ANTI BACTERIAL ACTIVITY OF DIFFERENT LEAF EXTRACTS OF *Chenopodium giganteum* USING TWO GRAM-POSITIVE AND TWO GRAM-NEGATIVE BACTERIA

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ABSTRACT

In this present research the Histological examination, physiological evaluation, phytochemical screening of leaf extracts of *Chenopodium giganteum* has been studied. Ethanol, aqueous, chloroform, and pet ether extracts of *Chenopodium giganteum* was prepared. Phytochemical screening shows presence of carbohydrates, proteins, amino acids, glycosides like saponin and flavonoids, tannins, phenols, alkaloids, and steroids. The physiological evaluation shows ash value %, acid insoluble ash % and water insoluble ash %, loss of drying was found to be 8.1%, extractive value of pet ether extract was found to be 2.3%, chloroform extract 4.5%, ethanol extract 7.2% and water 10.2%. In this study the antibacterial activities of four different extracts of leaves of *Chenopodium giganteum* against two gram-positive and gram-negative micro-organisms was carried out. Ethanol, aqueous, chloroform, and pet ether extracts of *Chenopodium giganteum* leaves was prepared. The antibacterial activity of different extracts was assessed and observed for their antibacterial activity against bacteria namely *Escherichia coli. Pseudomonas aeruginosa, Staphylococcus aureus* and *Lacto bacillus* using well plate method and were examined for the size of zone of inhibition and minimum inhibition method and were calculated for maximum and minimum concentration.

Keywords: Histological examination, Physiological evaluation, Phytochemical screening, Antibacterial, *Chenopodium giganteum.*

INTRODUCTION

The idea that some plants had healing properties or even included what we would now refer to as antimicrobial principles was widely believed long before humans learned about the presence of bacteria. Since ancient times, people have utilized plants to treat common infectious diseases, and some of these folk remedies are now used routinely to treat a variety of ailments. Plant oils and extracts have long been known to have antibacterial properties. However, few studies have directly comparative comparisons of a significant variety of oils and extracts. Numerous uses, such as the preservation of unprocessed and processed foods, pharmaceuticals, complementary medicine, and natural therapies, are based on the antibacterial activity of plant oils and extracts. For the investigation of the antimicrobial activity of plant extracts, essential oils, and the chemicals extracted from them, some broad principles must be established. The defining of common parameters, such as plant material, methodologies used, growth media, and microorganisms tested, is of vital importance. The selection of the plant material should be based on scientific standards.[1,2]

Standard in vitro minimum inhibitory and minimum bactericidal concentration results by themselves are insufficient for assessing antibacterial activity. The progression of antimicrobial activity is more specifically described by the rate of bacterial death, effect of increasing concentration, sub-MIC effects, and degree of suppression of bacterial growth after short exposure (post-antibiotic effect). Antibiotics such as aminoglycosides have a sustained post-antibiotic impact and concentration-dependent bactericidal action. Except for staphylococci, -lactam antibiotics

show higher time-dependent killing and no post-antibiotic effects. The majority of bacteriostatic antimicrobial drugs also result in post-antibiotic growth inhibition.Studies on several animal infection models reveal that -lactams are more effective with continuous dosage, whereas the efficiency of aminoglycosides is mostly independent of the dosing method, even when given once daily. Predicting the best dosing regimens requires knowledge of the kinetics of antibacterial action.[3]

MATERIALS AND METHODS

Collection, identification, and authentication of plant material

The plant Chenopodium giganteum leaf was collected from the surrounding areas of Meghalaya. It is dried under shade and made into coarse powder. The plant material collected was identified and authenticated by Scientist (Dr) S. Mutheeswaran, M.Sc, M.Phil, PhD, Xavier Research foundation, St Xavier's college, Tamil Nadu, India

Morphological characters

The fresh leaves of Chenopodium giganteum were examined for various macroscopical features like colour,odour and taste of leaves. other external morphological characters like venation, surface, base, margin, size and shape of leaves were also studied. The air-dried plant material was then, pulverized into a coarse powder and used for research work.[4]

Physicochemical constants

The physical constants like ash and extractive values help in establishing the pharmacopoeial standards of the drug. Physico-chemical constants of C. giganteum leaf parts were determined for loss of drying, Ash value and Extractive value, as per the method described in Pharmacopoeias and reported previously.[5,6]

Preparation of extracts

Successive Solvent extraction: Leaf parts of Chenopodium giganteum was dried and milled to a coarse powder. One kg of fresh plant material was grounded and defatted using petroleum ether. About 50g of the air dried powdered defatted plant material is then extracted subsequently with chloroform, pet ether, ethanol and water in a Soxhlet apparatus. Each time before extracting with the next solvent, the marc was air dried below 50°C. The extracts were filtered, the solvent was evaporated at room temperature and accurate weight of the extracts was taken. The extractive value (%) was calculated with reference to air dried drug.

Preliminary Phytochemical screening

The phytochemical constituents have played a major role as the basic source for the establishment of several pharmaceutical industries. Phytochemical screening carried out by the methods referred from [7,8]

Antibacterial activity of Chenopodium giganteum

Preparation of Luria Bertani Broth medium

LB broth is a commonly used nutritionally rich media for culturing bacteria. For 1 liter of preparation, suspend 10 gm tryptone, 5 gm yeast extract and 10 gm sodium chloride in 800 ml of distilled water in a Duran bottle. Shake the bottle to dissolve all the reagents. Then further add distilled water in a measuring cylinder to ensure accuracy to make a total of 1 liter. Autoclave it for 20 mins at 121 °C. After cooling swirl, the flask to ensure mixing and the LB is ready for use.

Preparation of LB agar plates

For 1 liter of preparation, suspend 10 gm tryptone, 5 gm yeast extract, 10 gm sodium chloride and 15 gm agar in 800 ml of distilled water in a Pyrex bottle. Shake the bottle to dissolve all the reagents. Then further add distilled water in a measuring cylinder to ensure accuracy to make a total of 1 liter. Autoclave it for 20 min at 121 ° C. Leave the solution to cool to approximately about 55 °C or warm it enough to be held in hand without burning. Prepare the petri dishes in a sterile environment and then carefully pour a thin layer of the solution to cover the bottom of the plate. Try to avoid any bubbles on the plates. Leave the plates to set and then it is ready for use.56

Preliminary test for antibacterial activity

The antimicrobial activity of pet ether, chloroform, ethanol, and water extracts of *Chenopodium giganteum* were evaluated against two strains of gram negative (Escherichia coli, Pseudomonas aeruginosa) and two strains of gram-

positive microorganisms (Staphylococcus aureus and Lacto bacillus) by cup diffusion method. Approximately 106 colony-forming units (CFU) of the microorganisms were inoculated on Luria Broth (LB) agar plate, and then different concentrations of AgNPs and AuNPs (50, 25, 10, 5, 1, 0.5, 0.25, 0 µg/ml) were loaded in the wells present in the LB agar plate. All the LB plates were incubated in the incubator at 37 °C overnight. After incubation period, the plates were observed for the zone of inhibition.

Evaluation of antibacterial effectiveness using Minimum Inhibitory Concentration method

The antimicrobial effectiveness of Pet ether, chloroform, ethanol and water extracts of Chenopodium giganteum were evaluated by Minimum Inhibitory Concentration (MIC) which was determined by broth dilution method against four bacteria including two strains of gram negative (Escherichia coli, Pseudomonas aeruginosa) and two strains of gram positive (Staphylococcus aureus and Lacto bacillus). In 5 ml LB broth, 100 µL of AgNPs and AuNPs from different concentrations (50, 25, 10, 5, 1, 0.5, 0.25, 0 µg/ml) and 100 µL of each test microorganisms were added. The cultures were then incubated at 37 °C for 24 hours with shaking. AgNPs and AuNPs with inoculum in Luria broth media were used as positive control. The results were then monitored by measuring the mean optical density OD at 600 nm.[9]

RESULTS AND DISCUSSION

Morphology of Chenopodium giganteum leaves

The morphological data showed the colour of young leaves was found to be pink, magenta colour and older leaves are green with smooth undersurface. It has odour and bitter characteristic taste. The shape of leaves was extremely variable as simple, deltoid, ovate to lanceolate, upper entire, rhomboid, lower toothed or irregularly lobed. The size of leaf is about 1-3 cm; petioles were of 1-2 cm in length and as long as thick blade. Its length varies from 9 to 4.5 cm broad having dentate margin. It has acute apex and upto 5 cm base.

whole plant

leaves

seeds

Fig 1: Morphological characters of leaves of Chenopodium giganteum

Determination of Physicochemical constants

The total ash, acid-insoluble ash and water-soluble ash value and loss of drying of leaves of *Chenopodium giganteum* was evaluated and the results are mentioned in table 1.

Table 1: Physicochemical constants and leaf constants of leaves of *Chenopodium giganteum*

Loss of drying	Total ash	Acid insoluble ash	Water soluble ash
8.1%	15.12%	7.46%	9.26%

Extraction of plant material

The appearance and yield of different extracts of *Chenopodium giganteum* were evaluated and the results are mentioned in table 2.

Table 2: Yield of extracts obtained from successive extraction of leaves of *Chenopodium giganteum.*

Plant name	**Type of Extract**	**Appearance/ State**	**Yield (% w/w)**
Chenopodium giganteum leaves	Pet ether	Yellowish green / Semisolid	2.3%
	Chloroform	Greenish black/ Semisolid	4.5%
	Ethanol	Dark green, black/ Semisolid	7.1%
	Water	Dark Brown black/ Semisolid	10.2%

Preliminary phytochemical screening of extracts

Petroleum ether and chloroform extract revealed the presence of steroids whereas ethanol and aquous extracts indicated the presence of flavonoids, carbohydrates, saponins, proteins, alkaloids phenols, steroids, and tannins respectively (Table 3).

Table 3: Preliminary phytochemical screening of leaf extracts of *Chenopodium giganteum*

Chemical tests	***Chenopodium giganteum* leaf extracts**			
	Pet ether	Chloroform	Ethanol	Water
Proteins & Amino acid	-	-	+	+
Carbohydrates	-	-	+	+

Steroids	+	+	-	-
Phenols	-	-	+	+
Saponins	-	-	+	+
Flavonoids	-	-	+	+
Alkaloids	-	-	+	+
Tannins	-	-	+	+

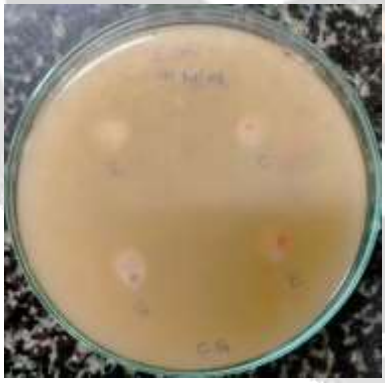
Antimicrobial activity

Preliminary test for antibacterial activity

The antibacterial activity of different concentrations of extracts was proved from the zone of inhibition against gram-negative (Escherichia coli, Pseudomonas aeruginosa) and gram-positive microorganisms (Staphylococcus aureus and Lacto bacillus). Extracts showed a clear zone of inhibition in all the plates.

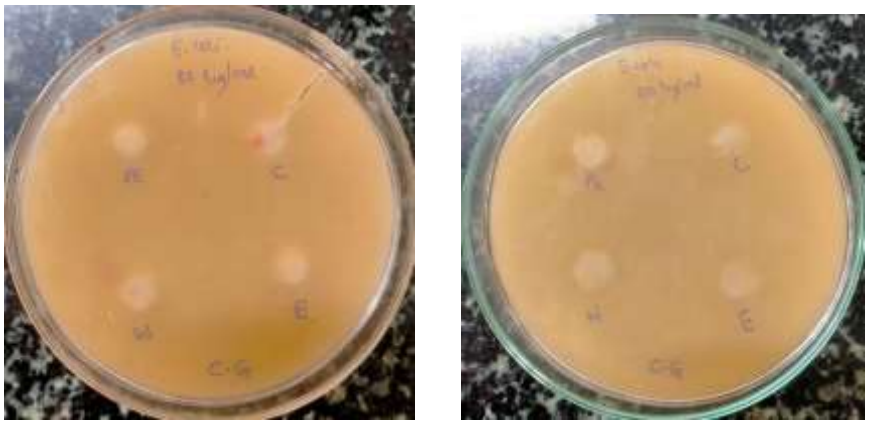
10µg/ml

20µg/ml



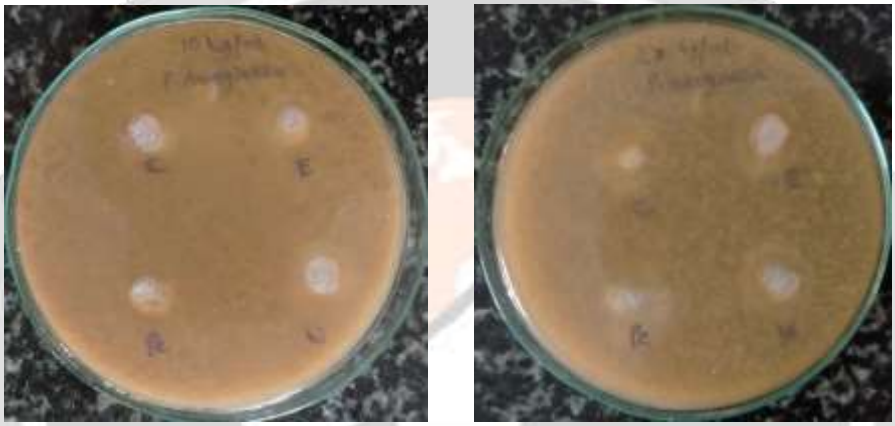
40µg/ml

60µg/ml

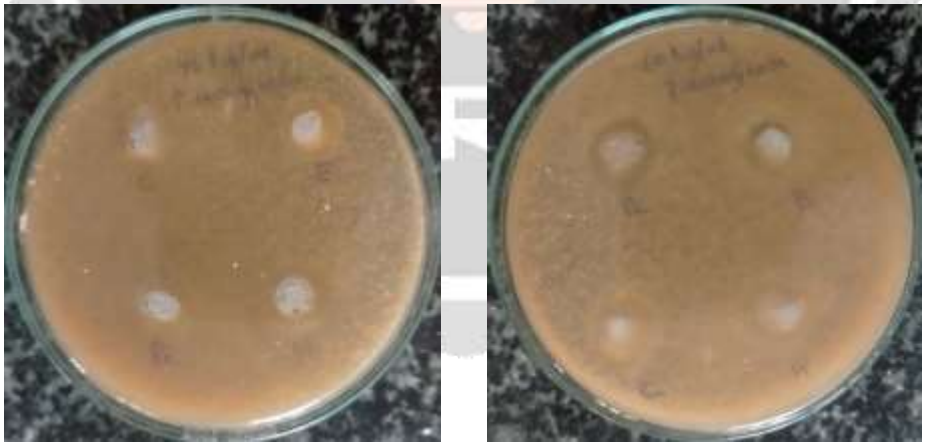


80µg/ml 100µg/ml

Fig 2: Zone of inhibition of different concentration of extracts against E. coli



10µg/ml 20µg/ml



40µg/ml 60µg/ml

80µg/ml

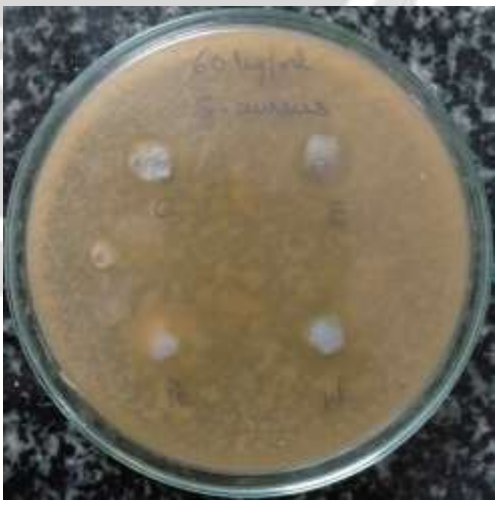
100µg/ml

Fig 3: Zone of inhibition of different concentration of extracts against p. aeruginosa

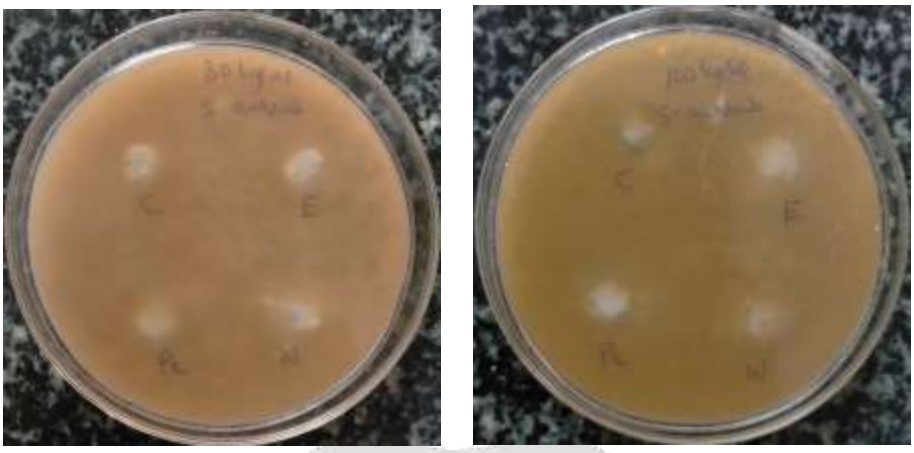
10µg/ml

20µg/ml

40µg/ml

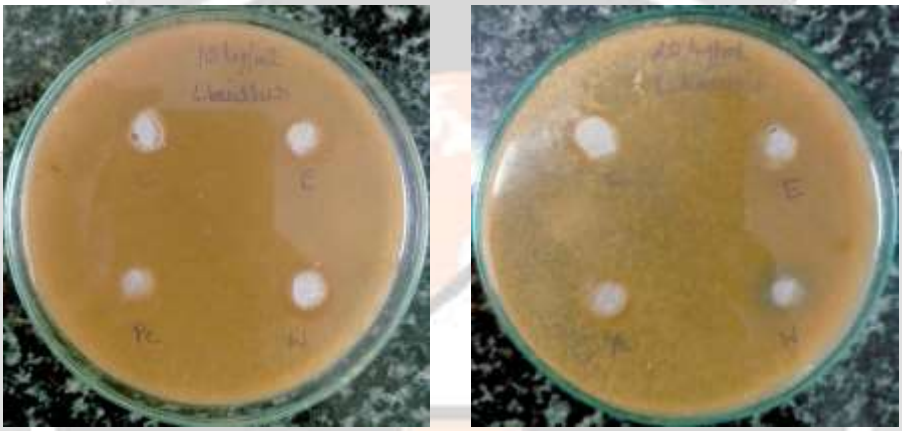


60µg/ml



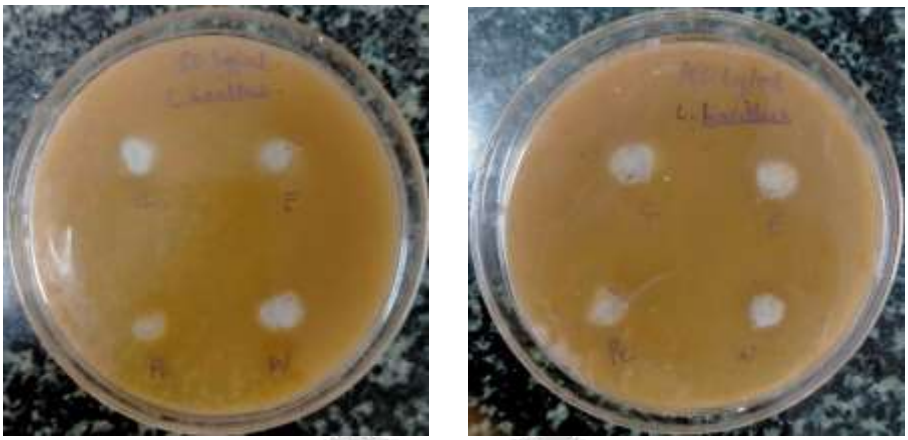
80µg/ml 100µg/ml

Fig 4: Zone of inhibition of different concentration of extracts against s. aureus



10µg/ml 20µg/ml

40µg/ml 60µg/ml

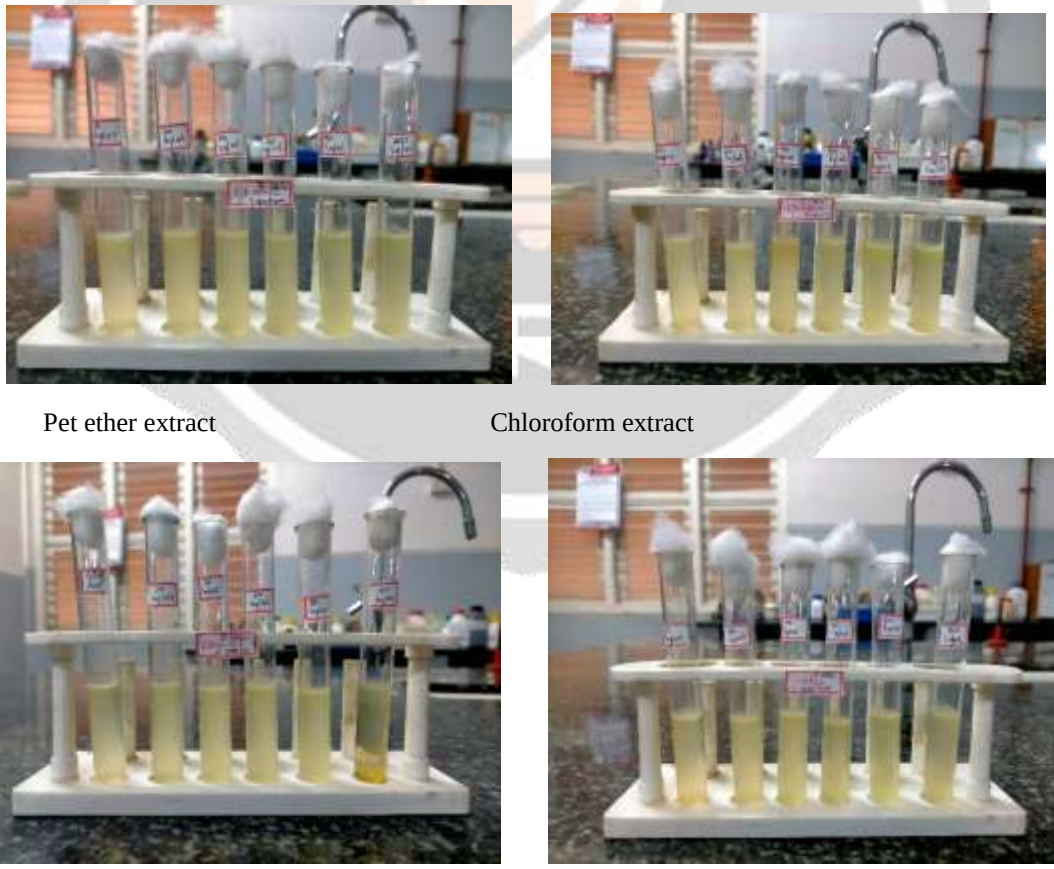


80µg/ml 100µg/ml

Fig 5: Zone of inhibition of different concentration of extracts against Lactobacilli

Evaluation of antibacterial effectiveness using Minimum Inhibitory Concentration method

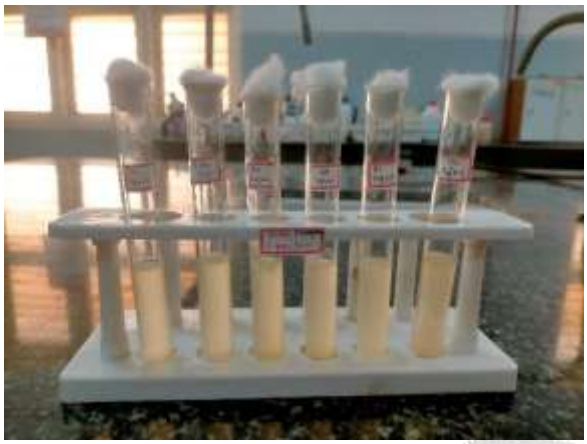
To study the antimicrobial effectiveness of extracts, a bacterial concentration of high CFU (106/ml) were treated with varying concentration of extracts from 10 to 100 µg/ml. When the concentration of extracts was increased, the bacterial concentration was found to decrease. At a concentration of 100 µg/ml of extracts, the growth of the microorganisms of gram-negative (Escherichia coli, Pseudomonas aeruginosa) and gram-positive microorganisms (Staphylococcus aureus and Lacto bacillus) was completely inhibited, which indicated that the minimum inhibitory concentration of extracts.



Pet ether extract Chloroform extract

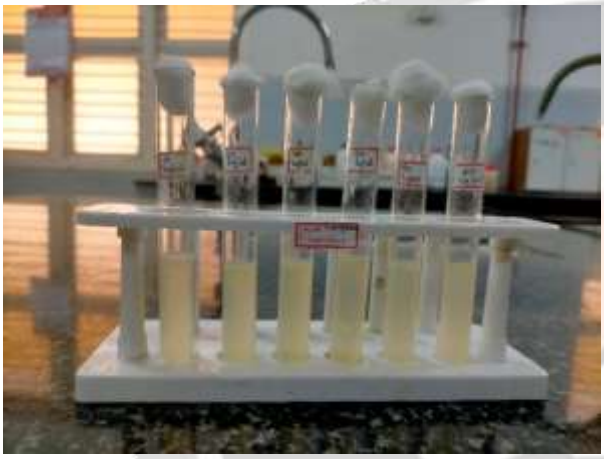
Ethanol extract Water extract

Fig 6: Minimum inhibitory concentration of E. coli on different concentration of extracts

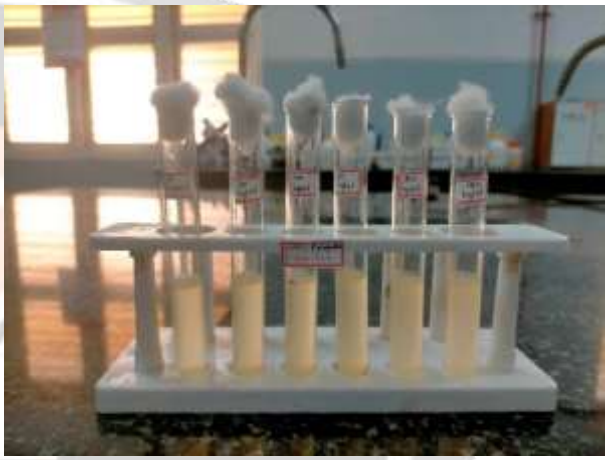


Pet ether

Chloroform



Ethanol

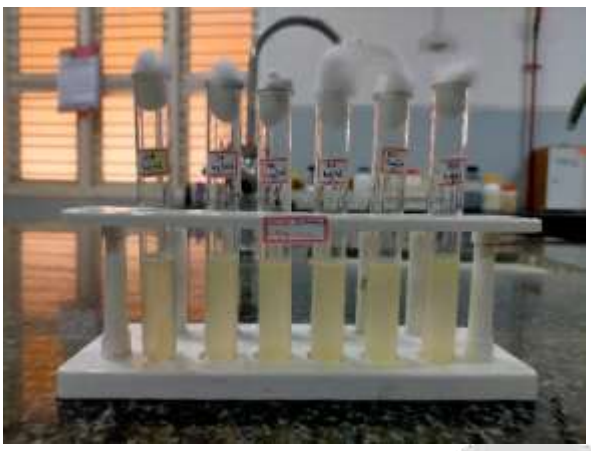


Chloroform

Fig 7: Minimum inhibitory concentration of P. aeruginosa on different concentration of extracts

Pet ether

Chloroform



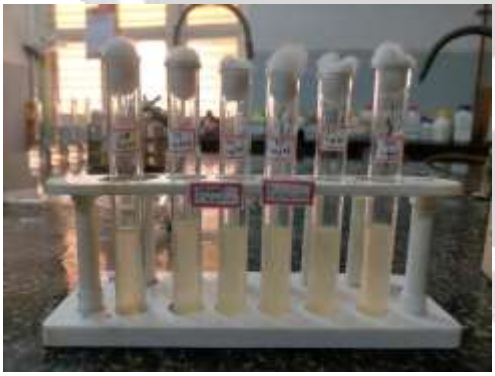
Ethanol

Water

Fig 8: Minimum inhibitory concentration of S. aureus on different concentration of extracts

Pet ether

Chloroform



Ethanol

Water

Fig 9: Minimum inhibitory concentration of L. bacillus on different concentration of extracts

CONCLUSION

The present work deals with the study of plants *Chenopodium giganteum* for Pharmacognostic characterization, determination of their physiochemical parameters and phytochemical screening of the crude extracts. The selected plants were authenticated, and the macroscopic studies were performed as the first step towards establishing their identity and purity. Physicochemical studies were carried out as per Ayurvedic and Indian Pharmacopoeia (I.P., 1996) such as ash value, acid insoluble ash values and extractive values. The important phytoconstituents were present as depicted in phytochemical screening which are well known for their medicinal potentials. Leaves extracts

of Chenopodium giganteum were then evaluated for their antimicrobial potential against Lactobacillus, E. coli, P aeruginosa and S. aureus using zone of inhibition method and minimum inhibitory concentration method. Water, ethanol, chloroform, and pet ether extracts of leaves of *Chenopodium giganteum* have been found to be quite effective against gram-positive and gram-negative bacteria namely Lactobacillus, E. coli, P aeruginosa and S. aureus. The higher the concentration of the extract the more the activity of the gram-positive and gram-negative bacteria is inhibited.

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