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"Antimicrobial Activity of *Cymbopogon citratus* against Pathogenic Bacteria"

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Abstract:
Lemongrass is an herb which belongs to the gramineae family. Scientifically it is called *Cymbopogon citratus*. The prefix Lemon owes to its typical lemon like odour, due to presence of citral a cyclic monoterpene lemongrass has phytoconstituents such as tannins, flavonoids, alkaloids etc. Secondary active metabolites of a number of components have also been implicated in the varied pharmalogical effects of this plant. The current Study aimed to extract cymbopogon citratus leaf using various solvents with view to determine the phytochemical constituents and antimicrobial activity against various bacteria *E. Coli, P. acne, p. aeruginosa, S. aureus, S. typhi.*

Keywords: Cymbopogon, solvents, antibacterial.

INTRODUCTION

Lemongrass is an aromatic medicinal grass belonging to the genus Cymbopogon. Lemongrass possesses various antimicrobial properties. Lemongrass is a native tall sedge / grass (Rangari vinod, 2009). Lemon grass belongs to the family Poaceae/Gramineae with diverse medicinal Value grown in many parts of tropical and Subtropical South east Asia. and Africa. It was grown in India a century back and now commercially cultivated in different parts of India.It is an oral antitumor drug for the cancer (Parekh and Chanda, 2007).

People now-a-days are more aware on health issue due to the emergence of new diseases. Treatment using plant based medicine appears to be an alternative approach due to the adverse effects associated with the use of Synthetic drugs (Mirghani *et al.,* 2012), The plant also contains 1-2 percent essential 60 oil on a dry basis with wide variation of chemical composition as a function of habitat, (Ademuyiwa and Grace, 2015). The extract of lemon grass leaves (dried) with cold, hot water and different solvents like ethanol, methanol were Screened for its antimicrobial activity against various pathogenic bacteria.

Lemongrass Soap helps to improve the skin by reducing acne and pimples. It helps tone the muscle and tissue. It is an effective deodorant. Lemongrass oil used by healthcare providers to treat digestive problems and high blood pressure. It is used as an insect repellant. lemongrass oil eliminates dandruff and purifies skin, (Amruta 2018).

MATERIAL AND METHODS

Collection of Sample:

The samples were taken from the patients visiting MSB pathological labs, Khan hospital, Khamgaon and Government Medical College, Akola.

The samples were collected -

- By Swabbing the surface of an infected wound by sterile swab.
- By collecting pus sample.
- By collecting urine sample.
- By collecting contaminated water sample.

- By Collecting blood samples from the site of injury.

Isolation and Identification:

The collected samples (blood, pus, urine, dental plaque) were inoculated on the nutrient media. The colonies that appeared on the plate were categorised. on the basis of characters and microscopy. These selected colonies were then inoculated on selective media.

Identification of isolated bacteria by Conventional Methods:

The bacteria isolated from clinical samples were subjected to cultural and morphological characterization. Other characteristics were determined by various biochemical tests such as Indole test, Methyl Red test, Voges Proskaur test, Citrate test , Sugar fermentation test and various enzyme test such as catalase, amylase, urease. The growth of bacterial isolates were also observed on Eosin Methylene Blue (EMB) Agar, Mannitol Salt Agar (MSA), Cetrimide Agar, Bismuth Sulphate Agar, Brain Heart Infusion (BHI) Agar.

Extract preparation:

Dried Samples were grinded and sieved.

a) Ethanol Extract:

powdered sample were extracted with of ethanol. Keep the mixture in air tight glass bottles. The powdered sample soaked in the solvent for 3 days. Shake the solvent one's in a day. After 3 days filtered the solvent through cotton cloth. Solvent is heated up to its boiling point in the boiling water bath. As vapours are condensed stored the solvent in amber bottles.

b) Methanol Extract:

Powdered sample were extracted with methanol. Keep the mixture in air tight glass bottles. The powdered sample soaked in the solvent for 3 days. Shake the solvent one's in a day. After 3 days filtered the solvent through cotton cloth. Solvent is heated up to its boiling point in the boiling water bath. As vapours are condensed Stored the solvent in amber bottles.

c) Cold water and Hot water Extract:

Repeat the same procedure as per given above.

Phytochemical Analysis:

The following methods were used by qualitative Phytochemical analysis of lemongrass extract. (Babatude *et. al.*, 2019).

1) Test for Carbohydrate-

1 ml of Molish's reagent was added to 2m1 of lemongrass extract after which a few drops of concentrated Sulphuric acid was added. A purple colouration depicts that carbohydrates. are present.

2) Test for Tannins-

2 ml of 5% ferric Chloride was added to 1ml of extract. A greenish black colouration depicts that tannins are present.

3) Test for saponins-

2 ml of distilled water was added to 2 ml of extract and shaken for 15 minutes. Foam formation indicates that saponins are present.

4) Test for Flavonoids-

5 ml of dilute NH3 Solution was added to 1 ml of extract prior to the addition of concentrated Sulphuric acid. A yellow colouration depicts that flavonoids are present.

5) Test for Alkaloids -
2 ml of concentrated HCl was added to a 2 ml of extract before a few drops of Mayer's reagent were added. A greenish colouration depicts that alkaloids are present.

6) Test for Terpenoids-
2 ml of chloroform and concentrated H2SO4 was added 0.5 ml of extract. A red brown colouration at the interface depicts that terpenoids are present.

7) Test for phenols -
2 ml of distilled water and few drops of 10% ferric chloride was added to 1 ml of extract. A green colouration depicts that phenols are present.

8) Test for Glycosides-
3 ml of chloroform and 10% NH3 Solution was added to a 2 ml of extract. A pink colouration depicts that glycosides are present.

9) Test for protein.-
Two drops of 3% copper Sulphate and few drops of 10% sodium hydroxide were added to 1 ml of extract. Violet or red colour formation indicates that protein are present.

10) Test for steroids-
1 ml of extract was dissolved in 10ml of the chloroform and equal volume of sulphuric acid was added by the side of the test tube. If the upper layer turns red and fluorescence that steroid are present.

11) Test for Coumarins-
8 ml of to 10% NaOH was added to a 2 ml of aqueous plant extract and yellow colouration depicts that coumarins are present.

Antimicrobial Activity of *cymbopogon citratus* Leaf Extract.

Antimicrobial activity using plant extract was evaluated by the Agar well diffusion method on Muller Hinton Agar or Nutrient Agar. Bacterial isolates were uniformly swabbed onto the individual plates using sterile cotton swabs, pour the plant extract solution into well which was made with the help of sterile cork-borer. After incubation at 37°c for 24 hours, the zone of inhibition was measured in millimeters.

NATURAL PRODUCT FORMATION: LEMONGRASS SOAP

All the ingredients which are used in the preparation of Lemongrass natural soap like rose water, glycerine, fresh lemongrass leaves, glycerine soap base, carrier oil, mixing spoon, moulds are purchased from the local market of Jatharpeth Akola.

Method of soap preparation:

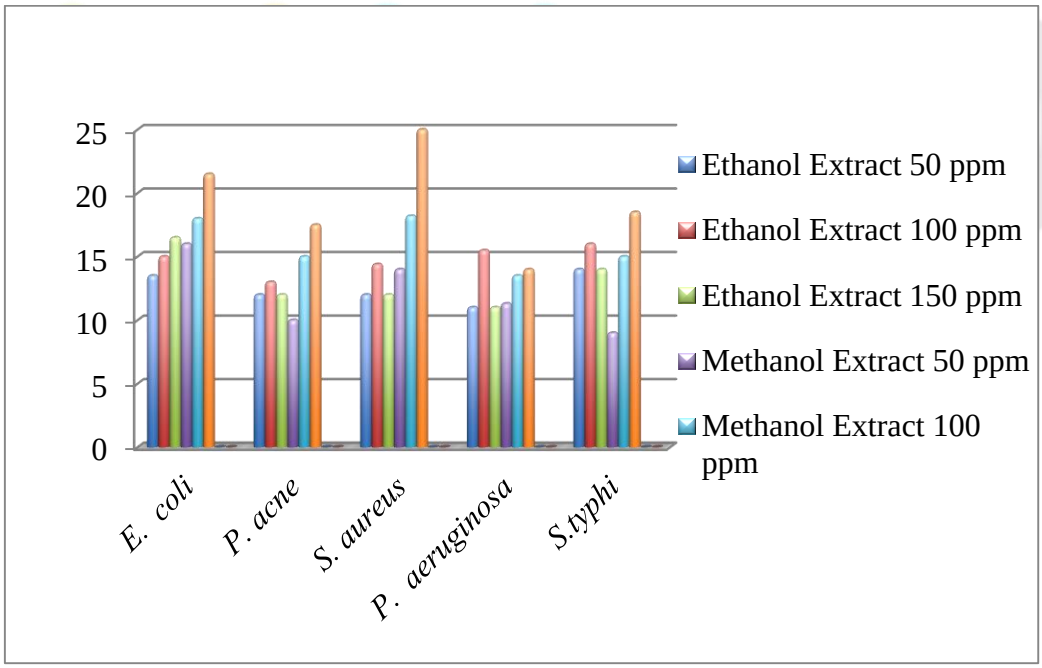
- Cut glycerine soap base into small pieces.
- Boil the base soap pieces and let it melt.
- Stir it for a few seconds.
- Add chopped fresh lemon grass leaves into the melted soap base.
- By gently Swirling, the right amount of rose water in mixture. , glycerine, carmer oil was added
- Cool it slightly for sometime and pour it in a mould.
- Allow the mixture to set and place the moulds in a freezer for at least 30 minutes.
- Remove the mould and soap is formed.

RESULT AND DISSCUSSION:

Antimicrobial Activity against different extract of Lemongrass

Sr.No	Isolates	Zone of inhibition in mm							
		Ethanol Extract			Methanol Extract			Cold Water Extract	Hot Water Extract
		50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm		
1	*E. coli*	13.5	15.0	16.5	16.0	18.0	21.5	R	R
2	*P. acne*	12.0	13.0	14.3	10.0	15.0	17.5	R	R
3	*S. aureus*	12.0	14.4	19.0	14.0	18.2	25.0	R	R
4	*P. aeruginosa*	11.0	15.5	16.0	11.3	13.5	14.0	R	R
5	*S. typhi*	14.0	16.0	18.0	9.0	15.0	18.5	R	R

Where R= Resistant



RESULT AND DISCUSSION

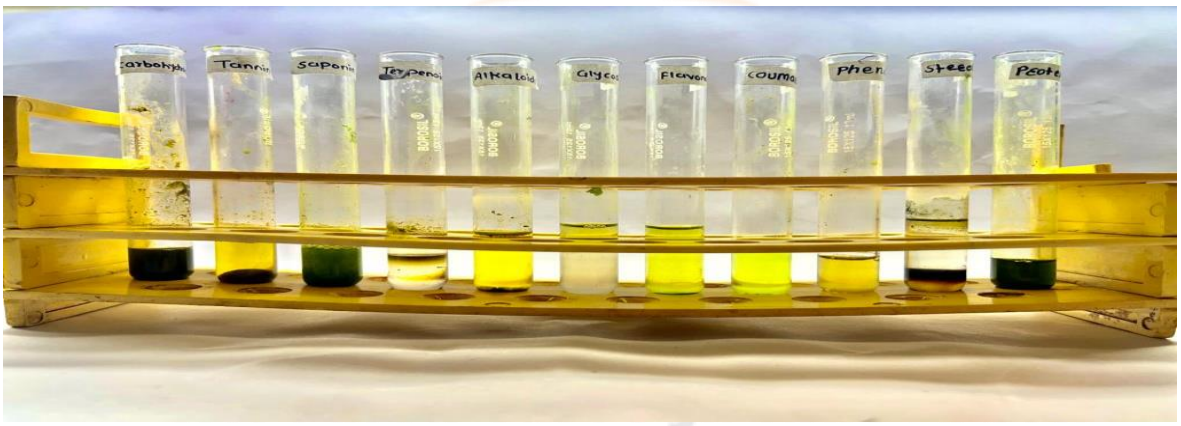
In ethanol, extracted various concentration were taken. Among all concentration it was found that, in case of *S. aureus* at 150 ppm Concentration maximum zone of inhibition was observed i.e. 19.0 mm which was followed by. 18.0 mm for *S. typhi* and 16.5 mm for *E. coli* while *P. aeruginosa* showed 16.0 mm and *P. acne* showed 14.0mm.

In methanol extract various concentrations were taken. Among all concentrations it was found that in case of *S. aureus* at 150 ppm concentration maximum zone of inhibition was observed i.e. 25.0mm which followed by 21.5 mm for *E. coli* and 18.5 mm for *S. typhi* . While *P. acne* showed 17.5 mm and *P. aeruginosa* showed 14.0mm.

For other concentrations of Ethanol and Methanol extract showed moderate to good results. There ware no zone of inhibition observed in cold water and hot water extract.

Phytochemical Analysis :

Sr. No.	Test	Lemon Grass Extract
1	Carbohydrate	Negative
2	Tannins	Positive
3	Saponins	Positive
4	Flavonoids	Positive
5	Alkaloids	Positive
6	Terpenoids	Positive
7	Phenols	Positive
8	Glycosides	Negative
9	Protein	Positive
10.	Steroids	Positive
11.	Coumarins	Positive



RESULT AND DISCUSSION

In present study the results are compatible with the Nwachukwu *et. al*., (2008) in Nigeria. It is found that the phytochemical studied of *Cymbopogon citratus* contained Tannins, Saponins, Alkaloids, Phenol, Steroid, Flavonoids, Proteins, Coumarins and Carbohydrate. The extract showed an inhibitory effect on some microorganisms. Asaolu *et al*., in 200g had also reported the similar result of ethanolic extract of *Cymbopogon citratus* collected from Nigeria contained alkaloids, phenols, tannins, flavonoids and Saponins.

Composition of Soap :

Ingredients	**Quantify**	**Uses**
Aloe vera gel	0.7 gm	Hydrating agent, anti wrinkles
Glycerine	0.1 ml	Humectants
Rose water	20 ml	Flavoring, cooling agent
Fragrance carrier oil	2 ml	Fragrance purpose

Result of Natural product Formation:

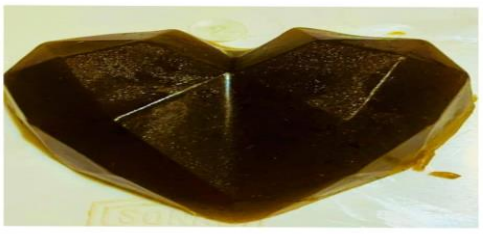
The prepared formulations underwent satisfactory results for colour, odor, consistency, pH, washability and grittiness

Physical examination of formulations.

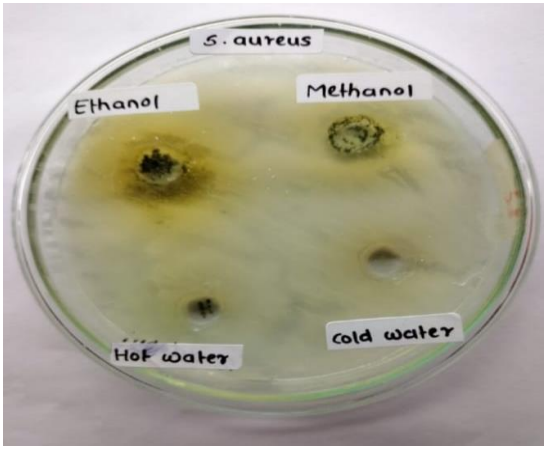
Sr.No.	Physical Parameters	Inference
1	Colour	Green/Dark green
2	Odour	Pleasant/Sweet
3	pH	8 to 10
4	Grittiness	No gritty particles
5	Washability	Good
6	Feel on Application	Smooth

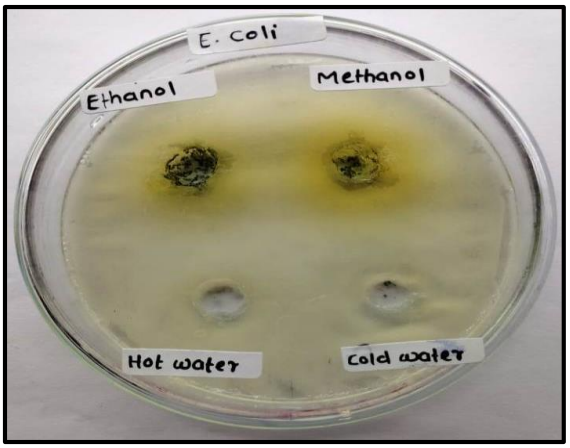
The natural lemongrass Soap was green or Dark green in colour with pleasant or Sweet odour. The pH was found to be 8 to 10. No gritty particle found. It was given good washability and it felt smooth when applying on skin.

Natural Product Lemongrass Soap

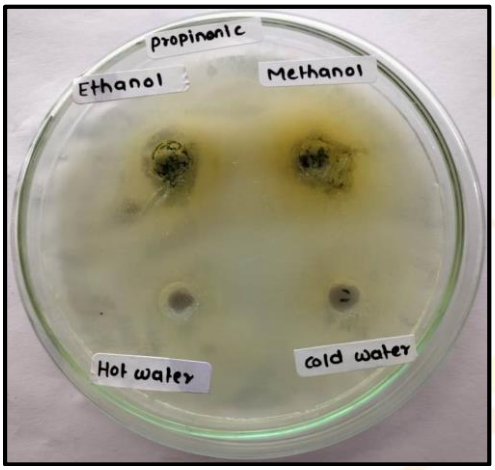


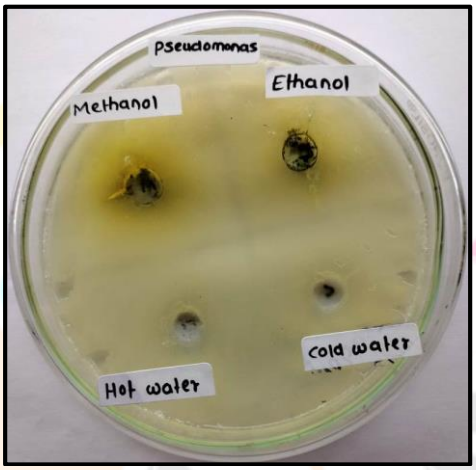
Antimicrobial Activity of Lemon Grass Extract in Different solvent against Different Isolates



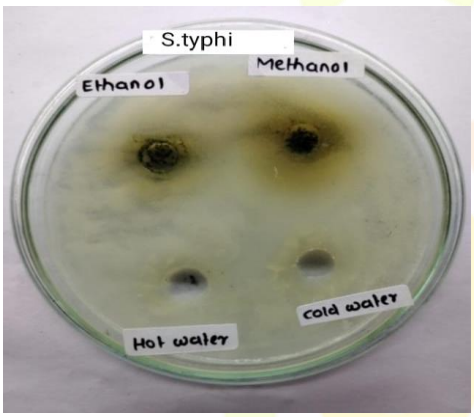


Antimicrobial activity of different solvent against *S. aureus* and *E.coli*





Antimicrobial activity of different solvent against P. acne and *P. aeruginosa.*



Antimicrobial activity of different solvent against *S. typhi.*

CONCLUSION

Cymbopogon citratus is a fragrant grass that contains a wide range of bioactive compounds with a wide range of therapeutic properties. The therapeutic value of lemongrass could be used in the future in herbal medicine. lemongrass leaf extracts is highly effective in controlling different types of pathogenic microorganism. Lemongrass has bioactive compounds such as alkaloids, flavonoids tannins etc. The most important advantages of lemongrass are as follows

- It can help to prevent teeth and rubber diseases such as Periodontitis.
- *C. citratus* has good activity in anti fungal, antimicrobial, anti-inflammatory, antioxidant.
- Treats respiratory disorders
- Promotes healthy skin
- Treats stomach disorders
- Natural remedy for digestive issues.
- Alleviates stress and anxiety

• Control blood sugar level.

Summarising all result we have no doubt that lemongrass is an interesting and promising alternative to classical antibiotics and should be more seriously considered as the therapeutic agents.

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