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Desmodium Gangeticum Plant Extracts Possess Growth Inhibition Properties against Bacteria

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ABSTRACT

Desmodium gangeticum has been demonstrated to have multiple medicinal properties. Isolated studies compared the method of preparation solvent for such activity. We propose the property should be dependent on the part of plant as they accumulate different concentration of active molecules. This study investigated the antimicrobial potential of Desmodium gangeticum (DG) extract made from leave shoot and roots against Escherichia coli K-12 strain (MG1655). The broth microdilution method was used to determine the antimicrobial potential of the extract, where the extract was serially diluted in LB liquid and incubated with the tested strain in each well of the microplate. The growth inhibition was measured by increased absorbance at 600 nm. The minimum inhibitory concentration (MIC) of the DG extract was found to be 10 mg/ml, which exhibited inhibitory effects against E. coli. The results of agar disc diffusion assay showed that the DG extract was effective in inhibiting the growth of E. coli, suggesting its potential as an antimicrobial agent. The study highlights the use of DG extract as a natural alternative to conventional antimicrobial agents for controlling E. coli infections. Further research is needed to identify the active compounds responsible for the antimicrobial activity and to determine the safety and efficacy of DG extract in vivo.

Key words: Desmodium gangeticum, Ant-microbial activity, Growth inhibition, E. coli.

I. INTRODUCTION

World faced a big pandemic situation due to COVID-19 and everybody is looking for therapeutics that can deliver many medicinal properties without side effects and as much as possible to be natural and available nearby. This is the time again to rethink and revise our natural plant resources which are rich reservoirs of medicine around us. Among many well known plant with medicinal value is Desmodium gangeticum and it is one of many well known plants for various medicinal properties. The plant part of its decoction are already being used in commercial Ayurvedic supplements like Dashmoola(Aparna et al., 2012). Many local preparations of the district tribe use this plant or its part in decoction or crude extracts or raw plant part like leave for treatments of injury, fever, and inflammation. Ancient text from Indic literature like Charak Samhita, Mahabharata mention this plant as Shalparni or shalykarni for its rich wound healing property. Desmodium gangeticum is a medicinal plant that has been traditionally used in Indian Ayurvedic medicine for the treatment of various diseases, including respiratory infections, liver disorders, and snakebites. In recent years, there has been a growing interest in exploring the pharmacological properties of Desmodium gangeticum, particularly its antimicrobial activity.

In recent years, infections have become an increasing therapeutic problem as antibiotic resistance continues to grow. As a result, the screening of antimicrobial activity has become crucial due to its significant medicinal relevance. Higher plants are known to contain antifungal and antimicrobial substances, which have served as a source of inspiration for the development of novel drug compounds. This has led to the contribution of plant-derived medicines towards improving human health. The use of phytomedicine, as seen in traditional medical practices such as Unani and Ayurvedic systems, provides a natural blueprint for the development of new drugs. Natural products derived from microbial sources have also been explored and utilized for their antimicrobial potential. With the potential of developing antimicrobials from higher plants, the development of phytomedicine to combat microbes appears promising

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Although the plant has well noted application in synergy with several other plants in commercially available preparation and traditional system of medicine, the species particularly growing in this region is under risk of loss and has not come into attention. This has been primarily due to less scientific evidence and attention about the potential of D. gangeticum which can range from simple antimicrobial properties to therapeutics for nervous system. However, medicinal properties show very similar pharmacology in Ayurvedic medicines (Buddhadev, Vaghela and Shukla, 2013). Other species of the genus has been explored for several excellent properties worldwide, for example for the ability to modulate brain functions (N'gouemo et al., 1996). Therefore, requires proper exploration about its known medicinal uses, and what are the important bioactive molecules, primary or secondary metabolites present in the plant responsible for its medicinal properties and possible mechanism of action.Thisstudy aims to investigate the antimicrobial activity of DG extract from its various part leaves, stems, root and plant as whole.

II. MATERIALS AND METHODS

Preparation of crude extract

Roots, stems, leaves and flowers of the plant were cleaned and disinfected by alcoholic spray and then through washing with water, the seeds and whole-body parts were grinded to make aqueous and alcoholic extract of the plant in 100 ml of solvent. At end the extractswere filteredwith Whatmann filter paper No. 1 and aliquot in 5ml tubes were be taken for further study.

Antimicrobial Potential Assay

Microplate reader-based assays were used to determine the antimicrobial activity of compounds. These assays are based on the ability of antimicrobial compounds to inhibit the growth of microorganisms in culture. In this method, the antimicrobial compounds were serially diluted in a liquid growth medium, and a standardized amount of bacterial or fungal cells were added to each well of the microplate. The microplate was then incubated at a suitable temperature for a specified time period, and the growth of microorganisms in each well was determined by measuring the absorbance or fluorescence using a microplate reader. Another example is the disc diffusion assay, in which a filter paper disc containing the antimicrobial compound is placed on a solid agar medium that has been inoculated with the microorganism. The microplate is then incubated, and the inhibition zone around the disc is measured using a caliper or ruler. The size of the inhibition zone correlates with the antimicrobial activity of the compound. In this study we have used bothbroth based as well as agarose diffusion assay based

Microplate Antimicrobial potential assay

The protocol involves testing crude extracts against Escherichia coli using the method of micro dilution in Multi-well microplates. First, the bacterial strain is prepared by growing it overnight in LB medium and diluting it the next day. Then, a DG extract is prepared and serial dilution is performed in a microplate. Inoculums of the tested strain are added to each well, and the microplate is kept at 37°C for 20 hours. Absorbance is measured using a plate reader at 600 nm to determine the inhibition of bacterial growth. A separate sterilized microplate is used to transfer bacterial cultures, which are incubated overnight and OD is measured using a microplate reader. Finally, the mean and standard error of the mean (SEM) are calculated for each sample at each time point.

Statistical analysis

We did each measurement three times. We used Microsoft Excel 2007 to organize the data. Next, we will analyze the data using power analysis and descriptive statistics. The information will be shown and organized in tables as an average with measures of uncertainty. Checking the normality and homogeneity of variances will be done to see if the data points are suitable for the study. We will study the data by using ANOVA to see if there are any important differences ($p<0.05$) among different conditions. If needed, we will study and find out why something happened. If systematic changes show significant results, we conducted a meta-analysis.

III. RESULTS

Effect of DG extract on zone of inhibition in E. coli

We investigated the antibacterial properties of aqueous and ethanolic extract of DG against E. coli organisms grown on agar plates. The extracts were placed in triplicate on the corners of the agar plate, and a blank disc dipped in ultrapure sterile water was used as a control.The zone of inhibition was found to be negligible for the control

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disc, while the positive control of Penicillin antibiotics showed the maximum zone of inhibition. The DG extracts exhibited significantly high antibacterial properties and showed a zone of inhibition of 18-20mm (Fig. 1).

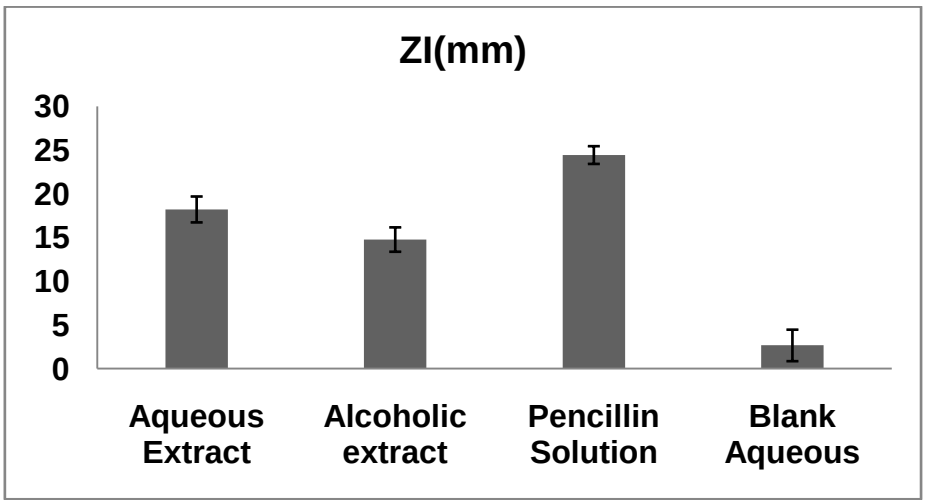

Fig. 1: Bar graph comparing the mean zone of inhibition for different treatments. The figure shows a bar graph comparing the mean zone of inhibition (ZI) for aqueous and ethanolic extracts of DG and Penicillin antibiotics. The graph clearly illustrates that Penicillin antibiotics had the highest zone of inhibition, followed by the aqueous extract of DG and then the ethanolic extract.

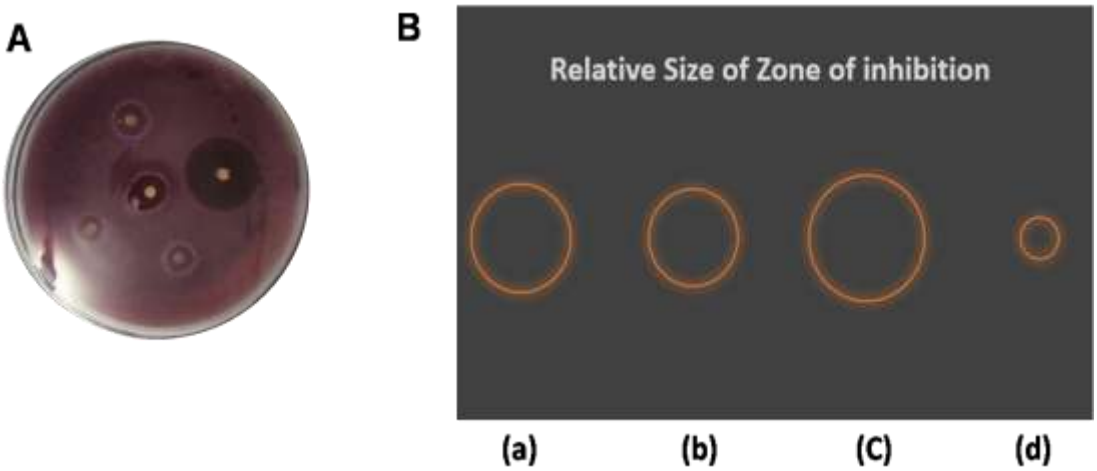

Fig.2: (A). An Illustration of schematic circle showing the relative size of circular zone. (B). Schematic circle illustrates the relative size of the circular zone of inhibition. The circle for Penicillin antibiotics is the largest, followed by the ethanolic extract of DG and then the aqueous extract. Left to right: (a) aqueous extract, (b) ethanolic extract, (c) antibiotics, (d) blank.

Antimicrobial Potential of Desmodium gangeticum Extract

The antimicrobial potential of the extract was determined using the broth microdilution method in multiwell microplates. The extract was serially diluted in LB liquid, and the diluted extract was inoculated with the tested strain in each well of the microplate. The microplates were incubated at 37°C for 20 hours, and the inhibition of bacterial growth was determined by measuring the increased absorbance in a plate reader at 600 nm. At the MIC concentration of 5 mg/ml, the DG extract was able to inhibit the growth of E. coli, indicating its potential as an antimicrobial agent (Fig. 3).

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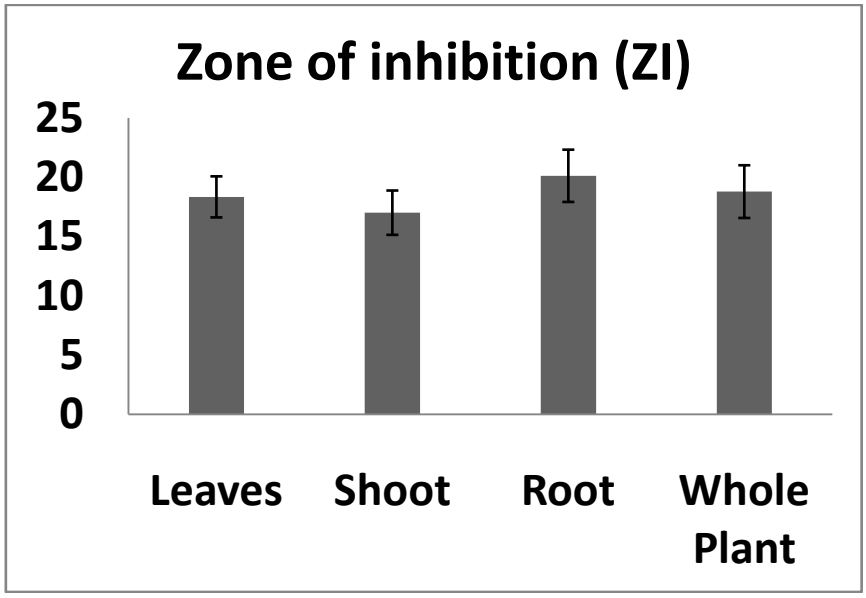

Fig. 3: Bar graph comparing the mean zone of inhibition for different extracts of D. gangeticum plant. The figure shows a bar graph comparing the mean zone of inhibition (ZI) for leaves, shoots, roots and whole plant extracts of DG. The graph clearly illustrates that roots had the highest zone of inhibition, followed by whole plant, leaves and shoots extract.

IV. DISCUSSION

Desmodium gangeticum is a very important medicinal plant that also grows well as herb in the climatic condition of the region. This is easily available to locals and tribes moreover it have been well noted for uses in wound healing and some more treatments. The medicinal values are planned to be evaluated in the study proposed. The study results will not only explore the possible candidates in plants for therapeutic use, it will also evaluate and try to decipher the mechanisms of phytochemicals of the plant responsible for the same. The study will result into important and immediate source of information about the plant and simultaneously it will explore the medicinal properties of the plant in vitro as well as in silico.

The plant D. gangeticum is anti-inflammatory, analgesic, and serve as beneficial effects in brain functions(Changdar et al., n.d.). Some of the properties were shown to specific compound present in the plant (Ghosh and Anandakumar 1983). The plant has gained major importance due to further studies support other medicinal properties to be present in the herb (Vedpalet al., 2020). Its decoction is used externally to clean wounds and ulcers while the whole plant is considered to be anti-helminthic, antiamnesic ((Joshi and Parle 2006). Desmodium is also an important crop in many countries, and its antimicrobial properties have potential applications in agriculture and food preservation. For example, Desmodium extracts have been found to have activity against post-harvest decay in fruits and vegetables, which can help to extend the shelf life of these foods and reduce food waste.

Antimicrobial activities of the plant has not been significantly reported however since the plant shalykarni or Salparni or srikarni is known to have analgesic, anti-inflammatory, diuretic properties it has been used in case of several health and disease condition (Jayasurya et al., 2017). Several studies have investigated the antimicrobial potential of Desmodium gangeticum. For example, a study published in the Journal of Ethnopharmacology investigated the antimicrobial activity of Desmodium gangeticum against several bacterial strains, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Ourresults confirm that that Desmodium gangeticum exhibited significant antibacterial activity against all the tested strains. Another study investigated the antimicrobial activity of Desmodium gangeticum against several fungal strains, including Candida albicans, Aspergillus niger, and Trichophyton mentagrophytes (Rahman and Parvin 2014). The study found that Desmodium gangeticum exhibited significant antifungal activity against all the tested strains.

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Desmodium is a genus of plants in the legume family, and some species of Desmodium are used for their medicinal properties. To evaluate the antimicrobial potential of DG extracts, we used E. coli as the research organism. In the disc diffusion assay, although the DG extract did not exhibit comparable antibiotic potential to commercially available antimicrobials, it significantly inhibited the growth of the organism in a zone that was thicker than the blank and placebos. Furthermore, the minimum inhibitory concentration was found to be 87 mg/1 ml concentration, which could be useful in the further development of herbal bacteriostatic agents. However, it is important to note that further studies are required to investigate the effects of DG extracts on other organisms. Findings of our study are in line with several findings which shows that The plant has demonstrated various pharmacological activities, including anti-leishmanial(Mishra et al., 2005), immunomodulatory, antioxidant, anti-inflammatory, antinociceptive, cardioprotective, antiulcer (Dharmani et al., 2005).

The antimicrobial active substrates are still away from the study but the finding of the study indicated strong evidence to explore such component in Desmodium gangeticum. Which could be any of chemical constituents, including alkaloids such as tryptamines and phenylethylamines, pterocarpanoids like gangetin and desmodin, phospholipids, sterols, flavone, and glycosides? Biologically active natural products from plants have attracted many of research professionals throughout globe in recent pandemic situation. Plants are rich source of triterpenoids, quinones and flavonoids alkaloids steroids, tri-terpenoids and troplones. Desmodium gangeticum is no exception to it. The antimicrobial activity of Desmodium gangeticum is attributed to the presence of various bioactive compounds, such as flavonoids, alkaloids, and phenolic compounds, which have been shown to possess antimicrobial properties.Overall, the available literature suggests that Desmodium gangeticum has significant antimicrobial potential and could be used as a natural source of antimicrobial agents. However, further research is needed to identify the active compounds responsible for the antimicrobial activity and to understand their underlying mechanisms of action.

Several studies have used microplate reader-based assays to investigate the antimicrobial activity of Desmodium gangeticum. Microplate reader-based assays are widely used due to their simplicity, reproducibility, and high-throughput capabilities (Karthikeyan, Selvam et al. 2012). These assays can be used to screen a large number of compounds quickly and efficiently and can be adapted to various microorganisms and antimicrobial compounds. However, it is important to note that these assays have limitations and should be used in conjunction with other methods to fully evaluate the antimicrobial activity of a compound. For example, a study published in the journal "Evidence-Based Complementary and Alternative Medicine" investigated the antimicrobial activity of Desmodium gangeticum against various bacterial strains, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, using a microplate reader-based assay. The study found that Desmodium gangeticum exhibited significant antibacterial activity against all the tested strains, with minimum inhibitory concentrations ranging from 2.5 to 10 mg/mL.

However in our study we report higher concentration possibly this is due to the aqueous nature of extract.Another study published in the journal "Pharmacognosy Research" investigated the antifungal activity of Desmodium gangeticum against various fungal strains, including Candida albicans and Aspergillus niger, using a microplate reader-based assay. The study found that Desmodium gangeticum exhibited significant antifungal activity against all the tested strains, with minimum inhibitory concentrations ranging from 1.25 to 5 mg/mL however if the time period for treatment was increase this does not significantly influences the MIC(Chen et al., 2022)

When we looked at how different bacteria reacted, we found that the water extract from D. gangeticumbacteria was effective.Desmodiumgangeticum was really good at killing bacteria, just like the best antibiotics like penicillin. The findings show that these plants can be used as traditional medicine and some of their extracts contain substances that can kill bacteria and be used as new drugs to fight infections.

In conclusion, Desmodium is a highly valuable plant with significant antimicrobial potential. Its ability to kill or inhibit the growth of microorganisms, including bacteria, fungi, and viruses, makes it a promising candidate for use in various applications, including medicine, agriculture, and food preservation. Further research is needed to fully understand the mechanisms underlying the antimicrobial properties of

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Desmodium, but the evidence to date suggests that this plant has great potential for improving human and environmental health.

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