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### RESEARCH ARTICLE

#### PRELIMINARY STUDIES ON PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTS OF MEDICINAL PLANT LAWSONIA INERMIS.

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#### Abstract

Medicinal plants comprise bioactive compounds called phytochemicals which are used for curing of various human diseases. Phytochemicals are non-nutritive, chemical compounds that occur naturally on plants and have diverse protective properties. These compounds are produced by plants for their protection. Recent research demonstrates the protective role of such compounds against human diseases in several medicinal plants. In this present study the compositional analysis of the medicinal plant *Lawsonia inermis* was documented. Solvent extraction of leaf samples with Chloroform, Acetone, Ethanol, Methanol, and water extracts of leaf samples revealed the phytochemical constituents in the plants. In this study, antimicrobial activity was performed by disc diffusion and well diffusion method. The maximum activity was showed in methanol extraction against pathogens. The main objective of the research work was to check the presence or absence of the phytochemical constituents in the selected medicinal plant.

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#### Introduction:-

Medicinal plants offer a great numbers of highly effective drugs and they remain as an attractive option for discovery of new molecular entities for a long time, due to their largely untapped chemical diversity (Malherbe *et al.*, 2012). Virtually, 80% of the world's population relies on traditional medicines for primary health care, mostly involving plant extracts. In India, almost 95% of the prescriptions were based on traditional systems of Unani, Ayurveda, Homeopathy and Siddha (Savithramma *et al.*, 2011). Eventually, the presence of phytochemical constituents in medicinal plants is useful for healing as well as for curing of human diseases (Nostro *et al.*, 2000).

Typical phytochemical compound that possess antioxidant activity include saponins, glycosides, alkaloids, tannins, and their derivatives, ascorbic acid, flavanoid and many sterols. As antioxidant, these spices are capable of removing free radical, chelate metal catalyst, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidaze (Nisa *et al.*, 2013).

Phytochemicals are chemical compounds that naturally occurring in the plant parts such as flower, buds, fruits, barks, leaves, vegetables and roots to have defense mechanism and protect the plants from various diseases. They are also found in spices, and medicinal plants; and work in conjunction with other plant components as defensive mechanisms for the plants against diseases and many external attacks (Chang *et al.*, 2006). In humans, many phytochemicals have been found to be protective and preventive against many degeneratives diseases and

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pathological processes such as in ageing, neurodegenerative disorder, atherosclerosis and inflammation (Kawoand Kwa, 2011). In addition to protective effects, they contribute to the plant's color, aroma and flavor. Phytochemicals are found to be accumulated in different parts of the plants, such as in the roots, stems and leaves. Phytochemicals are categorized as primary and secondary constituents in which proteins, chlorophyll and common sugars are included in primary constituents and terpenoid, alkaloids and phenolic compounds are grouped into secondary constituents (Krishnaiah *et al.*, 2007), Terpenoids exhibit various important pharmacological activities (Mahato and Sen, 1997), Alkaloids are used as anaesthetic agents and are found in medicinal plants (Hérouart *et al.*, 1988). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anti cancer property (Saidulu *et al.*, 2014).

Once such medicinal plant commonly known as henna, mehendi available in tropical and subtropical areas has a long history of traditional medicine in India due to its diverse uses and appreciable role in ayurvedic and natural herbal medicines (Lavhate and Mishra, 2007).

Henna is a flowering plant, having a height of 5 meters, natal to subtropical and tropical regions of world including South Asia, Africa, and oases of Sahara Dessert and even in northern regions of Australia. Leaves of henna plant are entire, opposite, sub-sessile, oval-shaped and smooth (Ashnagar *et al.*, 2011). Leaves have length of 2–3 cm with 1–2 cm width (Basirian Mina *et al.*, 2012). Henna shrub is highly branched and has greyish-brown barks (Chang *et al.*, 2006). Main chemical constituents of henna are Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, gallic acid and tannic acid (Nisa *et al.*, 2013). Henna is known to be used as a cosmetic agent for dyeing hair, nails and skin (Kawoand Kwa, 2011).

In traditional medicine, henna plant is used to treat many diseases like oedema, bronchitis, menstrual disorder, rheumatism, hemorrhoids and even in jaundice, leprosy, pain, spleen enlargement, dysentery and skin problems (Rahmoun *et al.*, 2010; Mahato and Sen, 1997; Bhuvaneshwari *et al.*, 2002; Warriar *et al.*, 1995). Henna can also be used as an astringent and antihemorrhagic agent and is also known for its hypotensive, cardio inhibitory and sedative effects (Rahmoun *et al.*, 2010). In addition, henna is reported to show some other properties including hypoglycemic (Syamsudin and Winarno, 2008), immunostimulant (Mikhaeil *et al.*, 2004), hepatoprotective (Chaudary *et al.*, 2012), anti-inflammatory (Singh *et al.*, 1982), tuberculostatic (Sharma, 1990), anti-cancer and antioxidant properties (Kamal and Jawaid, 2010). *Lawsonia inermis* is a small tree or large shrub growing to six meters high. It has lateral branches with leaves that grow in pairs, two or four centimeters. Throughout India, plant parts commercially cultivated in Punjab, Gujarat, Madhya Pradesh, Rajasthan and Tamilnadu (Olopade *et al.*, 1992). Leaf is deciduous shrub with lateral branches often ending in spines; leaves are simple, opposite, entire, lanceolate, petioles very short or absent. Flower is white or rose coloured fragrant in large terminal pyramidal paniced cymes, stamens 8 in 4 pairs inserted on the calyx tube. Fruits is globose capsules, tipped with the style and supported by the persistent calyx, seeds numerous, smooth, pyramidal. Genus *Lawsonia* consists of one species, *Lawsonia inermis* (Henna, mehendi, shudi, Madurang, Mendi, Manghati, Madayantika and goranti), (Gupta, 2003). It is biennial dicotyledons herbaceous herb. The origin of henna plant is North Africa and south west Asia. It is grown for ornamental as well as dye plant. It is much branched glabrous shrub or small tree (2 to 6 m in height). Leaves are small, opposite in arrangement along the branches, sub-sessile, about 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, elliptic to broadly lanceolate leaves with entire margin, petiole short and glabrous and acute or obtuse apex with tapering base. Young branches are green in colour and turn red with age. Bark is greyish brown, unarmed when young but branches of older trees are spine tipped. Inflorescence is a large pyramid shaped cyme. Flowers of henna are small about 1cm across, fragrant, white or rose coloured with four crumbled petals. Calyx is 0.2 cm tube and 0.3 cm spread lobes. Fruit is a small brown coloured round capsule. Fruit opens irregularly and splits into four sections at maturity and is many seeded. Seeds are about 3mm across, numerous, smooth, pyramidal, hard, thick seed coat with brownish coloration (Chauhan and Pillai, 2007).

Henna plant has several ethno botanical uses. It is widely used as medicinally and cosmetically. Henna leaves consist of 0.5-1.5% lawsone which is responsible for orange red dye color. It has an orange red dye and leaf paste or powder is used for decorating hands, nails and feet. It can also be used as a hair dye. Henna plant leaves are used to cure jaundice, skin diseases, venereal diseases, smallpox, and spermatorrhoea. Henna plant leaves, flowers, seeds, stem bark, roots are used as agent to treat ailments as leprosy, fever, eucorrhoea, diabetes, cardiac disease, rheumatoid arthritis, headache, ulcers, diahorrea, hepatoprotective and coloring (Vasudevan and Laddha, 2003). Based on this

view, the present study was aimed to analyze the chemical constituents present in the solvent extracted leaves of Henna using biochemical studies.

### Phytochemical Analysis

#### Plant Description - Classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Myrtales
Family	:	Lythraceae
Genus	:	<i>Lawsonia</i>
Species	:	<i>inermis</i>

#### Vernacular Names

English	:	Henna,
Hindi	:	Mehanti, Hena
Malayalam	:	Mailanci, Mayilanci
Sanscript	:	Medhini, Madayantika
Tamil	:	Mailenanti, Marutani
Telungu	:	Goranta



**Plate 1:-**Morphology of *Lawsonia inermis* L.

### Methodology:-

#### Collection and processing of plant material:

The leaves of *Lawsonia inermis* (Plate 1) were collected from the local area for its freshness, healthy and free from any deformation. The collected leaves were transferred immediately to the laboratory for analysis. The samples were shadow dried for a week and blended into powder by mixture blender. Then it was passed through the sieve to get the equal size particles. The powder was kept in air tight container at moisture free environment for further analyses.

### **Plant Extract Preparation using Hot Method**

The powdered leaves were extracted with different solvents by hot method using soxhlet apparatus. Briefly, 10 gm of powdered plant leaves were taken in clean sterile soxhlet apparatus and extracted with 150 ml of different solvents such as Chloroform, Acetone, Ethanol, Methanol, and water (low polar to high polar). After extraction, the extracts were dried at room temperature until reaches the solid (powder) form. Subsequently, the extracts were dissolved in Dimethyl sulfoxide (DMSO) to the concentration of 10 µg/ml for further analysis.

### **Phytochemical Analysis**

The preliminary phytochemical analyses of the different extracts were determined using the standard methods (Roopashree *et al.*, 2008; Obasi *et al.*, 2010; Audu *et al.*, 2007; Debiyi and Sofowora, 1978; Sofowora, 1993; Trease and Evans, 1989).

#### **Alkaloids**

Plant extracts were dissolved individually in dilute Hydrochloric acid and filtered.

**Mayer's Test:** Plant extracts were treated with Mayer's reagent (Potassium Mercuric Iodide). Appearance of yellow coloured precipitate indicates the presence of alkaloids.

**Wagner's Test:** Plant extracts were treated with Wagner's reagent (Iodine in Potassium Iodide). Appearance of brown/reddish precipitate indicates the presence of alkaloids.

**Dragendroff's Test:** Plant extracts were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Appearance of red precipitate indicates the presence of alkaloids.

**Hager's Test:** Plant extracts were treated with Hager's reagent (saturated picric acid solution). Formation of alkaloids confirmed by the formation of yellow colored precipitate.

#### **Amino Acids**

**Ninhydrin Test:** To the plant extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

#### **Phenolic Compounds**

**Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Appearance of bluish black colour indicates the presence of phenols.

#### **Reducing sugar**

**Benedict's test:** Plant extracts were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

#### **Terpenoids**

**Copper acetate Test:** Plant extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Appearance of emerald green colour indicates the presence of diterpenes.

#### **Glycosides**

Plant extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

**Modified Borntrager's Test:** Plant 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. Appearance of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

#### **Sapanins**

**Froth Test:** Plant extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Appearance of 1 cm layer of foam indicates the presence of saponins.

**Foam Test:** 0.5 gm of plant extract was shaken with 2 ml of water. Appeared foam produced persists for ten minutes it indicates the presence of saponins.

#### **Steroids**

**Salkowski's Test:** Plant extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Formation of golden yellow colour indicates the presence of triterpenes.

**Libermann Burchard's test:** Plant extracts were treated with chloroform and filtered. The plant extracts were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Appearance of brown ring at the junction indicates the presence of phytosterols.

#### **Tanins**

**Gelatin Test:** To the Plant extract, 1% gelatin solution containing sodium chloride was added. Appearance of white precipitate indicates the presence of tannins.

#### **Flavonoids**

**Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Appearance of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

**Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

#### **Anthraquinones**

1ml of plant extract is boiled with 10% HCL for few minutes in a water bath. It is filtered and allowed to cool. Equal volume of  $\text{CHCl}_3$  is added to the filtrate few drops of 10% Ammonia are added to the mixture and heat. Formation of rose pink colour indicates the presence of anthraquinones.

#### **Screening of Antibacterial Activity**

The pathogenic cultures of bacterial strains were used. The bacterial strains were obtained from clinical laboratory. Five pathogens were chosen for the present investigation. The cultures were prepared separately in nutrient broth. The culture was stored at  $4^\circ\text{C}$  and it was taken just before performing the experiment. Name list of the strains used in the present study,

1. Staphylococcus aureus
2. Escherichia coli
3. Pseudomonas aeruginosa
4. Proteus vulgaris
5. Streptococcus mutans

#### **Disc Diffusion method (Perz *et al.*, 1990)**

Antibacterial activity of the extracts was tested by Disc-diffusion method. 0.2 ml bacterial cultures (4hrs) were uniformly spread on solidified nutrient agar medium. The filter paper discs prepared with plant extracts were carefully placed over the spreaded cultures by using sterile needle and incubated at  $37^\circ\text{C}$  for 24hrs. After the incubation period, the plates were examined for inhibitory zones (including the diameter of the disc).

#### **Well diffusion assay**

It was used to test the antagonistic activities of *Lawsonia inermis* extract against various test organisms. The selected strains of bacteria were inoculated in to 5ml of sterile nutrient broth, and incubated at  $37^\circ\text{C}$  for 16-18 hrs. The cotton swab and agar plates were prepared. Using the sterile cotton swabs, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. The well was created on the agar plates by using sterilized cork borer with 10mm diameter. 100 $\mu\text{l}$  of different solvents extract of *Lawsonia inermis* were transferred into each well. The plates were incubated for 24 hrs at  $37^\circ\text{C}$  and examined for clear inhibitions zone around the well. The diameter of inhibition zones was measured in mm and the results were recorded (Baues *et al.*, 1996).

#### **Results and Discussion:-**

The herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (WHO, 2001). In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new antimicrobial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of the commonly used antibiotics (Abebe *et al.*, 2003). Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. It is believed that plant chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their antimicrobial activities (Cowan, 1999).

The results of the present study revealed the presence of phytochemicals in the extract of *Lawsonia inermis*. It plays a major role in pharmacological activity spectrum. In modern days people looking for nontoxic medication so it can be used as traditional medicine and without any toxicity and side effects. Important medicinal phytochemicals such as terpenoids, reducing sugar, flavanoids and alkaloids were present in the samples. By considering these results we can carry out further experiment to get more medicinal and industrial products.

The secondary metabolites mainly attribute the antimicrobial activity to plants (Gonzalez-Lamothe *et al.*, 2009). The active constituents of these secondary metabolites include phenolic compounds and tannins (Edwin, 1996). The result of the phytochemical analysis shows that the experimental plant is rich in phytochemicals. The phytochemical screening of medicinal plant *Lawsonia inermis* is shown in (Table 1). Due to the presence of several chemical components it can be used as analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostic and anticancer properties. It clearly shows that the plant *Lawsonia inermis* is considered as a valuable source for traditional and industrial products.

The results obtained from the phytochemical test shows correlation with further studies in some ways and contradiction in some other ways. In the present investigation, the phytochemical screening of ethanolic extract of *Lawsonia inermis* shows the presence of phytochemicals such as Aminoacids, Phenolic compounds, Reducing sugar, Terpenoids, Saponins and Steroids out of eleven for which screening has been performed. Similarly, the methanolic extract shows the presence of Alkaloids, Aminoacids, Phenolic compounds, Reducing sugar, Terpenoids, Glycosides, Flavonoids, Saponins, Steroids and Saponins. This indicates that most of the active compounds in the plant might be soluble in particular solvent than other solvents (Choudhary *et al.*, 2010).

In similar studies, Raja *et al.*, 2013 confirmed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids in methanolic extract of the *Lawsonia inermis* leaves. Upadhyay *et al.*, 2011 shows Tannin, Saponins, Naphthaquinone, Flavanoid, Steroids Terpenoid and Cardioglycosides in aqueous extract of leaves. Kawo and Kwa, in 2011 reported the presence of Tannins, Saponins, Sterols and Carbohydrates in methanol extract. Arun *et al.*, 2010 reveals the presence of Flavonoids Alkaloids, Tannins and quinines in methanolic extract. Singh *et al.*, 2014 reported the presence of Alkaloid, Glycoside, Hydrolysable Tannins, Flavonoids, Steroids, Proteins, Carbohydrates and Saponins in hydroethanolic extract of plant. The studies of Chaudhary *et al.*, 2010 have reported that leaves of *Lawsonia inermis* contain carbohydrates, proteins, flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes and fatty acids. Basirian Mina *et al.*, 2012 detect the presence of Steroids, Flavonoids and Tannins in ethanolic extract. Jeyaseelan *et al.*, 2012 demonstrate the presence of Tannins, Terpenoids, Flavonoids and glycosides in ethanolic extract.

The present study has been undertaken to find out the effectiveness of the different extracts of *Lawsonia inermis* leaves against some bacterial pathogens. Out of five strains selected three strains showed good antimicrobial potential against our plant extract.

The antimicrobial potency of *Lawsonia inermis* leaf extracts were tested against some bacterial pathogens and was quantitatively assessed for the presence or absence of zone of inhibition. The results relative to antibacterial activity were observed by measuring the diameter of the zone of inhibition (Figure 1). By disc diffusion method the activity of methanol extracts of *Lawsonia inermis* against *Pseudomonas aeruginosa* the zone of inhibition obtained at 75% ( $3.3 \pm 3.16$ mm) followed by 100% ( $2.6 \pm 2.16$ mm), 50% ( $2.2 \pm 2.4$ mm) and 25% ( $1.7 \pm 2.1$ mm) showed maximum activity when compared to all the three strains (Table 2). The activity of methanol extracts of *Lawsonia inermis* leaves against *Staphylococcus aureus* showed minimum activity ( $1.4 \pm 2.01$ mm) at 25% concentration and maximum activity ( $2.9 \pm 1.9$ mm) at 100% level. The activity of methanol extracts of *Lawsonia inermis* against *Streptococcus mutans* showed minimum activity ( $1.4 \pm 1.51$ mm) at 25% followed by 50% ( $2.4 \pm 3.23$ mm), 75% ( $2.6 \pm 5.12$ mm) and 100% ( $2.8 \pm 1.54$ mm). The ethanol extracts of *Lawsonia inermis* leaves against *Staphylococcus aureus* showed minimum activity ( $2.1 \pm 1.21$ mm) at 25% concentration and maximum activity ( $3.1 \pm 2.2$ mm) at 100% level (Table 3). The ethanol extracts of *Lawsonia inermis* leaves against *Streptococcus mutans* showed minimum activity ( $2.0 \pm 1.31$ mm) at 25% concentration maximum activity ( $3.5 \pm 2.1$ mm) at 100%. The ethanol extracts of *Lawsonia inermis* leaves against *Pseudomonas aeruginosa* showed maximum activity ( $2.6 \pm 1.61$ mm) at 25% concentration and maximum activity ( $4.1 \pm 2.41$ mm) at 100%. The present investigation has been undertaken to find out the effectiveness of the methanol and ethanol extracts of *Lawsonia inermis* against some pathogenic microorganism such as *Staphylococcus aureus*, *Streptococcus mutans*, and *Pseudomonas aeruginosa*.

### Conclusion:-

By observing the results of phytochemical analysis, thus we conclude that *Lawsonia inermis* consists of high medicinal and therapeutic sources. A drug can be developed by undertaking the modern drugs isolated from henna plant. Earlier literature indicated that medicinal plants are the back bone of the traditional medicine. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The important phytochemical properties identified by our study in the local plant of *Lawsonia inermis* will be helpful in the coping different diseases. *Lawsonia inermis* samples demonstrated antibacterial activity against all isolates but the highest susceptibility was against *Pseudomonas aeruginosa* of this particular region. Thus we concluded that the medicinal plants could be used as drug to treat the infections caused by bacteria without any side effects.

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### Conflicts of Interest

The authors declare that they have no conflict of interest.

**Table 1:-**Preliminary phytochemical analysis of plant *Lawsonia inermis*

Phytochemicals	Methanol	Chloroform	Ethanol	Acetone	Ethanol
Alkaloids	+	+	+	+	-
Amino acids	+	-	-	-	+
Phenols	+	+	+	+	+
Reducing sugar	+	+	+	-	+
Terpenoids	+	-	+	+	+
Glycosides	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	+	-	+
Steroids	+	+	+	+	+
Tanins	+	-	+	+	-
Anthraquinones	-	+	-	+	-
+ = indicates presence of phytochemicals - = indicates absence of phytochemicals					

**Table 2:-** Antibacterial activity of methanolic extracts of *Lawsonia inermis* (mm)

S.No	Organisms	Zone of inhibition(mm)			
		25%	50%	75%	100%
1	<i>Staphylococcus aureus</i>	1.4±2.01	2.4±3.0	2.7±2.7	2.9±2.3
2	<i>Streptococcus mutans</i>	1.4±2.0	2.4±3.1	2.6±4.0	2.8±4.4
3	<i>Pseudomonas aeruginosa</i>	1.7±2.1	2.2±2.4	2.6±3.16	3.3±2.16

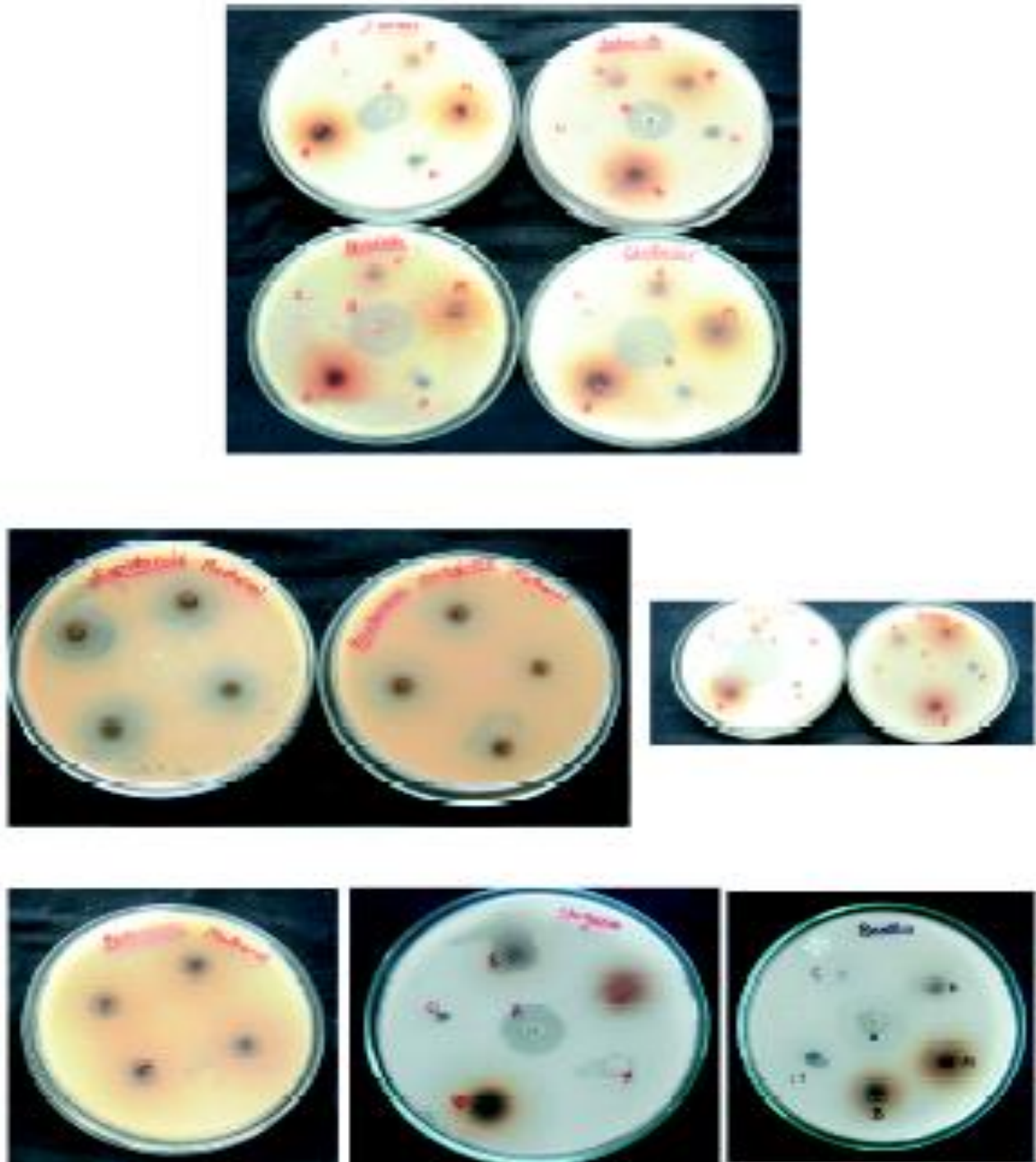
Value are triplicate and represented as mean ± standard deviation

**Table.3** Antibacterial activity of ethanolic extracts of *Lawsonia inermis* (mm)

S.No	Organisms	Zone of inhibition(mm)			
		25%	50%	75%	100%
1	<i>Staphylococcus aureus</i>	2.1±3.21	2.3±3.0	2.8±2.7	3.1±6.2
2	<i>Streptococcus mutans</i>	2.0±4.31	2.4±3.1	3.1±4.0	3.5±4.1
3	<i>Pseudomonas aeruginosa</i>	2.6±2.1	2.8±2.4	3.6±6.4	4.1±8.61

Value are triplicate and represented as mean ± standard deviation

Figure 1:-Antimicrobial activity of plant extracts Lawsonia inermis





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