Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 4 Issue 11 November 2020

Research Article

Evaluation of Anti-Bacterial Activity of *Mansoa alliaceae* Leaves

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Received: September 22, 2020

Published: October 08, 2020

Abstract

The *Mansoa alliaceae* leaves contain many medical applications. The plant leaves contain anti-inflammatory activity, anti-oxidant activity, anti-arthritic activity, anti-rheumatic activity, anti-septic activity, anti-fungal activity and anti-bacterial activity. The present study was aimed to determine the anti-bacterial activity of ethanolic extract of *Mansoa alliaceae* leaves.

Keywords: Anti-Bacterial Activity; Mansoa alliaceae; Anti-Oxidant Activity

Introduction

According to world health organization, many of people use medicinal plants to treat their diseases. In olden days *Mansoa alliaceae* leaves used to treat many of the diseases in European countries. *Mansoa alliaceae* plant contains many medical uses like bacterial activity, fungicidal activity, arthritic activity, inflammatory activities. In the phytochemical studies the leaves contains flavonoids, alkaloids, glycosides, carbohydrates etc.

Materials and Methods

Materials

Collection, authentication and treatment of plant material

The leaves of plant *Mansoa alliaceae* belonging to the family *bignoniaceae* to were collected from surroundings of Tirupathi, Andhra Pradesh, India in the month of June. The plant material was authenticated.

Extraction

1. The leaves of *Mansoa alliaceae* were shade dried for 7 days. The leaves were weighed about 100 gm and subjected for extraction with ethanol by soxhlet apparatus for 72 hrs.
2. Concentrate each extract by distillating off the solvent and then evaporating to dryness on the water-bath.
3. Weigh the extract obtained and calculate its percentage in terms of the air-dried weight of the plant material. Also note the consistency of the extract.

Figure 1: Extraction by soxhlet apparatus.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedure. The ethanolic extract of *Mansoa alliaceae* was tested for the presence of phytoconstituents viz. carbohydrates, proteins, alkaloids, flavonoids, saponins and glycosides.

Test for carbohydrates

- **Molisch test:** To 1 mg powder, two drops of alcoholic solution of alpha naphthol were added. The mixture was shaken and 1 ml of concentrate sulphuric acid was added slowly along the sides of the test-tube, the test tube was cooled in ice water and allowed to stand. A violet coloured ring at the junction indicates the presence of carbohydrates.
- **Benedicts test:** To 1 mg of powdered drug 0.5 ml of benedicts reagent was added. The mixture was heated on boiling water bath for 2 minutes. A red green or yellow colored precipitate indicates the presence of sugar.

Test for alkaloids

- **Dragendroff's test:** To the 2 ml of test solution add 2 ml of Dragendroff's reagent (Potassium bismuth iodide solution) Reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids

- **Shinoda test:** To the test solution add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet crimson red are occasionally green to blue color appears after few minutes.

Test for saponins

- **Froth formation test:** Place 2 ml solution of drug in water in a test tube, shake well, stable froth foam is formed.

Test for glycosides

Borntragers test: Boil the test material with 1ml of sulphuric acid in a test tube for 5 minutes and filter while hot. Cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red color is produced in the ammonical layer.

Test for terpenoids

Liebermann-Burchard test: 2 - 3 ml of extract, 2 - 3 drops of dry chloroform was added and several drops of acetic anhydride solution followed by 2 ml of glacial acetic acid were added. The above solution was made warmed and cooled. 2 - 3 drops of conc. H_2SO_4 was added along with the sides of the test tube.

Test for fats and oils

- **Spot test:** An extract drop was placed on filter paper and the stain was observed.
- **Saponification test:** To small quantity of each extract 0.5N alcoholic potassium hydroxide was added along with a drop of phenolphthalein and heated on water bath for 2 hours. Formation of soap takes place.

Test for gums and mucilage

- Diluted extract was added to the test tube containing alcohol with continuous stirring results in precipitation.
- Extract treated with Ruthenium red results, red color.

Test for resins

To 5 - 10 ml of extract acetic anhydride was added, heated followed by cooling. After cooling 0.5 ml of H_2SO_4 was added results in bright purplish red changes to violet color rapidly.

Evaluation of anti-bacterial activity

Bacterial strains

The antibacterial potency of leaves of *Mansoa alliaceae* was evaluated using four bacterial strains viz., two strains of Gram negative *Escherichia coli*, *Pseudomonas aeruginosa,* two strains of Gram positive *Staphylococcus aureus* and *Bacillus subtilis*.

Inoculums preparation

Each bacterial strain was sub cultured overnight at 35°C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water and diluted to attain viable cell count of 10^7 CFU/ml.

Determination of minimum inhibitory concentrations (MIC's) of the effective plant extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24h of incubation. The most effective plant extracts which exhibiting a strong antibacterial activity at 10 mg/ml was manipulated to determine their MIC using cup plate method and evaluate their efficiency in controlling bacterial strains. Different concentrations of the effective plant extract (1.25, 2.5, 5.0, 10.0, 12.5 and 15.0 mg/ml) were prepared separately by dissolving 50 mg in DMSO (Dimethyl sulfoxide), sterilized through Millipore filter and loaded in cups. Nutrient

Agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the strains. The plates were kept in the fridge at 5°C for 2h. then incubated at 35°C for 24h. The inhibition zones were measured by Vernier caliper and recorded against the concentrations of the effective plant extracts.

Antibacterial activity of Ethanolic leaves extract of *Mansoa alliaceae*

The Cup plate method is used to evaluate antimicrobial activity of the each plant extract. The plant extract residues (50 mg) were re-dissolved in DMSO (Dimethyl sulfoxide), sterilized through Millipore filter (0.22 µm) then loaded in cups prepared by sterile cork borer to obtain final concentration of 10 mg/disc. Ten ml of agar medium was poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 10^5 CFU/ml of medium. The cups were loaded with plant extract concentration of 10, 20, 40 and 60 mg/ml were placed on the top of Nutrient Agar plates. The cups loaded with 5 µg of Amoxycillin was used as positive control. The plates were kept in the fridge at 5°C for 2h to permit plant extracts diffusion then incubated at 35°C for 24h. The presence of inhibition zones were measured by Vernier calliper, recorded and considered as indication for antibacterial activity.

Results

Extraction: The leaves of *Mansoa alliaceae* were subjected for drying for a period of 7 days. After drying the powder is made extracted with the solvent ethanol and the extract obtained is subjected for the preliminary phytochemical screening.

Preliminary phytochemical screening

Preliminary phytochemical analysis revealed the presence of phytoconstituents carbohydrates, proteins, alkaloids, flavonoids, saponins and glycosides (Table 1).

S. No.	Test	Ethanolic leaf extract of *Mansoa alliaceae*
1	Carbohydrates	+
2	Protein	+
3	Alkaloids	+
4	Flavonoids	+
5	Saponins	-
6	Glycosides	+

Table 1: Preliminary phytochemical screening for *Mansoa alliaceae*.

+: ≡ Presence, –: ≡Absence.

Evaluation of antibacterial activity

S. No.	Extract	Strain	Zone of inhibition (mm)							
			Concentration (µg/ml)							
			Standard (5 µg/ml)	Control	1	10	20	30	40	50
1.	Ethanolic leaf extract of *Mansoa alliaceae*	*E. coli*	18 ± 0.1 mm	0	No Zone	5.0 ± 0.2 mm	8.0 ± 0.1 mm	12.0 ± 0.2 mm	15.0 ± 0.1 mm	17.0 ± 0.2 mm
		P. aeruginosa	19 ± 0.2 mm	0	No Zone	4.0 ± 0.1 mm	6.0 ± 0.2 mm	8.0 ± 0.1 mm	9.0 ± 0.2 mm	12.0 ± 0.1 mm
		S. aureus	17 ± 0.1 mm	0	No Zone	5.0 ± 0.2 mm	9.0 ± 0.1 mm	13.0 ± 0.2 mm	14.0 ± 0.1 mm	15.0 ± 0.2 mm
		B. subtilis	16 ± 0.2 mm	0	No Zone	6.0 ± 0.1 mm	8.0 ± 0.2 mm	11.0 ± 0.1 mm	13.0 ± 0.2 mm	14.0 ± 0.1 mm

Table 2: Zone of inhibition of ethanolic extract of *Mansoa alliaceae* against selected both gram negative and gram positive bacteria.

Zone of inhibition of different extracts

Ethanolic leaf extract of *Mansoa alliaceae* on the strain *E. coli*

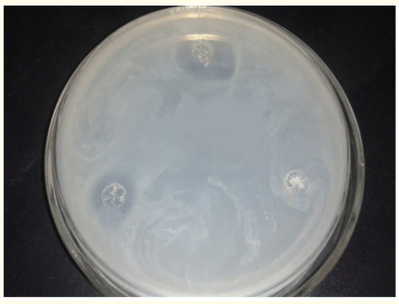


Figure 2

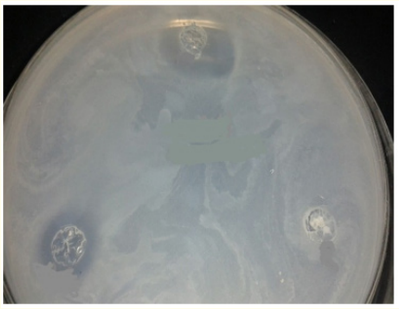
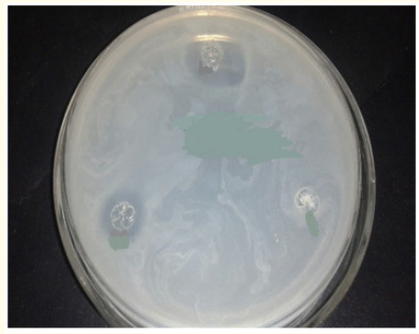


Figure 3



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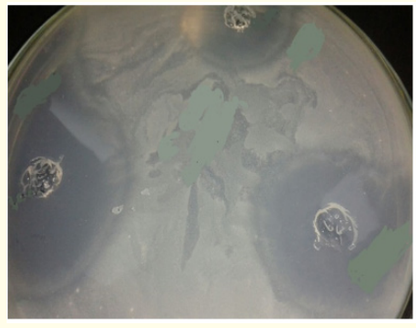

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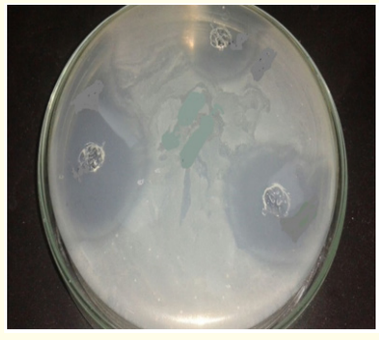

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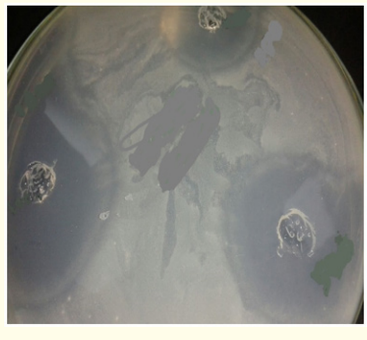
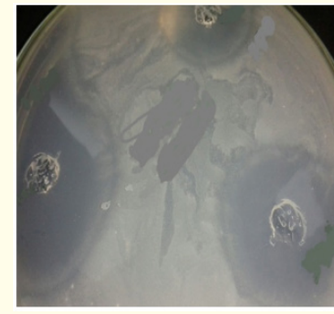


Figure 7

Zone of inhibition of different extracts

Ethanolic leaf extract of *Mansoa alliaceae* on the strain *P. aeruginosa*



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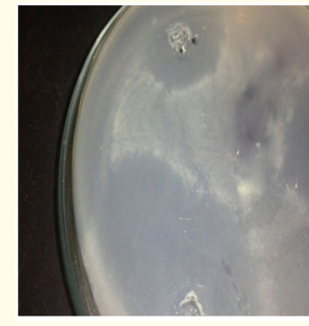


Figure 9

Figure 10

Figure 11

Figure 12

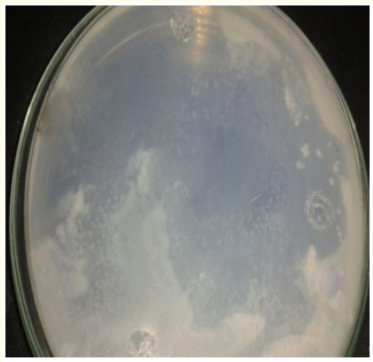


Figure 13

Zone of inhibition of different extracts

Ethanolic leaf extract of *Mansoa alliaceae* on the strain *S. aureus*

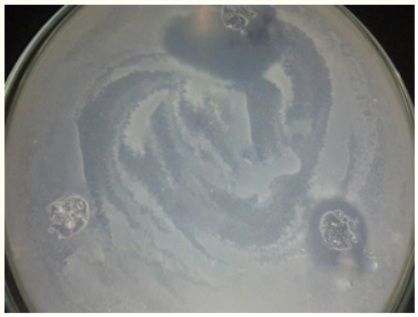


Figure 14

Figure 15

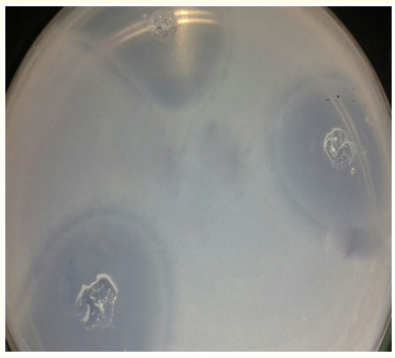
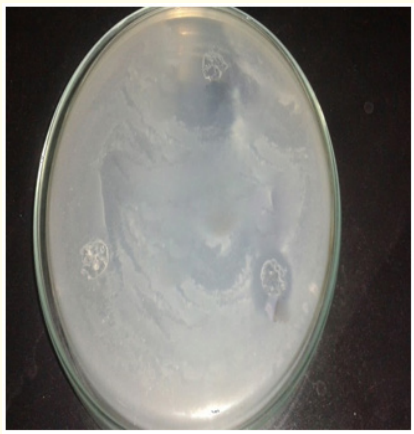


Figure 16



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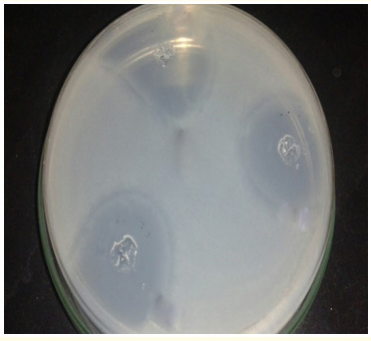

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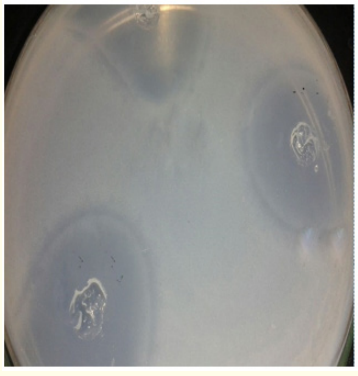
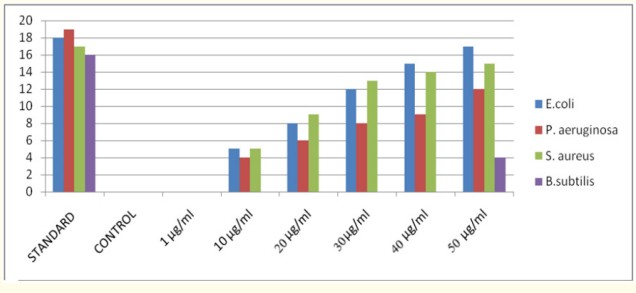


Figure 19



Graph 1: Zone of inhibition of ethanolic extract of *Mansoa alliaceae.*

Extract	Name of the Organism	Results Obtained
Ethanolic Extract of *Decaschistia crotonifolia*	*Escherichia coli*	+ve
	Staphylococcus aureus	+ve
	Pseudomonas aeruginosa	+ve
	Bacillus subtilis	+ve

Table 3: Antibacterial activity of leaf extract of *Mansoa alliaceae* against gram negative and gram positive bacteria.

Discussion

The antimicrobial study of ethanolic leaf extract of *Mansoa alliaceae* revealed that the ethanolic extract shown the antimicrobial activity against *E. coli, S. aureus* and *P. aeruginosa* and *B. subtilis* in concentrations of 10, 20, 30, 40 and 50 µg/ml but it shown very slight antimicrobial activity against *B. subtilis* in the concentration of 50 µg/ml [1-15].

Conclusion

Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects. This situation force scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of side effects that are often associated with synthetic antimicrobials.

The antibacterial study of ethanolic leaf extract of *Mansoa alliaceae* revealed that it shown the antibacterial activity against *E. coli, S. aureus, P. aeruginosa* and *B. subtilis*.

Acknowledgement

Authors are sincerely thankful to V.V institute of pharmaceutical sciences, Gudlavaalleru for providing necessary facilities for carry out research work.

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