

## Comparative Testing of Antibacterial Activity of Aqueous Extract of *Bergenia Ligulata* Rhizomes and Ethanolic Extract of *Butea Monosperma* Flowers for Herbal Gel Formulation

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**Abstract:** The aim of this research was to formulate a gel comprising of the both extracts of *Bergenia ligulata* rhizomes and *Butea monosperma* flowers that would aid in wound healing by exhibiting antibacterial activity at the site of wound infection if any. As per the literature survey conducted it was found that aqueous extract of *Bergenia ligulata* rhizomes and ethanolic extract of *Butea monosperma* flowers have good potential of antibacterial activity. Hence this antibacterial activity was studied with the help of agar well-diffusion assay method, against the micro-organisms-*S. aureus*, MRSA, *Pr. vulgaris*, and *E.coli*. Both these extracts were obtained through Soxhlet extraction process and this process was optimized to get maximum yield of extraction. By agar gel well- diffusion assay at the concentration of about 100µg/mL both the extracts exhibited maximum zone of inhibition. This concentration was helpful in deciding the dose for topical gel formulation.

**Keywords:** antibacterial activity, Soxhlet extraction, agar gel well-diffusion assay, *Bergenia ligulata*, *Butea monosperma*

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### I. Introduction

India has a rich heritage of medicinal herbs. With the advent of new technology and innovations in pharmaceutical arena, these medicinal herbs can now be formulated into different herbal formulations. Herbal medicines are still mainly used by 70-80% of the world's total population, mostly in the developing countries.[5] According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two or three times. There are about 10,000 plant species which are being used in Indian System of Medicines (ISM) traditional medicines in Indian subcontinent and out of these 450-500 species are mostly used in over 85% of Ayurvedic, Unani and Siddha formulation and about 40 species are used in modern drugs.[6] Drugs were shortlisted and selected by carrying out review of literature of Research articles and other official books like The Ayurvedic Pharmacopoeia of India. *Butea monosperma* flower, belonging to family Fabaceae, is explored to possess wound healing, anti-oxidant, anti-bacterial and anti-inflammatory activities.[9]The flower contains mainly flavonoids butrin 1.5%, isobutrin coreopsin, isocoreopsin and butein.[7]*Bergenia ligulata* rhizome, belonging to family Saxifragaceae is explored to exhibit anti-bacterial activity. The Phytoconstituents present in *Bergenia ligulata* rhizomes are gallic acid and tannic acid.[5] These polyphenolic compounds are mainly responsible for antibacterial activity that prevent bacterial infections like sepsis and others arising during wound healing. Bergenin 0.9% is also a major phytoconstituent acting as a strong antibacterial agent. [4]Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts or a drug.[5]

### II. Material And Methods

#### Plant Materials and Preparation of Extracts:

The rhizomes of *Bergenia ligulata* and flowers of *Butea monosperma*, were purchased from local Kalbadevi market supplier Manilal and Lallubhai sons. and co. in Aug. 2016 and authenticated from Agharkar Research Institute, Pune, India. The rhizomes and flowers were cut into small pieces and dried at controlled temperature 45<sup>0</sup>C and powdered. The powder was then extracted with ethanol [2] under soxhlation to give ethanolic extract of flowers of *Butea monosperma* and similarly with water [8,1]under soxhlation to give aqueous extract of rhizomes of *Bergenia ligulata*. These extracts were filtered and evaporated to dryness with a dryer.

#### Preliminary Phytochemical Screening:

The ethanolic extract of *Butea monosperma* flower and aqueous extract of *Bergenia ligulata* rhizomes; were subjected to a preliminary phytochemical screening for the detection of various plantconstituents i.e. phytoconstituents.

#### Chemicals:

Muller and Hinton (MH) Agar butts, ethanol A.R grade, distilled water.

**Procedure for Antibacterial Agar well-diffusion Assay:**

20mL of sterile, molten & cooled to 45°C Muller Hinton (MH) agar butts were added to sterile big petri dishes containing 0.625mL of the 18-24 hr old test pathogens with O.D 1 and the plates were rotated clockwise and anticlockwise so as to distribute the culture uniformly in the medium. The plates were kept undistributed for 10 to 15 mins in order to set the medium properly. Then using a sterile cork-boarer with internal diameter 6mm and external diameter 8mm; four wells were punched in the medium one in each quarter of the plates. 80µL of the antibiotics, herb extract control soframycin etc were then added to each of the well and the plates were kept in the refrigerator for ½ hr for prediffusion of the sample. Then the plates were incubated at 37°C for 24 hr., after which the zones of inhibition were read by the zone reader supplied by the Hi-media.

**Extraction**

The yield of ethanolic extract of *Butea monosperma* flowers and aqueous extract of *Bergenia ligulata* rhizomes obtained were **6.76 % w/w** and **4.84 % w/w** respectively. The ethanolic extract of *Butea monosperma* flowers showed presence of phytochemicals namely alkaloids, flavonoids, glycosides, tannins, phenolic compounds and aqueous extract of *Bergenia ligulata* rhizomes showed the presence of phytochemicals namely flavonoids, glycosides, tannins, phenolic compounds.

The *Bergenia ligulata* and *Butea monosperma* extracts, Soframycin cream (std) and the gel were dissolved in DMSO where as Penicillin (5 U/ml) conc. was prepared in Buffer pH6.8 and streptomycin (100 mcg/ml) conc. in buffer pH7.0.

**III. Results And Discussion**

As shown in the table 1.0 DMSO shows negligible zone of inhibition, ethanolic extract of *Butea monosperma* flower exhibited better antibacterial activity in comparison with soframycin cream. Aqueous extract of *Bergenia ligulata* rhizomes also showed antibacterial activity except against MRSA .The formulated gel was also found to have very good antimicrobial activity, except for MRSA where it was comparatively less. The antibiotics definitely have a better role to play in treatment of infections.

The results of zone of zones of inhibition of the drug / chemical /antibiotic used are as shown in the table 1.0

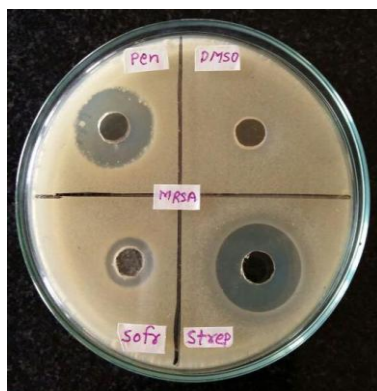
**Table no. 1** Results showing zone of inhibition of test microorganisms against both the extracts and formulated gel

Test organisms used	Drug / chemical /antibiotic used (Diameter of zone of inhibition in mm)						
	DMSO	<i>Bergenia l</i> extract	Soframycin cream	<i>Butea m</i> extract	Gel	Penicillin	Streptomycin
<i>S. aureus</i>	1.33 ± 0.000	24.67 ± 1.247	14.33 ± 0.471	21.67 ± 1.247	30.33 ± 1.247	38.33 ± 1.247	20.67 ± 1.247
MRSA	1.33 ± 0.471	16.00 ± 0.816	13.33 ± 0.471	0.67 ± 0.471	11.67 ± 0.943	23.67 ± 1.247	22.33 ± 0.471
<i>Pr. vulgaris</i>	1.33 ± 0.471	21.00 ± 1.700	14.00 ± 0.816	11.67 ± 0.471	25.67 ± 1.700	39.67 ± 2.055	17.00 ± 1.633
<i>E. coli</i>	1.00 ± 0.000	16.33 ± 1.247	13.67 ± 0.471	14.00 ± 1.633	15.00 ± 0.816	29.00 ± 0.816	17.67 ± 0.471

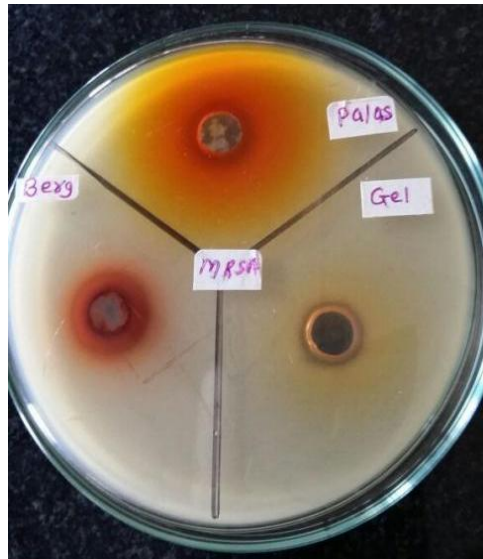
**Key:** *S. aureus*= *Staphylococcus aureus* MRSA= Methicillin resistant *Staphylococcus aureus*

*Pr. vulgaris*= *Proteus vulgaris* *E. coli*= *Escherichia coli*

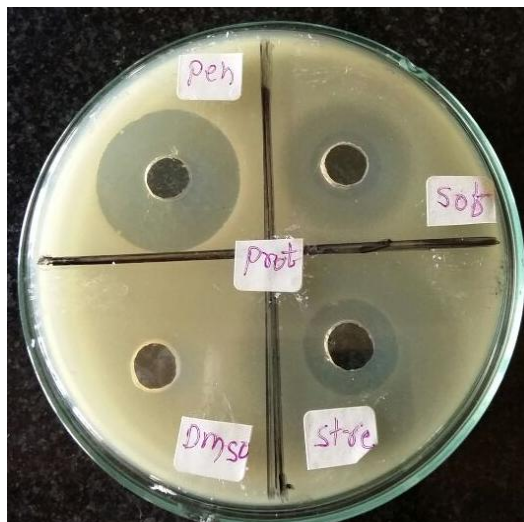
The results of antibacterial activity of penicillin, DMSO, soframycin and streptomycin and that of *Butea. m* extract, *Bergenia. l* extract, and gel against MRSA, *S aureus*, *Proteus vulgaris* and *E.coli* are shown in figures from 1.1 to 1.8



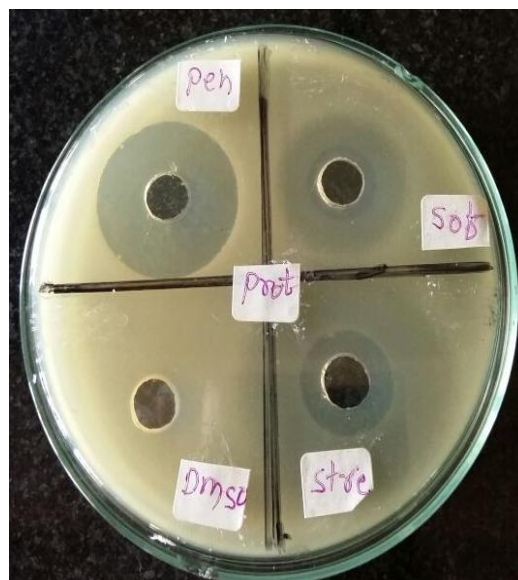
**Fig No. 1.1:** Antibacterial activity of penicillin, DMSO, Soframycin and streptomycin against MRSA



**Fig No. 1.2:** Antibacterial activity of *Butea. m* extract, *Bergenia. l* extract, and gel against MRSA



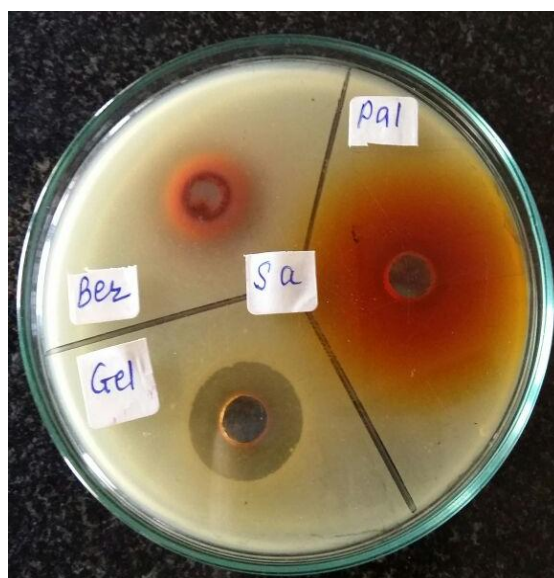
**Fig No. 1.3:** Antibacterial activity of penicillin, DMSO, Soframycin and streptomycin against *P. vulgaris*



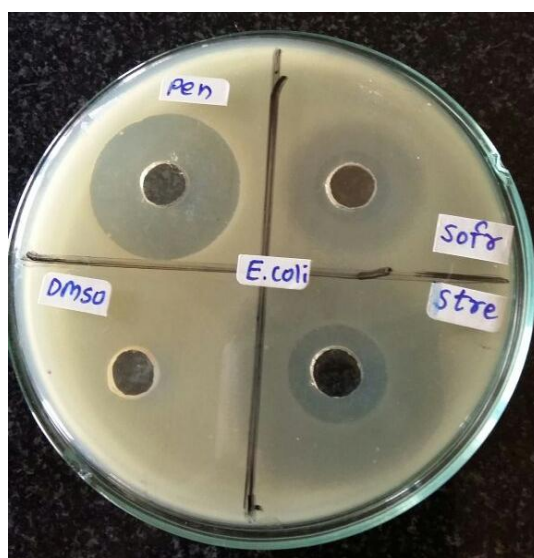
**Fig No. 1.4:** Antibacterial activity of *Butea. m* and *Bergenia. l* extract, and gel against *P. vulgaris*



**Fig No. 1.5:** Antibacterial activity of penicillin, DMSO, Soframycin and streptomycin against *S. aureus*

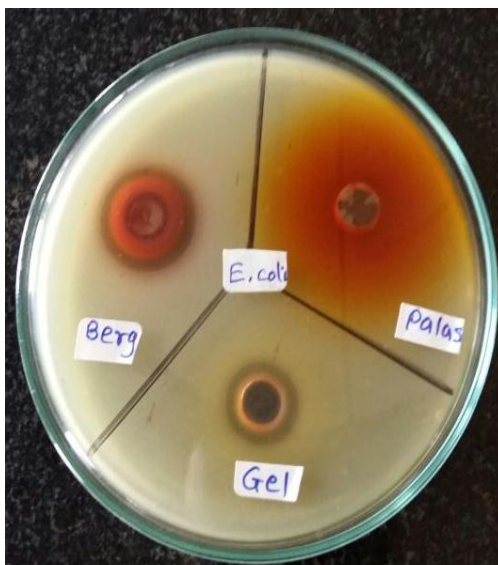


**Fig No.1.6:** Antibacterial activity of *Butea. m* and *Bergenia. l* extract, and gel against *S. aureus*



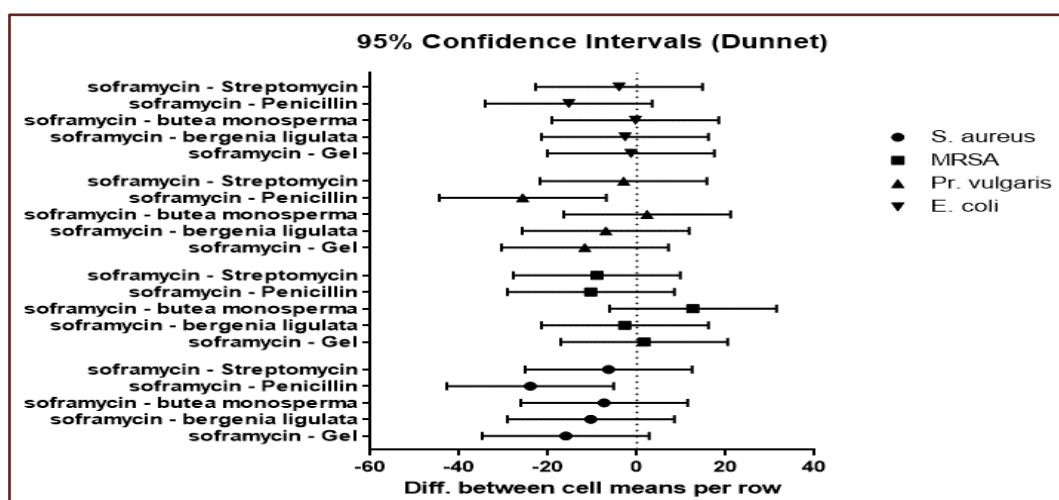
**Fig No. 1.7:** Antibacterial activity of penicillin, DMSO, Soframycin and streptomycin against *E. coli*





**Fig No. 1.8:** Antibacterial activity of *Butea. m* extract, *Bergenia. l* extract, and gel against *E. coli*

Dunnets' statistical method with help of GRAPHPAD PRISM SOFTWARE VERSION 6.6; was applied to test whether the data obtained and results were statistically significant or not. The results of the same are as follows: The Dunnets' test applied exhibited the result that the results obtained were statistically significant. [10] Results of Dunnets' statistical test for antibacterial assay are as shown in fig 1.9 below.



**Figure No.1.9:** Results of Dunnets' statistical test for antibacterial assay

#### IV. Conclusion

The above studies have demonstrated that both the ethanol & aqueous extract of *Butea monosperma* flowers and *Bergenia ligulata* rhizomes respectively have antibacterial properties. Although as expected aqueous extract of *Bergenia ligulata* rhizomes has more antibacterial activity. Further studies to elucidate the exact antibacterial mechanism of *Bergenia ligulata* rhizomes especially and *Butea monosperma* flowers need to be explored. *In-vivo* studies also need to be done to correlate with the *in-vitro* results.

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