



# ANTIMICROBIAL ACTIVITY OF PLANT OF MOLLUGO OPPOSITIFOLIA AND ITS PHYTOCHEMICAL SCREENING

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## 1. Abstract

The plant of *Mollugo oppositifolia* Linn is used in the treatment like skin diseases, increase appetite, cures kapha, piles, leukoderma, tonic of intestine, urinary infection, fever, cough, liver problem. Due to its excellent properties and potent constituents' activities like free radical scavenging and antioxidant activities, hepato protective effect, antiprotozoal activity, immunomodulating activity. Thus, the extract of *Mollugo oppositifolia* Linn (family: Molluginaceae) leaves was examined using the antimicrobial activity by agar well diffusion method. Here the chloroform, pet. ether and ethanolic extracts of *Mollugo oppositifolia* Linn was tested at 20mg/ml concentration and evaluated against organism of staphylococcus aureus and proteus vulgaris. Ciprofloxacin was used as standard. The level of effect was determined by measuring zone of inhibition at room temperature for 48hrs and diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. Analytical method of FTIR used to evaluated the extract of *Mollugo oppositifolia* Linn. Result of the preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, steroids, glycosides, flavonoids, protein.

## KEY WORDS-

Anti-microbial activity, Diffusion, *Mollugo oppositifolia* Linn, Proteus vulgaris, Staphylococcus aureus.

## 2. Introduction

Herbal medicine, also known as phytomedicine or phytotherapy, involves the study and use of medicinal plants. It has limited scientific evidence for its safety and efficacy in modern herbalism. Medicinal herbs contain various chemical compounds like alkaloids, glycosides, and essential oils, leading to growing interest in their chemical composition and pharmacological activity. *Mollugo oppositifolia* L. has also been described under the names *Mollugo spergula* L. or *glinus oppositifolius*(L.) Aug. DC.(Aizoaceae) It is commonly known as carpet weed. It is an erect slender, much branched annual herb, up to 30 cm. high, commonly found in dry as well as moist areas. It is also having numerous applications in traditional medicine as aperients, antiseptic, emmenagogue. An infusion of the plant is given to women to promote the menstrual discharge. Leaves are bitter and antiperiodic; they are warmed after smearing with oil and applied to the ear to relieve earache. Antibacterial resistance is a global concern, limiting the effectiveness of current drugs. Plants have been investigated as potential sources of new antimicrobial agents due to their bioactive compounds. This study

aims to investigate the antibacterial potential of Cameroonian plants against multi-drug resistant phenotypes. Traditional medicine, including herbal remedies, is widely used in Asian and African countries. The chemical composition of plant-based medicines is gaining interest, and bioactive constituents have been isolated and studied for their effects.

### 3. Material and methods

#### 3.1. Plant collection

The plant of *Glinus oppositifolia* or *Mollugo oppositifolia* Linn were collected from the Harur, Dharmapuri district, Tamilnadu and were authenticated by Dr. N.Suresh Head and Assistant Professor of Botany, P.D.R.T Padmavathi arts and science college (women) Sekkampatti, Harur, Salem, tamilnadu.

#### 3.2. Preparation of extracts

##### 3.2.1. Petroleum ether extracts

The shade dried material (200 mg) was extracted with petroleum ether (6080) in Soxhlet apparatus. After the completion of the extraction, the solvent was removed.

##### 3.2.2. Ethanol extracts

The mare left after the Petroleum ether extraction was dried and then extracted with Ethanol in Soxhlet apparatus. After the completion of the extraction, the solvent was removed.

##### 3.2.3. Chloroform extracts

The mare left after the Ethanol extraction was dried and then extracted with Chloroform in Soxhlet apparatus. After the completion of the extraction, the solvent was removed.

#### 3.3. Morphological characters

Morphology is the study of the form of an object while morphology is the description of that form where the material is known to occur in a particular form. Morphological and organoleptic characters viz., colour, odour, taste, shape, and size were observed and evaluated botanically.

#### 3.4. Preliminary phytochemical screening

The leaf powder and various extract of the plant was subjected to chemical test for identification of its different active constituent like alkaloids, carbohydrates, steroids, glycosides, flavonoids, protein, tri terpenoids, tannins and phenolic compounds.

#### 3.5. Extractive values

Extractive values help to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with various solvent. The extraction of any crude with particular solvent yields a solution containing different Phyto constituents. The composition of these Phyto constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample.

##### 3.5.1. Determination of alcohol soluble extractive

5 grams of powder was macerated with 100 ml of alcohol of the specified strength in closed flask 24 hours shaking frequently during 6 hours and allow standing for 18 hours. it was filtered rapidly taking precaution against loss of alcohol and 25ml of filtered was evaporated by dryness in third flat bottom shallow dish dried 1 50 °C and weighed. The percentage of all extractive was calculated with reference to the air-dried powder.

##### 3.5.2. Determination of water-soluble extractivity

About 5 grams of powder was added to 50 ml of water 80 °C in a stopper flask. It was shaken and allowed to stand for 10 minutes, cooled to 1 5 °C and to it 2grams of kieselghur was added and filtered, 5ml of the filtrate was transferred to a tarred and evaporating basin, 7.5cm in diameter, the solvent was evaporated on a water bath, drying was continued for half an hour, finally it was dried in a hot air oven for two hours and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried powder.

##### 3.5.3. Determination of loss on drying

About 1-2 grams of powdered leaves was accurately weighed in tarred dish in an oven at 1 00 °C to 1 50 °C. It was cooled in desiccators and again weighed. The loss on drying was calculated with reference to the amount of air-dried powder taken.

##### 3.5.4. Determination of total ash

About 2-3 grams of powder was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (45 °C) until free from carbon cooled and weighed. The percentage of ash was calculated with reference to the air-dried powder. The procedure was repeated to get constant weight.

##### 3.5.5. Determination of water-soluble ash

The total ash was boiled with 25 ml and was filtered through ash less filter paper. It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble

matter was weighed. The water-soluble ash can be calculated by subtracting the water insoluble matter from the total ash.

### 3.5.6. Determination of acid insoluble ash

The total ash obtained was boiled for five minutes with 25ml of 10% w/v dilute hydrochloric acid and filtered through the ash less filter paper. The filter paper was ignited in the silica crucible cooled and insoluble ash weighed.

## 3.6. Analytical evaluations

### 3.6.1. Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

Dried powder of ethanol, pet ether, chloroform extracts of *Mollugo oppositifolia* was used for FTIR analysis. The extract contains both polar and nonpolar components of the plant material, and 2 µl of the sample of the solutions was employed in FTIR for analysis of different compounds. 10mg of the dried extracts powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>

## 3.7. Pharmacological Studies

### 3.7.1. Antibacterial activity

Antibacterial activity of various extracts of *mollugo oppositifolia linn* was evaluated by using Agar Diffusion method. Each petri dish containing nutrient agar medium was inoculated with one bacterial culture by spreading the suspension of organism with a sterile glass rod with a bended tip. In each plate cups of 6mm diameter were made at equal distance using sterile cork borer. The extract of plans was tested. All plates were kept in the refrigerator for 30 mins to allow the diffusion of the sample to the surrounding agar medium. The petri dishes were incubated at 37°C for 24 hrs. The wells were created using a stainless-steel sterilized cork borer under aseptic conditions. The chloroform, pet ether and ethanol extract of *Mollugo oppositifolia linn* was tested at 20mg/ml concentration. Ciprofloxacin was used as standard. The plates were incubated at room temperature for 48 hrs and zones of inhibition were measured Diameter of the zones of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with standard.

## 4. Results

### 4.1. Pharmacognostic investigation

**Table 1. Pharmacognostic investigation**

S.no	Features	Observations
1)	Colour	Blackish green
2)	Odour	Odourless
3)	Taste	Bitter
4)	Consistency	Sticky

### 4.2. Preliminary phytochemical screening

Preliminary phytochemical screening of various extracts of *mollugo oppositifolia linn* showed the presence of alkaloids, carbohydrates, steroids, glycosides, flavonoids, protein, tri terpenoids, tannins and phenolic compounds in the extracts.

**Table 2. Phytochemical screening**

Tests	Successive extraction		
	Ethanol	Chloroform	Pet.ether
Alkaloids	+	+	+
Carbohydrates	+	+	+
Steroids	+	+	+
Glycosides	+	+	+
Saponins	+	+	-
Flavonoids	+	+	+
Tannins & phenolic compounds	-	+	-
Tri terpenoids	-	-	+

Protein	+	+	+
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‘+’ is present constituents

‘-’ is absent constituents

### 4.3. Physical evaluation

Physical evaluation of whole plant extracts of *mollugo oppositifolia linn* showed the report of extractive value and ash value.

**Table 3. Extractive value**

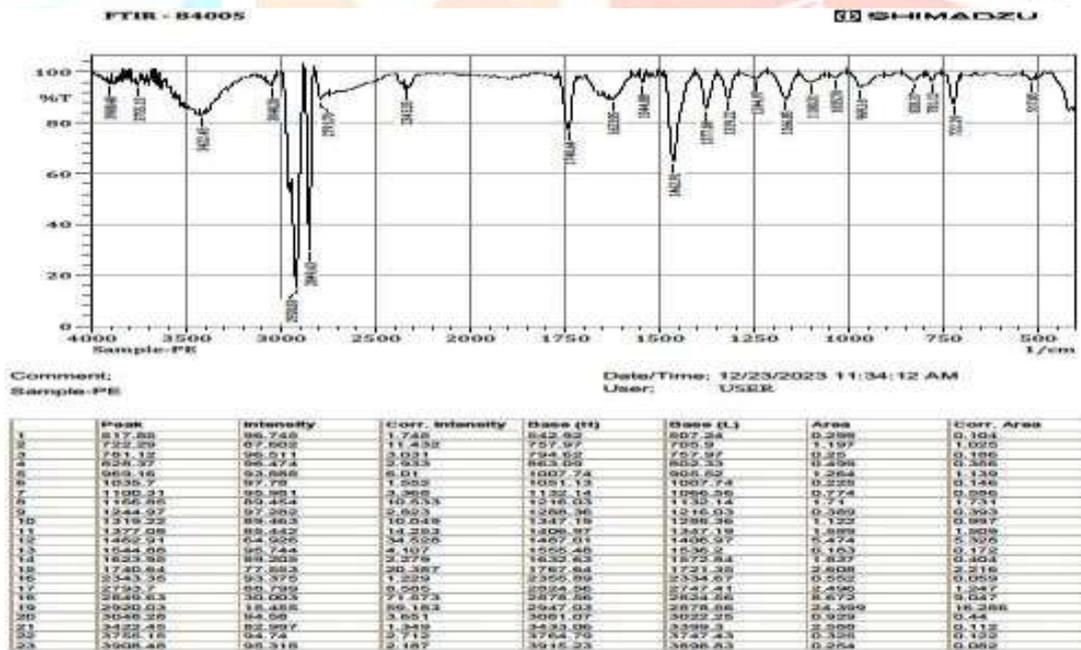
S.no	Parameters	Extractive values
1)	Alcohol soluble	12.73% W/W
2)	Water soluble	11.21% W/W

**Table 4. Ash value**

S.no	Types of values	Determined values
1)	Total ash value	10.81% W/W
2)	Water soluble ash value	5.43% W/W
3)	Acid insoluble ash value	6.21% W/W
4)	Loss on drying	8.5% W/W

### 4.4. Analytical evaluation

#### 4.4.1 FTIR analysis of for Pet. ether Extract



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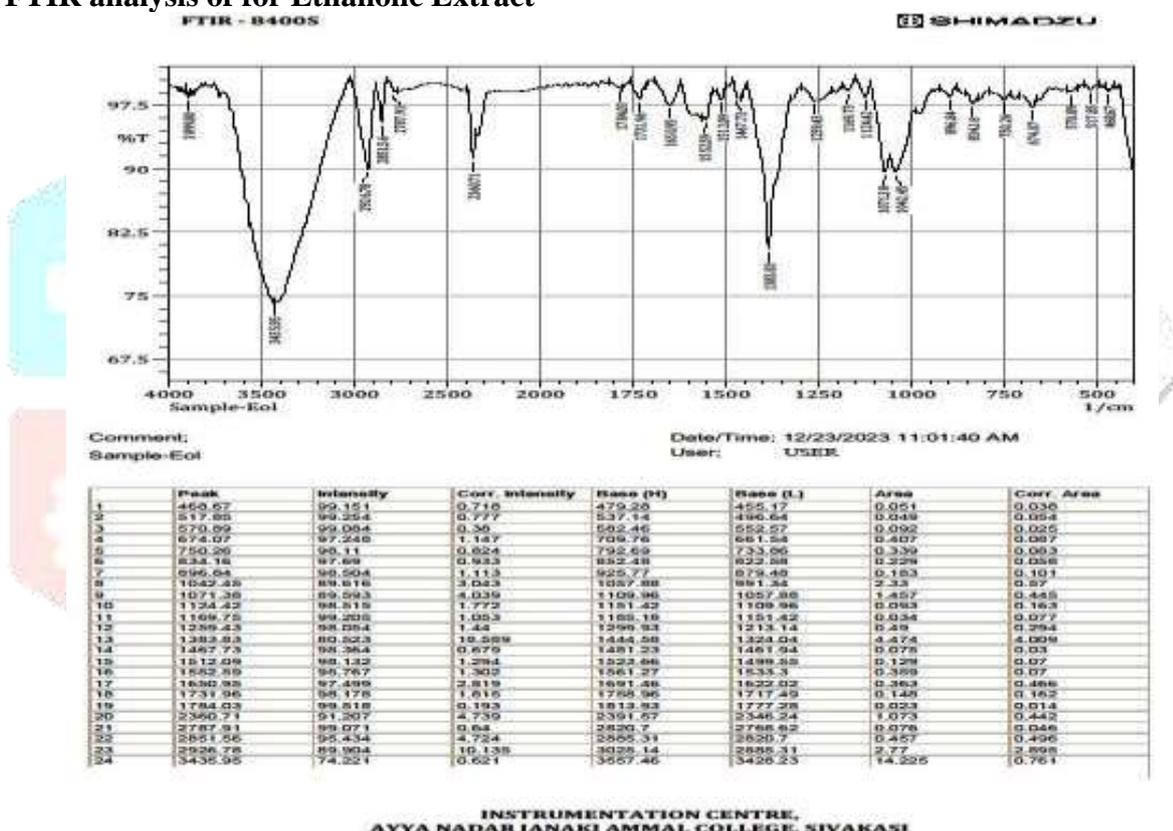
**Observation**

The given IR spectra of pet ether extract of *Mollugo oppositifolia* shows the presence of following functional group

**Table 5. Functional group in pet ether extract**

S.no	Functional group	Peak area
1	Hydroxyl group (OH)	3422 cm <sup>-1</sup>
2	Aliphatic group (C-H)	2920 cm <sup>-1</sup>
3	Ketone group (C=O)	1740 cm <sup>-1</sup>
4	Unsaturated group (C=C)	1623 cm <sup>-1</sup>
5	CH <sub>2</sub> type	1462 cm <sup>-1</sup>
6	C-H bond of Aromatic	722 cm <sup>-1</sup>

**4.4.2. FTIR analysis of for Ethanolic Extract**



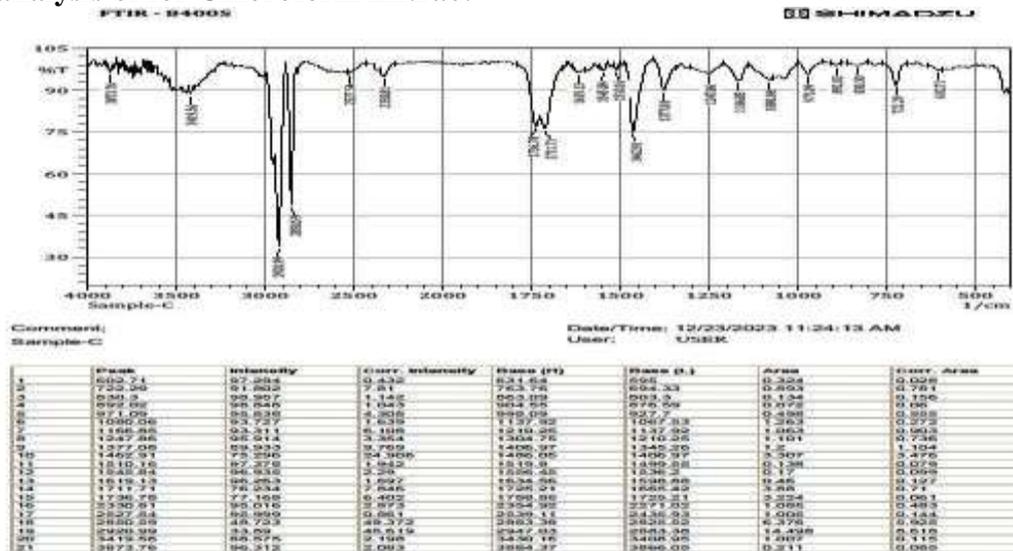
**Observation**

The given IR spectra of ethanolic extract of *Mollugo oppositifolia* shows the presence of following functional group

**Table 6. Functional group in Ethanolic extract**

S.NO	Functional group	Peak area
1	Hydroxyl group (OH)	3435 cm <sup>-1</sup>
2	Aliphatic group (C-H)	2926 cm <sup>-1</sup>
3	Ketone group (C=O)	1731 cm <sup>-1</sup>
4	Unsaturated group (C=C)	1650 cm <sup>-1</sup>
5	Carbohydrate (C-O)	1071 cm <sup>-1</sup>

#### 4.4.3. FTIR analysis of for Chloroform Extract



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#### Observation

The given IR spectra of chloroform extract of *Mollugo oppositifolia* shows the presence of following functional group

Table 7. Functional group in Chloroform extract

S.NO	Functional group	Peak area
1	Hydroxyl group (OH)	3419 $\text{cm}^{-1}$
2	Aliphatic group (C-H)	2920 $\text{cm}^{-1}$
3	Ketone group (C=O)	1736 $\text{cm}^{-1}$
4	Unsaturated group (C=C)	1619 $\text{cm}^{-1}$
5	CH <sub>2</sub> type	1462 $\text{cm}^{-1}$
6	C-H bond of Aromatic	722 $\text{cm}^{-1}$

#### 4.5. Anti-bacterial activity

The various extracts of *Mollugo oppositifolia* Linn. Showed the results of antimicrobial activity against organism like *Staphylococcus Aureus* and *Proteus Vulgaris*.

Table 8. Antibacterial effect of standard against organism

Name of the organism	Standard substance	Concentration	Zone of inhibition(mm)
<i>Staphylococcus Aureus</i>	Ciprofloxacin	20mg/ml	34mm
<i>Proteus Vulgaris</i>		20mg/ml	29mm

Table 9. Antibacterial effect in extract of Petroleum ether

Name of the organism	Test substance	Concentration	Zone of inhibition(mm)
<i>Staphylococcus Aureus</i>	Pet.ether extract	20mg/ml	20mm
<i>Proteus vulgaris</i>		20mg/ml	17mm

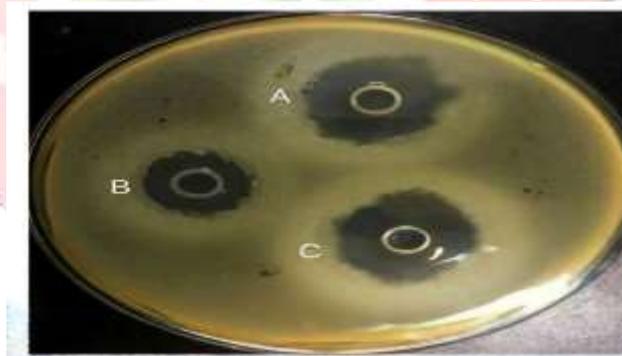
**Table 10. Antibacterial effect in extract of Ethanol**

Name of the organism	Test substance	Concentration	Zone of inhibition(mm)
Staphylococcus Aureus	Ethanolic extract	20mg/ml	25mm
Proteus vulgaris		20mg/ml	22mm

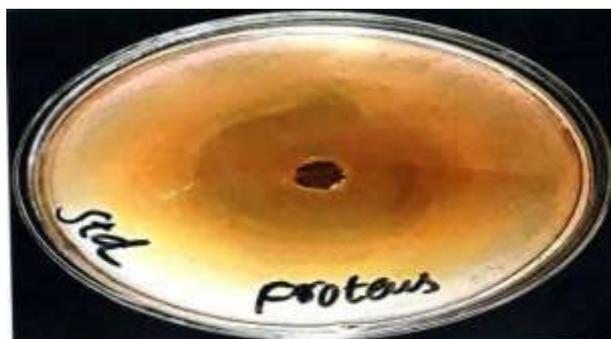
**Table 11. Antibacterial effect in extract of Chloroform**

Name of the organism	Test substance	Concentration	Zone of inhibition(mm)
Staphylococcus Aureus	Chloroform extract	20mg/ml	12mm
Proteus vulgaris		20mg/ml	13mm

#### 4.5.1 Anti-bacterial effect (Zone of inhibition)

**Figure 1:** Standard drug: Ciprofloxacin

**Figure 2:** A. Ethanolic extract  
 B. Pet.ether extract  
 C. Chloroform extract  
 Sample dissolved in DMSO (2%)



**Figure 3:** Standard drug: Ciprofloxacin



**Figure 4:** 1. Chloroform extract

2. Ethanolic extract

3. Pet. ether extract

Sample dissolved in DMSO (2%)

## Figures

**Figure 1&2:** Section shows antibacterial effect (Zone of inhibition) of standard and test substance against *Staphylococcus aureus* (Gram Positive)

**Figure 3&4:** Section shows Antibacterial effect (Zone of inhibition) of Standard and test substance against *Proteus vulgaris* (Gram Negative)

## 5. Discussion

### 5.1. Proximate values

Proximate values for the whole plant of *Mollugo oppositifolia* Linn are as follows: Alcohol soluble extractive value (12.73), Water soluble extractive value (11.21), Loss on drying (8.5), Total ash (10.81), Acid insoluble ash (6.21), Water soluble ash (5.43). These values are criterion to put the guidelines of identity and purity of crude drug.

### 5.2. Antibacterial activity

The antibacterial activity of the chloroform, pet ether and ethanolic extract of whole plant of *Mollugo oppositifolia* Linn of the plant was depends on their Phyto chemical composition. The diameters of the inhibition zones were measured in millimetre. The preliminary phytochemical components of the plants were studied various bioactive compounds were reported. The demonstration of antimicrobial activity against both gram positive and gram-negative bacteria maybe indicative of the presence of broad-spectrum antibiotic compounds. All extract has shown excellent antibacterial activity against gram negative and gram-positive organism. When compared to Chloroform, pet ether and ethanolic extract, the ethanolic extract showed the high antibacterial activity on gram positive and gram-negative bacteria. Results of zone of inhibition were presented in table. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. However, actual antibacterial ingredients need to be extracted and identified also its tolerable levels in the human body as well as any toxic effect on human and animal tissues are investigated accordingly.

The preliminary phytochemical components of the plants were studied various bioactive compounds were reported. The demonstration of antimicrobial bacterial activity against both gram positive and gram-negative

bacteria may be indicative of the presence of broad-spectrum antibiotic compounds. All extract has shown excellent antibacterial activity against gram negative and gram-positive organism.

## 6. Conclusion

The present study provided results to justify the traditional claim of herbs for anti-microbial activity. The screening of antimicrobial activity performed with chloroform, pet ether and ethanol extract of whole plant of *Mollugo oppositifolia* Linn proved that the plant having antimicrobial compounds. The current work will provide new reference data for the drug development and possesses the ability to inhibit pathogenic bacteria.

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