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Comparitive Study of Soxhlation and Maceration Extracts of Tabernaemontana Divaricta Leaves for Antibacterial Activity

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ABSTRACT

Tabernaemontana divaricata is commonly used for various illnesses. The young stems are used as dental caries, latex of leaves used as anti-inflammatory and infusion of roots are used as antipyretic. The leaves are collected from Husnabad village of Karimnagar district which were dried and made into pulverised then kept for maceration and soxhlation by chloroform and methanol as solvents. The preliminary phytochemical analysis was carried for these extracts and results showed the presence of carbohydrates, alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids, steroids, proteins and amino acids. These extracts were examined for their antibacterial activity against 4 pathogens bacteria by well diffusion method. The pattern of inhibition varied with the solvent used for extraction and microorganism tested. A comparative study had been done between the maceration and soxhlation extracts of leaves by using chloroform and methanol as solvents. Among these extracts, methanol soxhlation extract showed significant antibacterial activity against most tested bacteria. The most susceptible microorganism was Proteus vulgaris (ZOI-22mm) followed by Staphylococcus aureus (ZOI-17).

Keywords: *Tabernaemontana divaricata*, Antibacterial, Maceration, Soxhlation, Staphylococcus aureus, Lacto bacillus, Proteus vulgaris, *Enterobacter aerogenes*, ZOI.

INTRODUCTION

Herbal Medicine is the oldest form of medicine known to mankind. It provided us with food, medicine and cosmetics. The essential difference between herbal and conventional medicine is that, while the conventional medicine the most active constituent is extracted from the plant and then synthesized in the laboratory to make the drug, in herbal medicine extracts from the whole plant are used [1].

Bacterial infection is one of the most serious global health issues in 21st century. The emergency of bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanism of action to overcome these problems. Medicinal plants were used as excellent antibacterial agents because it poses a variety of chemical constituent in nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action [2].

Tabernaemontana divaricata belongs to family Apocynaceae, commonly called pinwheel flower, crape jasmine, East India rosebay and Nero's crown [3]. The stem exudes milky latex when broken, hence the name milk flower. Produces cymes of 4 to 6 salver form waxy, pure white flowers; in general, this spreading, bushy shrub grows to a height of 6 to 10 feet tall and 5 to 8 feet wide. It bears white, waxy summer flowers and has oblong leaves with wavy margins that are dark green above and pale green beneath [3].

The study of the reported activities the plant *Tabernaemontana divaricata* has the antibacterial activity but the method of activity is done by comparative study between maceration and soxhlation method where soxhlation extracts found to have better results than maceration extracts [4-6].

EXPERIMENTAL SECTION

Procurement of plant material

For the present study, *Tabernaemontana divaricata* leaves were collected in the month of January 2016 from Husnabad village of the Karimnagar district. The plant was identified and authenticated by BSI/DRC/16-17/Tech.05. The leaves were dried in shade and stored at 25°C. It was powdered, passed through sieve no.40 and stored in air tight bottles.

Drugs and chemicals

Streptomycin (Piramal healthcare limited), Dimethyl sulfoxide (Merck life sciences [p] Ltd, Chloroform (Finar limted), Methanol (Merck life sciences [p] Ltd), Agar medium (Himedia laboratories pvt. Ltd), Broth (Central drug house Pvt. Ltd) was used during the experiment.

Selection of bacterial strains

Medically important bacterial strains used in this study were Staphylococcus aureus, Lacto bacillus, Proteus vulgaris, & *Enterobacter aerogenes* which were procured from MTCC [IMTECH], Chandigarh, India.

Preparation of extracts

The weighed quanty oif powdered drug was taken and for maceration the drug was socked in the choloroform and methanol for 5 days and the soixhlatiuon procedure done as the standard procedure.

Antibacterial evaluation

Selection of bacterial strains: Medicinally important bacterial strains used in the study were Staphylococcus aureus, *Enterobacter aerogens*, Proteus vulgaris, *Lactobacillus*. These bacteria served as test pathogens for antibacterial activity.

Standard Reference antibiotic: The reference antibiotic used is streptomycin obtained from Hi-media.

Preparation of broth culture: For the preparation of broth culture for bacteria, the liquid media was prepared by the standard composition for broth culture. After the sterilization of media the bacterial strains were inoculated under laminar air flow. The incubation of inoculated media was carried out at 37°C for 48 hours [5].

Preparation and sterilization of media: The require amount of nutrient agar was weighed and dissolved in 500 ml of distilled water and dissolve the agar by heating then pulg with cotton and autoclave for 15 min (121°C, 15 lbs pressure) to sterilize the media [6].

Plating the media: Sterilized media was poured to the petri dish (pre-sterilized in oven for 2 hours at 200°C in order to avoid contamination). The plated petri dishes were kept on a flat platform to avoid non-uniform solidification of medium. All these operations were done in a sterile room which was fitted with laminar air flow.

Bacterial culture preparations: Bacterial cultures were inoculated in the freshly prepared nutrient broth (which are prepared prior and sterilized) and kept on rotary shaker for 24 hours and observed for growth (turbidity indicates the growth). One day old cultures are used for testing and determination of each extract.

Assay procedure: The assay procedure was carried out by well diffusion method. The sterilized agar media was poured in to petridishes, and kept aside for solidification. Then 100 µl of broth culture of bacterial solution was spread over the solid agar plate. By the use of sterile borer small bores were made over the plate and it was filled with test solution, standard solution and diluting solution respectively for each bacterial plate.
Then plates were kept under incubation for 48 hours at 37°C. The zone of inhibition was measured using scale in millimeters.

Antibacterial activity: The test microorganisms were seeded into media containing petri dishes, by spread plate method (100 µl. with 24 hours culturing of bacteria. The plates were kept for pre diffusion for 15 mins before use. Wells were then punched with sterile cork borer (6 mm diameter) and 50 µl of the chloroform and methanol extracts by both soxhlation and maceration (10 mg, 20 mg/ml in DMSO) were placed into each well. A negative control was maintained using 50 µl of DMSO in a well and 50 µl of standard antibiotic (streptomycin at 10 µl /ml) was the positive control. Triplicates were maintained for each extract. Finally the plates were incubated for 18-24 hrs at 37°C. The diameter of zone of inhibition was indicated by clear area which was devoid of growth of microbes was

measured [1].

RESULTS AND DISCUSSION

By the present study it was confirmed that leaf extract of *Tabernaemontana divaricata* have antibacterial activity. The different concentration of methanol and chloroform extract by both soxhlation and maceration process showed antimicrobial activity against the tested organisms Staphylococcus aureus, Psroteus vulgaris, *Lactobacillus*, *Enterobacter aerogens* (**Figures 1-12**).

Table 1: Comparative study between soxhlation and maceration extracts

Type of Bacteria	Diameter of zone of inhibition(mm)								
	Chloroform				Methanol				Strepto-mycin (standard)
	SLE		MLE		SLE		MLE		
	10 mg/ml	20 mg/ml	10 mg/ml	20 mg/ml	10 mg/ml	20 mg/ml	10 mg/ml	20 mg/ml	10 mg/ml
Enteroba-cter aerogens	11	11	10	10	11	14	12	12	17
Staphylococcus aureus	10	12	10	10	17	15	11	11	21
Lactobacillus	12	11	11	10	12	13	11	10	18
Proteus vulgaris	10	10	10	10	10	22	10	17	23

A comparative study between soxhlation and maceration extracts had been carried out. Unlike maceration, extracts obtained from soxhlation formed more zone of inhibition,Over all it has showed significant effect towards Staphylococcus aureus. Chloroform maceration extract (10 mg/ml) showed least zone of inhibition (*E. aerogens*-10 mm, *S. aureus*-10 mm, *Lactobacillus*-11 mm, *P. vulgaris*-10 mm). Methanol soxhlation extract (20 mg/ml) showed highest zone of inhibition (*E. aerogens*-14 mm, *S. aureus*-15 mm, *Lactobacillus*-13 mm, *P. vulgaris*-22 mm. The results are furnished in **Table 1**.

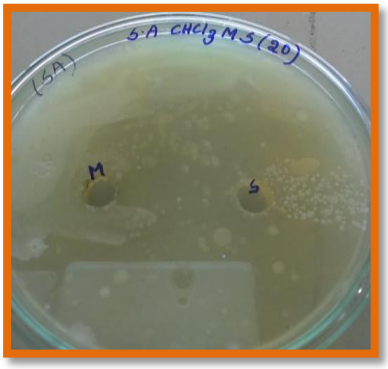

Figure 1: Staphylococcus aureus ($CHCl_3$)

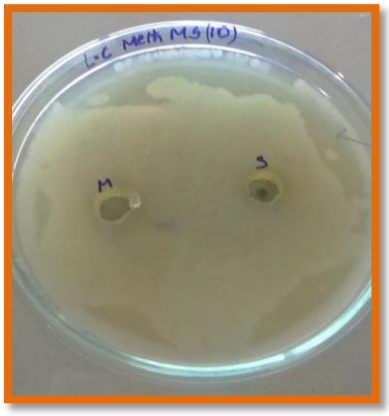

Figure 2: *Lactobacillus* (Meth MS)

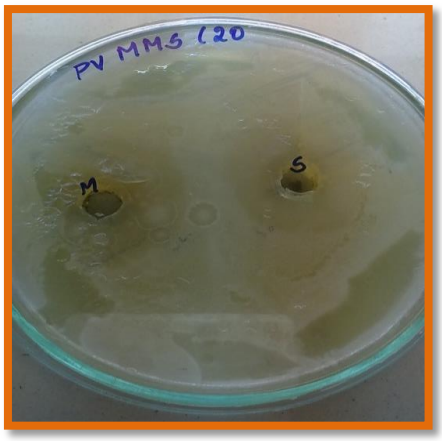

Figure 3: Proteus vulgaris (MMS)

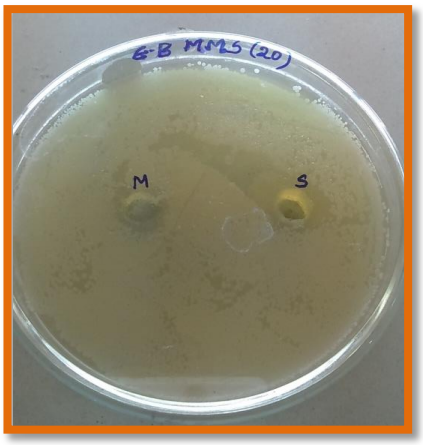

Figure 4: Enteroba-cter aerogens (MMS)

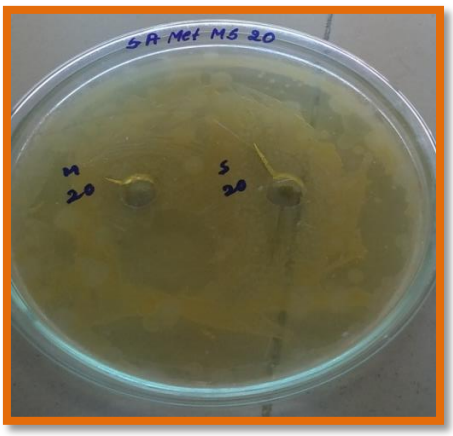

Figure 5: Staphylococcus aureus (Met MS)

Figure 6: Proteus vulgaris ($CHCl_3$ MS)

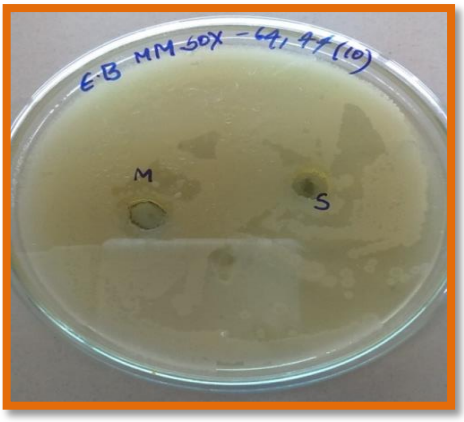

Figure 7: Enteroba-cter aerogens (MM Sox)

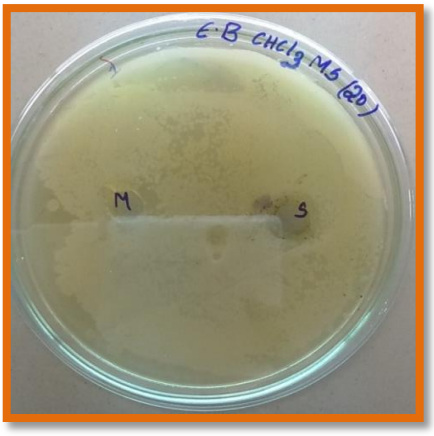

Figure 8: Enteroba-cter aerogens ($CHCl_3$)

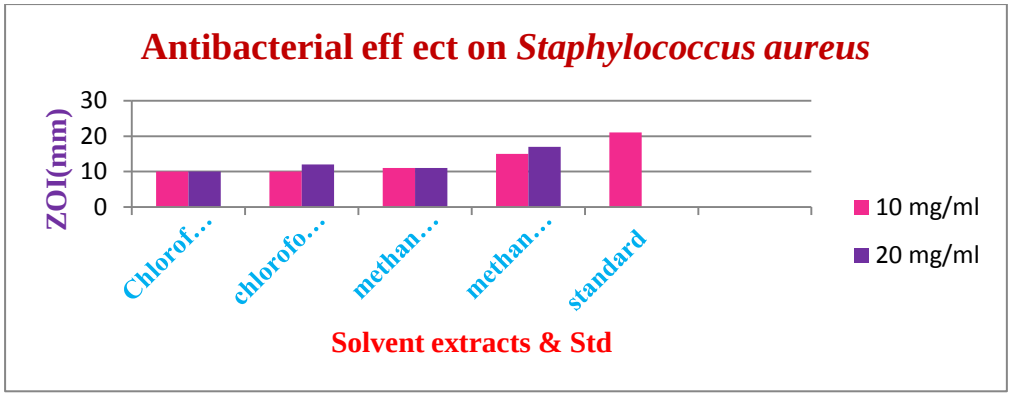

Figure 9: Antibacterial effect on *Staphylococcus aureus*

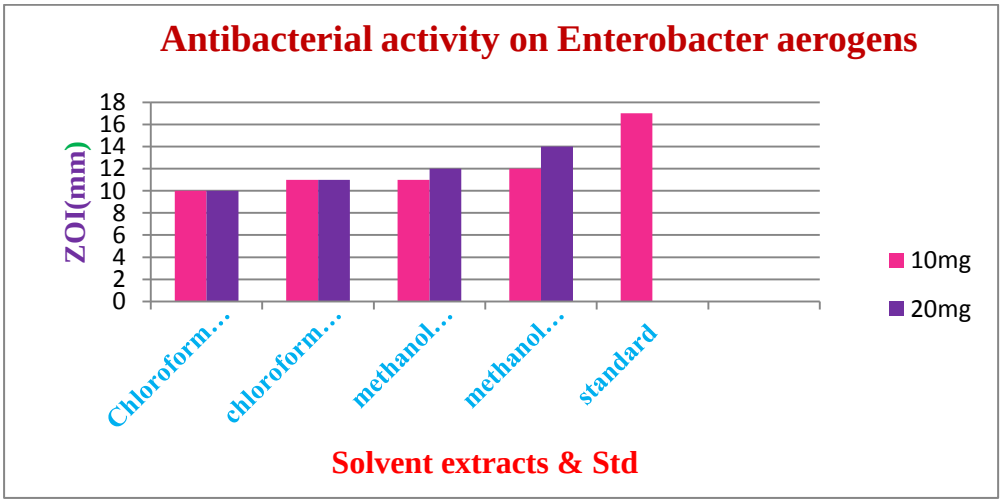

Figure 10: Antibacterial activity on *Enterobacter aerogens*

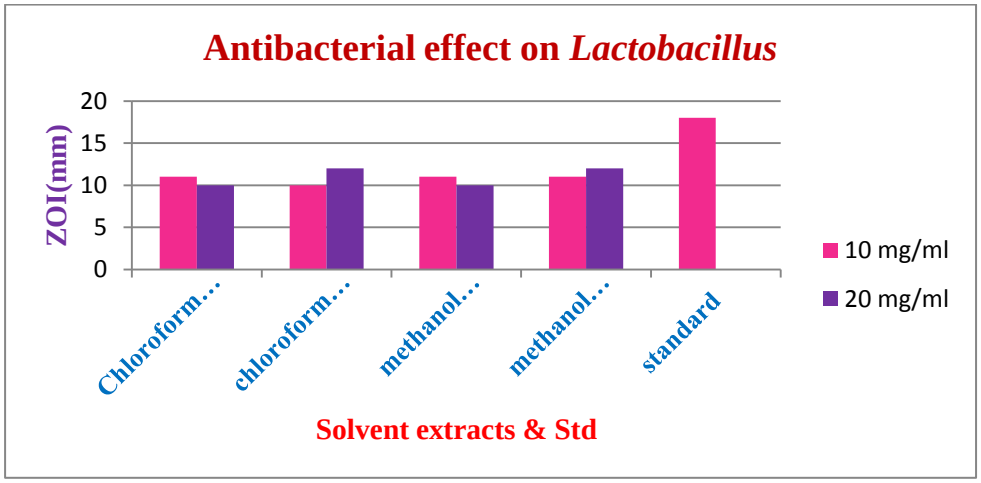

Figure 11: Antibacterial effect on *Lactobacillus*

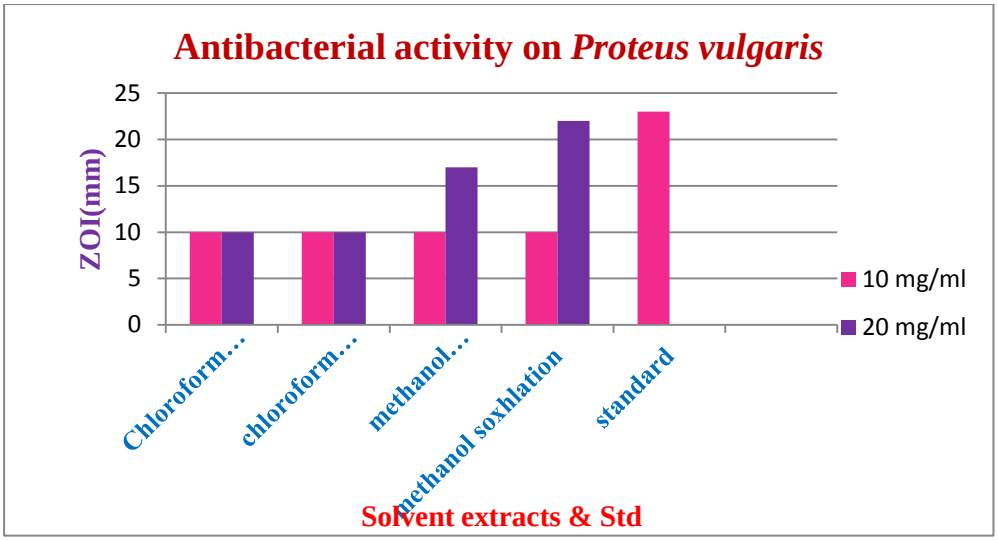

Figure 12: Antibacterial activity on *Proteus vulgaris*

CONCLUSION

The pharmacological screening of comparative study of soxhlation and maceration extracts of chloroform and methanol solvents of *Tabernaemontana divaricata* leaves had shown interesting results. All the extracts of *Tabernaemontana divaricata* were found to possess significant antibacterial activity. Compare to all the extracts of leaves methanolic soxhlation extract (20 gm/ml) had shown the more effective results than all extracts against most of the bacteria. The maximum ZOI was 22 mm shown by methanolic soxhlation extract against proteus vulgaris. Most of the extracts had shown appreciable effect. As most of the synthetic antibiotics are showing susceptibility to microbes and also showing various side effects, isolation of active principles from *tabernaemontana divaricata* will be advantageous to produce novel bioactive constituents.

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