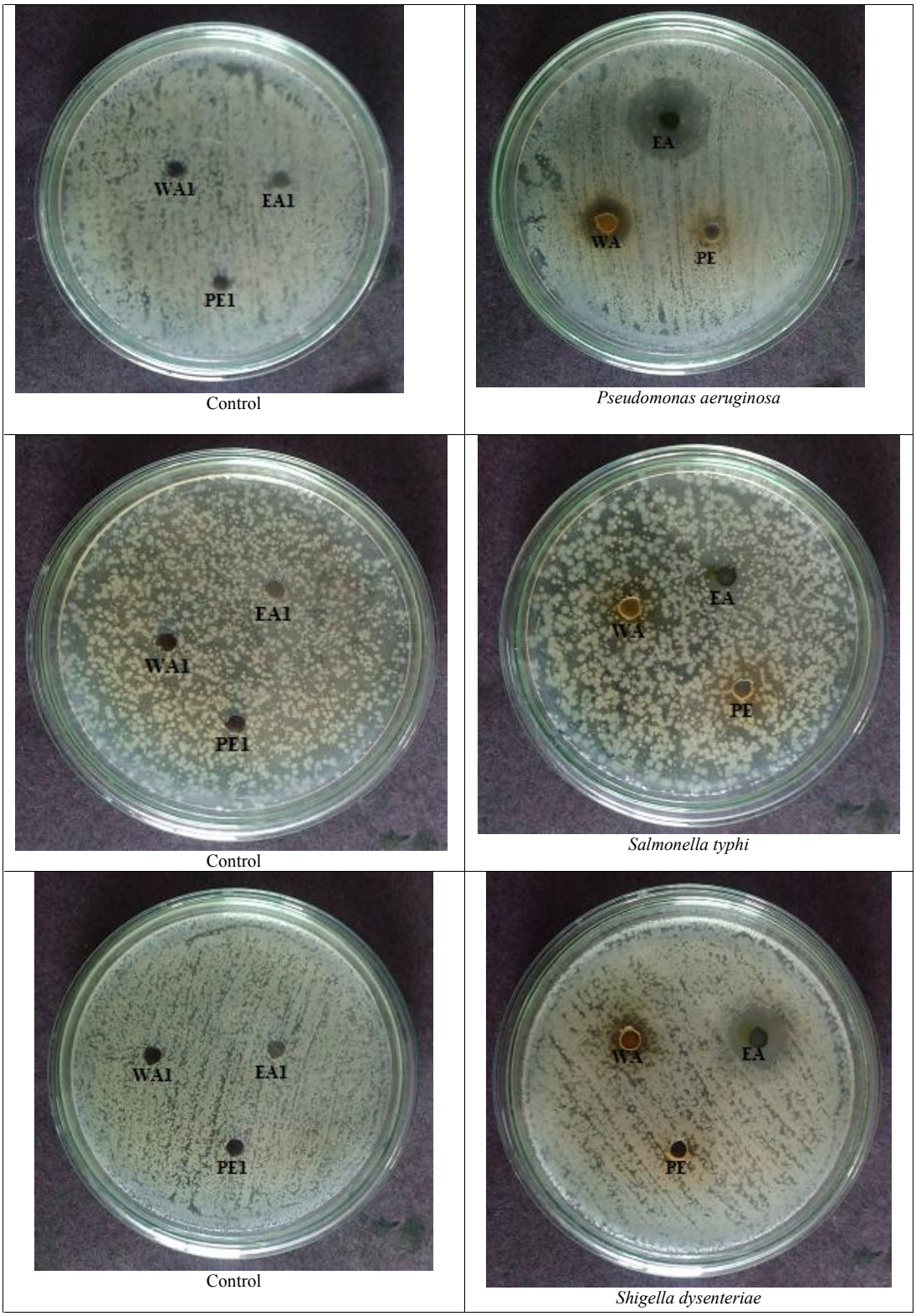

In agar well diffusion method, the ethyl acetate extract showed equal and more than 15mm mean zone of inhibition was documented against microorganisms were tested for minimum inhibitory concentration (MIC) by broth dilution technique. The result revealed that 600µg/ml was observed as MIC value against *Staphylococcus faecalis* and *Pseudomonas aeruginosa*,

1200µg/ml was documented against *Bacillus subtilis*, *Salmonella typhi* and *Shigella dysenteriae* , 2400µg/ml against *Corynebacterium diphtheria*. The result indicated that the ethyl acetate extract possesses good inhibitory activity against both gram positive and gram negative bacteria (Table. 2 & Plate 2-3). Similarly, lower MIC values of 0.25 mg/ml and 0.02 mg/ml for *Ocimum spp*. was reported by Akinyemi *et al* (2006). Phongpaichit et al. (2006) reported an MIC value of 32–512 ug $mL^{-1}$of ethyl acetate extract of endophytic fungi isolated from Garcinia sp. against MRSA. S. Antara Sen and Amla Batra (2012) also reported that the MIC value of crude aqueous extract of *Melia azedarach* was 33.7µg/ml against *Bacillus cereus*, 49.3µg/ml against *staphylococcus aureus*, 47.8µg/ml against *Escherichia coli* and 45.4µg/ml against *pseudomonas aeruginosa.*

**Table 2. Antibacterial activity of various solvent extracts of leaf of *Leucas aspera* against human pathogenic bacteria.**

| S.No | Human pathogenic bacteria | Leaf extract of *Leucas aspera* | | | Negative control | | |
|---|---|---|---|---|---|---|---|
| | | Zone of inhibition (mm) | | | | | |
| | | PE | EA | WA | PE1 | EA1 | WA1 |
| 1. | *Bacillus subtilis* | 10 | 18 | 12 | _ | _ | _ |
| 2. | *Corynebacterium diphtheria* | 10 | 20 | 13 | _ | _ | _ |
| 3. | *Staphylococcus faecalis* | 12 | 23 | 17 | _ | _ | _ |
| 4. | *Pseudomonas aeruginosa* | 12 | 23 | 16 | _ | _ | _ |
| 5. | *Salmonella typhi* | 9 | 15 | 11 | _ | _ | _ |
| 6. | *Shigella dysenteriae* | 10 | 21 | 12 | _ | _ | _ |

PE- Petroleum ether extract, EA- Ethyl acetate extract, WA- Aqueous extract, PE1- Petroleum ether, EA1- Ethyl acetate and WA- Aqueous.




PE- Petroleum ether extract, EA- Ethyl acetate extract, WA- Aqueous extract, PE1- Petroleum ether, EA1- Ethyl acetate and WA- Aqueous.

**Plate 2 . Antibacterial activity of leaf extract of *Lecus aspera* against selected human pathogenic bacteria**

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of micro-organism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MBC was determined by subculturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the Bacteria was taken as MBC. Moreover, it was noted that most of the antimicrobial properties in different plant part extractions shows, MBC value that is almost two fold higher than there corresponding MICs (Omar *et al*. 2010).

In the present exploration, the MBC value of 1200µg/ml was observed in ethyl acetate extract against *Staphylococcus faecalis* and *Pseudomonas aeruginosa*, 2400µg/ml against *Bacillus subtilis* and *Shigella dysenteriae*, 4800µg/ml against *Corynebacterium diphtheria* and *Salmonella typhi* respectively (Table 3) Similarly, the MBC values of 0.03 mg/ml for *Ocimum spp*. was reported by Akinyemi *et al* (2006).

Plants have developed natural defense mechanisms to protect themselves long before the man played an active role in protecting them. It is known that plants synthesize a variety of groups of bioactive compounds in plant tissues as secondary metabolites that have antibacterial activity to stop or inhibit the development of bacterial growth, each these groups presented variable mechanisms of action, for example, the toxicity of polyphenols in microorganisms is attributed to enzyme inhibition by oxidation of compounds (Zucker *et al*., 1972).

**Table 3. MIC and MBC values of leaf extract of *Leucas aspera* against against human pathogenic bacteria.**

| S.No | Human pathogenic bacteria | MIC and MBC values of leaf extracts of ***Leucas aspera*** | |
|---|---|---|---|
| | | MIC (ug/ml) | MBC (ug/ml) |
| 1. | *Bacillus subtilis* | 1200 | 2400 |
| 2. | *Corynebacterium diphtheria* | 2400 | 4800 |
| 3. | *Staphylococcus faecalis* | 600 | 1200 |
| 4. | *Pseudomonas aeruginosa* | 600 | 1200 |
| 5. | *Salmonella typhi* | 1200 | 4800 |
| 6. | *Shigella dysenteriae* | 1200 | 2400 |

The present study, the ethyl acetate extract of leaf of *Leucas aspera* showed an effective antibacterial activity against all the tested human pathogenic bacteria. The result revealed that the metabolites of *Leucas aspera* leaf are the potential source for the development of new antibacterial compounds.

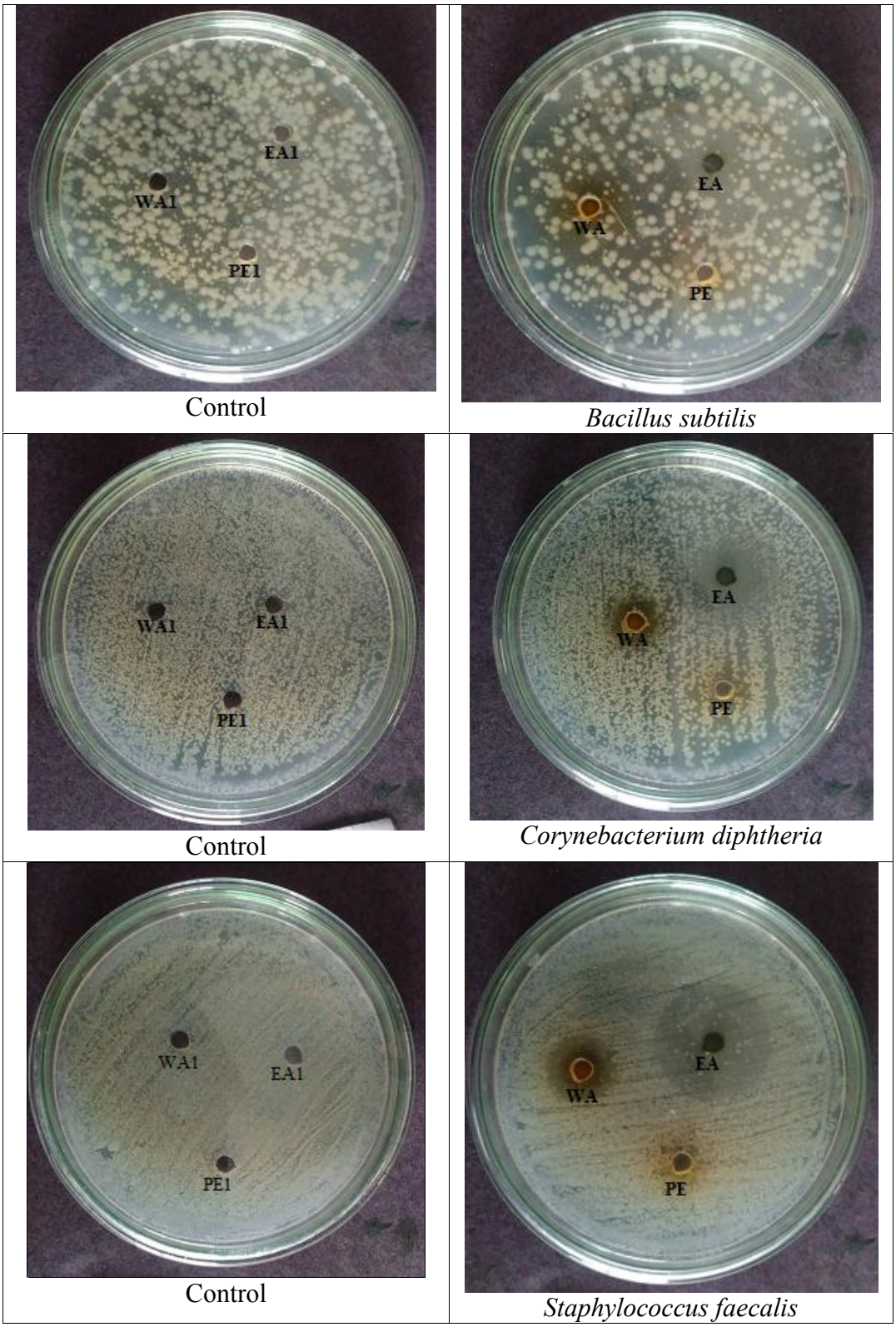



**Plate 3. Antibacterial activity of leaf extract of *Lecus aspera* against selected human pathogenic bacteria**

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## Conflict of interest statement

We declare that we have no conflict of interest.

## Reference

I. Ahmad, Z. Mehmood Z and F. Mohammad. "Screening of some Indian medicinal plants for their antimicrobial properities". *J. Ethnopharma,* 62.2 (1998):183-193.

K.O. Akinyemi, O.K. Oluwa and E.O. Omomigbehin. "Antimicrobial activity of crude extract of three medicinal plants used in south–west Nigerian folk medicine on some food borne bacterial pathogens. *African J. Traditional, Complementary & Alternative Medicines".* 3 (2006): 13-22.

M. Alam Morshed, A. Uddin, R. Saifur, A.Barua and A. Haque. "Evaluation of antimicrobial and cytotoxic properties of Leucas aspera and Spilanthes paniculata". *International J. Biosciences*, 1 (2011): 7-16.

Antara Sen and Amla Batra. "Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *melia azedarach". International J. Current Pharmaceutical Research.*, 4 (2012): 67-73.

J.E. Barrett. "Animal behavior models in the analysis and understanding ofanxiolytic drugs acting at serotonin receptors". In: Olivier B, Mos J, Slangen J. L. editors. "Animal models in psychopharmacology". Basel' Birkha¨user Verlag. (1991): 37-52

A. Berahou, A. Auhmani, N. Fdil, A. Benharref, M. Jana and C.A. Gadhi. "Antibacterial activity of *Quercus ilex* bark's extracts". *J. Ethanopharma*, 112.3 (2007): 426-429.

M. Cheesbrough. "Microbiological test: District Laboratory Practice in Tropical Countries". In: Cremer, A. and Evan, G. (eds). Cambridge University Press, UK. (2000).

J. Davies. "Inactivation of antibiotics and the dissemination of resistance genes". *Science*, 264. 5157 (1994): 375-382.

J. H. Doughari. "Antimicrobial activity of Tamarindus indica Linn". *Trop. J. Pharm. Res.,* 5 (2006): 597.

J. S. Gamble. "Flora of presidency of madras, London west Newm and Adlard". 2 (1935):1286-1290.

J. B. Harborne. "Phytochemical methods guide to modern technique of plant analysis". 3rd Edition, Chapmen all Hall, London, (1998).

K. Ilango, S. Ramya and G. Gopinath. "Antibacterial activity of *leucas aspera* spreng", *Int. J. Chem. Sci.,* 6.2 (2008): 526-530.

S. Lakshmi, G. Padmaja and P. Remani. "Antitumour Effects of Isocurcumenol Isolated from Curcuma zedoaria Rhizomes on Human and Murine Cancer Cells". *International J. Medicinal Chemistry*, 20 (2011):1-13

L. K. Madan and S. J. Sunil. "Evaluation of Anti-Inflammatory Effect of Ethanolic and Aqueous extracts of Curcuma Zedoaria Rosc Root". *International J. Drug Development & Research***,** 3 (2011): 360-365.

K. Moorthy, Aparna Aravind, T. Punitha, R. Vinodhini, M. Suresh and N. Thajuddin. "*In vitro* screening of antimicrobial activity of *wrightia tinctoria* (roxb)". *Asian J. Pharmaceutical & Clinical Research*, 5 (2012): 4-7.

K. Omar, A. Geronikaki, P. Zoumpoulakis, C. Camoutsis, M. Sokovic, A. Ciric, J. Glamoclija. "Novel 4-thiazolidinone derivatives as potential antifungal and antibacterial drugs". *Bioorg. & Med. Chem.*, 18 (2010): 426-432.

S. Phongpaichit, N. Rungjindamai, V. Rukachaisirikul and J. Sakayaroj. "Antimicrobial activity in cultures of endophytic fungi isolated from Garcinia species". *FEMS Immunol. Med. Microbiol.,* 48 (2006): 367-372.

K. Salmao, P. R. S. Pereira and L. C. "Campus, Brazilian propolis Correlation between chemical composition and antimicrobial activity". *Evidence-Based Complemen and Alternative Med.,* 5.3 (2008): 317-324.

Y. T. Snu, J. H. Shih, C. C. Charng, H. T. Ching, C. W. Chu and L. M. Jeng. "Composition and Antioxidant Properties of Essential Oils from *Curcuma* rhizome". *Asian J. Arts & Sciences*. 2.1, (2011): 57-66

G. E. Trease, and W. C. Evans. "Textbook of Pharmacognosy". 13th Ed. BalliereTindall, London." (1989).

Z. A. Zakaria, C. A. Fatimah, A. M. Mat Jais, H. Zation, E. F. P. Henie, M. R. Sulaiman, M. N. Somchit, M. Theamutha, and D. Kasturi. "The *in-vivo* antibacterial activity of *Muntingia calabure* Extracts". *International J. Pharmocology,* 2.4 (2009): 439-442.

M. L. Zucker, L. Hanskin and D. "Sands Factors governing pectate lyase synthesis in soft rot and non-soft rot bacteria". *Physiol. Plant Pathol.*, 2 (1972): 59-67.