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In-vitro antibacterial activities of leaf extracts of *Swertia chirata* and *Andrographis paniculata* antibacterial activity of two medicinal plants

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Abstract
Some medicinal plants have been used as phytomedicines for thousands of years. *Swertia chirata* C. B. Clarke (Gentianaceae), distributed in Nepal, and *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Acanthaceae), distributed in Gujarat, possess significant medicinal potential. These plants are considered important sources for the development of new drugs, and strategies such as combination chemotherapy, targeted therapy, and improved treatment adherence may help address challenges in cancer treatment. In this study, methanol, ethanol, and petroleum ether extracts of the aerial parts, including shoots and leaves, of both plants were evaluated for antimicrobial activity against various pathogenic bacterial strains. The results showed that the extracts exhibited varying degrees of antimicrobial effects. Ethanolic extracts showed superior antibacterial activity, warranting further studies to isolate, characterize, and elucidate the mechanisms of the active phytoconstituents.

Keywords: Swertia chirata, Andrographis paniculata, phytomedicines, medicinal plants, antimicrobial activity, ethanolic extract, methanolic extract

Introduction
Medicinal and aromatic plants have long been recognized as rich sources of potent bioactive phytoconstituents. These plants play a crucial role in traditional systems of medicine for the treatment of a wide range of diseases. Numerous studies have demonstrated that such phytomedicinal herbs exhibit diverse pharmacological properties, including anti-inflammatory, antioxidant, antifungal, antibacterial, antidiabetic, analgesic, antipyretic, anticancer, antidiarrheal, antiviral, and antimalarial activities. These biological effects are primarily attributed to the presence of various secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds (Ahirwal *et al*., 2011; Joshi and Dhawan, 2005; Mishra *et al*., 2009; Reena *et al*., 2001) [1, 2, 3, 4]. Some medicinal plants have been reported to exhibit hepatoprotective activity by enhancing the antioxidant status of the liver (Gupta *et al.*, 2005; Bhattacharjee and Sil, 2007) [5, 6]. Both *Swertia chirata* and *Andrographis paniculata* are traditionally used as bitter tonics in the management of liver disorders and are also known for their immunomodulatory properties, which help strengthen the immune system. During the COVID-19 pandemic, these plants were widely utilized in traditional remedies.

The antibacterial activity of these plants is attributed to multiple factors, including the presence of bioactive phytochemicals such as andrographolide and swertiamarin, along with the synergistic interactions among various compounds present in their extracts. In *Andrographis paniculata*, several active diterpenoids have been identified, and Pholphana *et al*. (2013) [7] reported that one of the most potent diterpenoids, AP6, is predominantly present in the leaves. Traditional herbal medicines have been used for centuries to treat a wide range of diseases. In recent years, there has been increasing scientific interest in the study of herbal medicines to validate their therapeutic potential. Research has demonstrated that many medicinal plants contain bioactive compounds capable of producing significant pharmacological effects, thereby supporting their use in modern healthcare system.

Materials and Method
Plant collection in winter seasons both mature plants, i.e., S. chirata were collected at the Nutan Ayurvedic Research Centre, Bhumel, Gujarat and A. paniculata were collected at the Shree Darshanam, Navli, Anand. S. chirata was collected from the same site and A. paniculata was collected at the Ajod Vruksh Mandir, Vadodara at a vegetative stage in the winter seasons.

Extraction Method
The extraction of phytoconstituents from Swertia chirata and Andrographis paniculata was carried out following the methodologies described by Harborne. The leaves, stems, and roots of *S. chirata*, along with the aerial parts of *A. paniculata*, were subjected to cold maceration as per Harborne to extract their bioactive constituents.The plant materials were first air-dried and finely powdered. Approximately 50 g of each powdered sample (leaves, stems, and roots) was extracted separately with 250 mL of ethanol, methanol, and petroleum ether in conical flasks. The mixtures were maintained at 37°C for 72 hours with continuous shaking using a mechanical shaker to ensure efficient extraction. After extraction, the mixtures were filtered through Whatman No. 1 filter paper. The filtrates were then concentrated using a rotary evaporator at 40°C to obtain crude extracts. The resulting dried extracts were carefully scraped, transferred into sterile containers, and stored under refrigerated conditions until further use.

Antimicrobial activity by agar well diffusion method

The effect of different concentrations of ethanol, methanol and petroleum ether extract of leaves, stems and roots of the plants on several bacterial strains can be studied using on agar well diffusion assay. The plant extract was added to the well in an agar plate that had been seeded with the bacterial strains. The plates were then incubated for some time and the diameter of the zone of inhibition around each well was measured. The larger the zone of inhibition the more effective the plant extract was at inhibiting the growth of the bacteria. In the process, 20 ml of sterile N. Agar with 0.2 mL broth culture of the test organisms was added to sterile Petri plates and allowed to solidify. Then, using a sterile Cork borer, a well of the agar was made in a plate at the desired location. 100 ml of different concentrations of the plant extracts (ethanol, methanol, and petroleum ether of leaf, stems and root ranging from 1000 to 5000 µg/mL were added into the well. The positive control well was filled with respective solvents (ethanol, methanol, and petroleum ether) and the negative control well was filled with distilled water. All the plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the zone of inhibition and, was noted after the next day. The result size of the zone of inhibition was an indication of the effectiveness. The Experiment was performed in triplicates.

Result and discussion

The results of the antibacterial activity of the ethanol extracts of the two plant species under study against eight different pathogenic bacterial strains are presented in graphs 1 and 2. The results of activity were analyzed using MS Excel 2021.

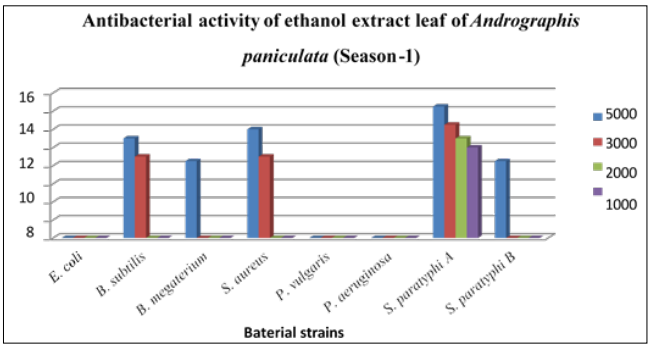

Fig 1: Antibacterial activity of ethanol extract leaf of *Andrographis paniculata* (Season-1)

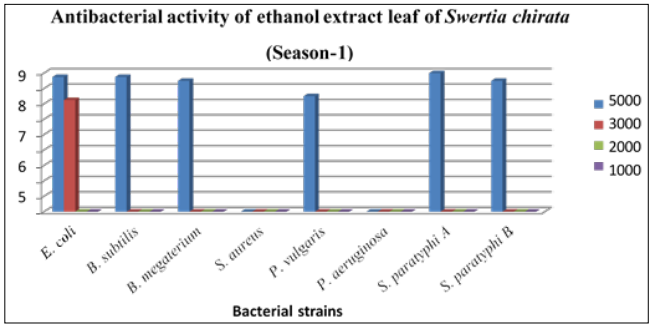

Fig 2: Antibacterial activity of ethanol extract leaf of *Swertia chirata* (Season-1)

The results clearly indicated in A. paniculata ethanolic leaf extract that the extract exhibited variable antibacterial activity against the tested organisms. Among all strains, the highest zone of inhibition was observed against *S. paratyphi A* with value 14.33mm with indicating strong susceptibility. This suggests that the ethanol extract is particularly effective against Gram-negative bacteria. Moderately high antibacterial activity was also recorded against *E. coli* and *B. subtilis*, showing that the extract possesses a broad-spectrum antimicrobial effect**.**

The results demonstrate that the extract exhibited moderate to selective antibacterial activity against the tested microorganisms. Among the bacterial strains, the highest zone of inhibition was observed against S. paratyphi A at higher concentration with value 9mm. An indication that the extract is more effective against Gram-positive bacteria. This may be due to the relatively simple cell wall structure, which facilitates the entry of bioactive phytochemicals. In the review carried out by Kumar *et al.*, (2010) [8], they expressed that Swertia is a very useful drug because of its medicinal uses. From our study, it is quite clear that both Swertia and Andrographis are biologically sensitive drugs that can be of immense use for treating various diseases.

Overall, the findings suggest that *A. paniculata* has promising antibacterial potential, although comparatively less potent than *S. chirata* under similar experimental conditions. Further studies focusing on purification of active compounds and mechanism of action are recommended.

Conclusion

Among the two, *A. paniculata* exhibited stronger and broader-spectrum antibacterial activity compared to *S. chirata*. The zones of inhibition observed in Fig. 1 were generally higher than those in Fig. 2, indicating a greater potency of *A. paniculata* extract. This suggests that *A. paniculata* contains a higher concentration or more effective combination of bioactive compounds responsible for antimicrobial action.

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