Phytochemical analysis and Evaluation of antimicrobial efficacy of different solvent fractions of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* L

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ABSTRACT

The rich diversity of phytochemicals and their powerful antimicrobial properties highlight their importance as a source for creating new antimicrobial agents capable of suppressing antimicrobial-resistant microbial strains. phytochemical analysis of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* (L) solvent extract was evaluated. The exhibited significant phenolic content in all extracts except the water extract of *G. maderaspatana* (L). Alkaloid content was dominant in methanolic and ethanolic extracts of *G. maderaspatana* (L). In contrast, terpenoids were equally distributed in methanolic and ethanolic extracts of *G. maderaspatana (L.) Poir* and *S. virginianum* (L). The methanolic extract of *S. virginianum* (L) represented maximum antifungal action in oppose to *Aspergillus flavus* ATCC 9643 and *Alternaria solani* ATCC 6663. Observations reveled statistically significant differences between the various solvents at *p*-value less than 0.05. Further investigation of the mechanisms of action and potential interactions of *G. maderaspatana* (L.) Poir and *S. virginianum* L-derived bioactive agents with current antimicrobial therapies accelerate the scientific progress towards curing infectious diseases.

Keywords: phytochemicals, solvent extract, Grangea maderaspatana (L) Poir, Solanum virginianum (L), antimicrobial therapies

INTRODUCTION

The development of antibiotic resistance in microorganisms is a sophisticated phenomenon that is influenced by different factors, e.g., excessive use of antibiotics (Laxminarayan *et al*., 2013), horizontal Gene Transfer (Frost *et al*., 2005), and natural selection (Blair *et al*., 2015). Moreover, environmental factors (such as pharmaceutical waste, animal farming, or untreated sewage, which leads to sub-lethal antibiotic concentrations in ecosystems) encourage antibiotic-resistant strains

in soil, water bodies, and agricultural lands (Kümmerer, 2009). Henceforth, the researchers are keen to explore plant-derived phytochemicals against antibiotic-resistant microbial strains. However, adequate infection control measures and hygiene must be followed to prevent infections in healthcare settings.

Phytochemicals are bioactive agents derived from plants that deliver many health benefits to humans. These agents include alkaloids, flavonoids, phenolics, saponins, and terpenoids. These bioactive agents are called plants' secondary metabolites, which play pivotal roles in plant defense mechanisms and serve human health by preventing and curing various diseases and disorders. The antimicrobial properties of plant-derived phytochemicals attracted scientific communities years ago to prevent and cure various diseases and disorders caused in living organisms. Alkaloids are nitrogen-containing compounds that exhibit antimicrobial, analgesic, anti-inflammatory, anticancer action and neurodegenerative disorders (Shah *et al*., 2014). Flavonoids are naturally polyphenolic compounds and are usually observed for antimicrobial, antioxidant, anti-inflammatory, and cardioprotective effects, diabetes control, and cancer treatments (Panche *et al*., 2016). Further, the phenolic compounds are extensively studied for their antioxidant properties and reported for antimicrobial potential, reducing the risk of cancers and cardiovascular diseases and preventing ageing-related disorders (Balasundram *et al*., 2006). Saponins are glycosides and are widely explored for controlling cholesterol levels, the effect of cancer, immune-boosting properties, antidiabetic potential and hepatoprotective properties (Güçlü-Üstündağ & Mazza, 2007). Terpenoids have been divulged for their anti-inflammatory, antiviral, antibacterial, and antimalarial benefits (Wagner & Elmadfa, 2003). These natural bioactive compounds have been exhaustively explored for antibacterial, antifungal, and antiviral activities.

G. maderaspatana (L.) Poir, a medicinal plant widely known in traditional medicine, has gained attention due to its potent bioactive phytochemicals with antimicrobial properties. Studies have shown that extracts derived from various parts of *G. maderaspatana* contain several secondary metabolites such as flavonoids, terpenoids, alkaloids, and saponins, which contribute to its antimicrobial efficacy against a wide range of pathogens (Sharma *et al*., 2018). Likewise, *S. virginianum* L. has also long been recognized in traditional medicine for its pharmacological properties, particularly its antimicrobial activity. The plant contains a variety of bioactive compounds, including alkaloids, flavonoids, saponins, and glycosides, which contribute to its therapeutic effects. Several studies have demonstrated the efficacy of these phytochemicals against a range of bacterial and fungal pathogens. The ability of phytochemicals to act synergistically enhances their overall antimicrobial potential, providing a broad-spectrum activity that can be harnessed for therapeutic applications. Natural antimicrobial agents open up avenues for developing plant-based therapies to combat drug-resistant infections and promote sustainable healthcare solutions. Therefore, the present research work was focused on the phytochemical analysis and evaluation of the antimicrobial efficacy of different solvent fractions of *G. maderaspatana* (L.) Poir and *S. virginianum* L.

MATERIALS AND METHODS

A fresh and healthy plant of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* L. were collected from the nearby forest region of Bilaspur City, Chhattisgarh, India in October 2023 for present research work. The plant was authenticated by Dr. U. Tiwari, Department of Botany, Govt. E. R. R. P.G. Science College, Bilaspur (C.G.).

Sample Preparation

The plants were shade-dried for two weeks and then oven-dried at 45^0C for 1 h. Dried plants were subjected to ground to make fine powder and stored in airtight plastic bags until used.

Preparation of Solvent Extract

The powder (1.0 g) was suspended in the 10 ml of solvent viz., water, methanol, and ethanol to make different solvent extract as per experimental design. The mixture was left for overnight and filtered with three layers of Whatman filter paper No. 1. The filtered extracts were then concentrated and stored at 4^0C until use.

Phytochemical Profiling

Phytochemicals profiling viz., phenols, tannins, steroids, alkaloids, flavonoids, saponins, and terpenoids) of water, methanolic, and ethanolic extract of *G. maderaspatana (L.) Poir* and *S. virginianum* (L) was qualitatively estimated as described by Pandey and Tripathi (2014).

Antimicrobial Profiling

Solvent extracts of *G. maderaspatana* (L.) Poir and *S. virginianum* L. were investigated for antibacterial activity against *E. coli* ATCC10536 and *Staphylococcus aureus* ATCC25923 whereas antifungal efficacy in oppose to *Aspergillus flavus* ATCC 9643 and *Alternaria solani* ATCC 6663. The nutrient broth was used to culture bacterial strains while potato dextrose broth was used for fungal strains. The agar well diffusion method was used for the assessment of the antimicrobial profile. 0.1 ml of each solvent extract was inoculated in agar wells prepared in Mueller-Hinton agar media. The test bacterial and fungal strains were spread gently by using an L-shaped glass rod aseptically. Gentamycin(10µg/ml) and Kanamycin (30µg ml^{-1}) were used as a positive control for bacterial strains whereas Gentamycin (10µg ml^{-1}) and Kanamycin (30µg/ml) were used for fungal strains. The bacterial and fungal strains were incubated at 37^0C (for 24 h) and 25^0C (for 48 h) respectively. All the experiments were carried out in triplicates. The inhibition zone around colonies after incubation was noted as diameter (mm).

Statistical Analysis

The data were tabulated and analyzed using MS Excel 2021. All the experimental data are expressed as mean ±SD. The p-value of <0.05 was considered a statistically significant result.

RESULTS AND DISCUSSION

The water, methanolic and ethanolic extracts of *G. maderaspatana* (L.) Poir and *S. virginianum* (L) were evaluated for phytochemical analysis. The phytochemical analysis of *G. maderaspatana* and *S. virginianum* indicated that methanol and ethanol extracts were the most

effective in extracting a diverse array of phytochemicals from the plants. The methanolic and ethanolic extracts of *G. maderaspatana* were rich in flavonoids, whereas the aqueous extract was contained alkaloids, phenols, and saponins. The methanolic and ethanolic extracts of *S. virginianum* was observed with elevated levels of alkaloids, phenols, steroids, flavonoids, and terpenoids. Present research highlighted the efficacy of methanol and ethanol in extracting bioactive molecules with potential medical applications. Alkaloids such as berberine and quinine have shown antibacterial efficacy by disrupting bacterial cell walls and impeding DNA replication (Cowan, 1999). Terpenoids like thymol and carvacrol have also been reported to disrupt microbial membranes, leading to the leakage of cellular contents and subsequent microbial death (Burt, 2004). Flavonoids have been unequivocally examined for antioxidant, anti-inflammatory, and anticancer potential (Kumar & Pandey, 2013). The present research work revealed that the flavonoid content was present in significant amounts in water, methanolic, and ethanolic extracts of *G. maderaspatana* (L.) Poir (Table 1; Fig. 1). Flavonoids such as quercetin and kaempferol have been found to interfere with microbial membrane integrity and promote cellular lysis (Cushnie & Lamb, 2005).

Extensive research has shown that environmental factors, viz., light, temperature, and nutrient availability, significantly influence the production of phytochemicals (Harborne, 1999). Present experimental analysis showed that the terpenoids were present in the methanolic and ethanolic extracts of *G. maderaspatana* (L.) Poir and *S. virginianum* (L). Zhao *et al*. (2021) mentioned that terpenoids have been widely deliberated for their antimicrobial and anticancer efficacy. High antibacterial activities against many pathogenic microorganisms have been observed in various phytochemical classes, e.g., alkaloids, flavonoids, terpenoids, tannins, and phenolic acids. In previous studies, alkaloids have been reported to have therapeutic potential for pain and hypertension (Roberts & Wink, 2020). Hence, a synergistic potential of plant phytochemicals in conjunction with conventional antibiotics could potentially be used for augmentation of antimicrobial efficacy, particularly against multidrug-resistant bacteria.

Table 1. Phytochemical profiling of different extracts of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* (L)

Phytochemicals	*Grangea maderaspatana* (L)			*Solanum virginianum* (L)		
	Water	Methanolic	Ethanolic	Water	Methanolic	Ethanolic
Phenols	+	++	++	++	++	++
Tannins	-	+	-	++	+	-
Steroids	-	+	+	-	++	+
Alkaloids	+	++	++	+	+	++
Flavonoids	++	++	++	-	++	-
Saponins	+	-	-	++	-	+
Terpenoids	+	++	++	+	++	++

Not present (-); color intensity low (+); Color intensity (++)

Methanolic extract of *G. maderaspatana* (L.) Poir exhibited maximum antibacterial action against *E. coli* ATCC10536 (22.8 ±0.41) and *Staphylococcus aureus* ATCC25923 (19.9 ±0.23). Methanolic extract of *S. virginianum* (L) represented maximum antibacterial activity in opposition to *E. coli* ATCC10536 (21.3 ±0.41) while ethanolic extract of *S. virginianum* (L) displayed maximum activity against *Staphylococcus aureus* ATCC25923 (18.5 ±0.54). Methanolic and

ethanolic extracts of *G. maderaspatana* (L.) Poir exhibited maximum antifungal action against *Aspergillus flavus* ATCC 9643 (20.4 ±0.37) and *Alternaria solani* ATCC 6663 (15.9 ±0.24) respectively. Further, the methanolic extract of *S. virginianum* (L) represented maximum antifungal action in oppose to *A. flavus* ATCC 9643 (22.5 ±0.36) and *A. solani* ATCC 6663 (16.1 ±0.35). Antibacterial profiles of different extracts of *G. maderaspatana* (L.) Poir and *S. virginianum* (L) are mentioned in Table 2 and Fig. 2. Observations reveled statistically significant differences between the various solvents at *p*-value less than 0.05.

Table 2. Antibacterial profile of different extracts of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* (L)

Plant Extracts & Standard antibiotics	Conc. (%)	Zone of inhibition (Mean value ± SD)					
		E. coli ATCC10536			*Staphylococcus aureus* ATCC25923		
		Methanol	Ethanol	Hot Water	Methanol	Ethanol	Hot Water
Negative Control	0	00	00	00	00	00	00
Grangea maderaspatana (L) Poir	25	7.1 ±0.17	6.9 ±0.15	5.1 ±0.16	5.6 ±0.09	5.8 ±0.12	4.3 ±0.08
	50	12.3 ±0.24	12.7±0.26	8.6 ±0.22	7.7 ±0.13	6.6 ±0.16	6.4 ±0.12
	75	18.2 ±0.32	15.4 ±0.37	11 ±0.21	13.2 ±0.19	10.8 ±0.11	8.6 ±0.24
	100	22.8 ±0.41	18 ±0.43	12.7 ±0.23	19.9 ±0.23	13.4 ±0.22	12.9 ±0.37
Solanum virginianum (L)	25	7.44 ±0.20	7.3 ±0.19	6.4 ±0.15	6.5 ±0.17	8.7 ±0.17	4.0 ±0.11
	50	12.6 ±0.21	11.4 ±0.22	9.3 ±0.18	13.4 ±0.28	9.2 ±0.19	4.7 ±0.18
	75	17.4 ±0.38	15.7 ±0.36	14.1 ±0.24	15.8 ±0.33	12.3 ±0.26	8.5 ±0.22
	100	21.3 ±0.41	19.6 ±0.43	16.4 ±0.29	17.6 ±0.39	18.5 ±0.54	13.1 ±0.57
Gentamycin (µg/ml)	10	24.3 ±0.62			26.8 ±0.53		
Kanamycin (µg/ml)	30	27.6 ±0.51			22.6 ±0.46		

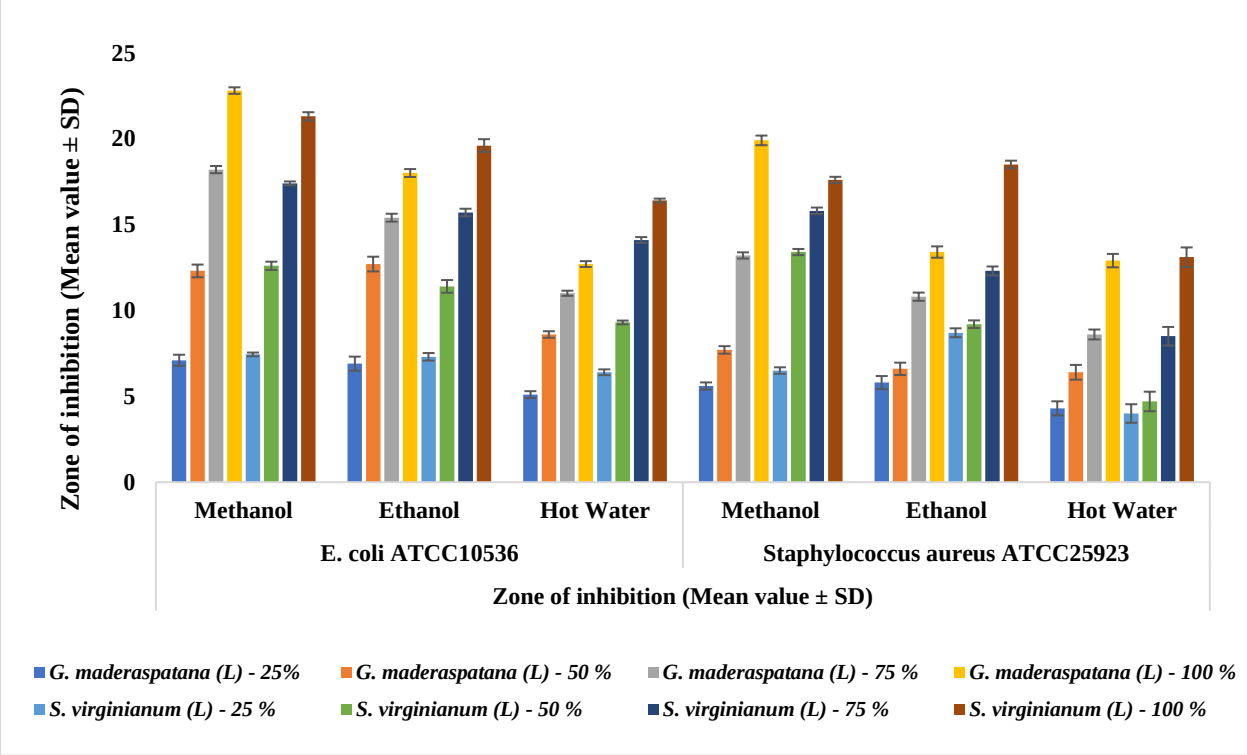

Fig. 1. Antibacterial profile of different extracts of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* (L)

The antibacterial potential of ethanol and methanol extracts of *G. maderaspatana* has been tested against *S. aureus*, *E. coli*, and *P. aeruginosa*. Observation divulged significant results regarding the same (Patel *et al*., 2020). This broad-spectrum activity suggests the potential of *G. maderaspatana* as a natural alternative to synthetic antibiotics, especially in the context of increasing antibiotic resistance (Chaudhary *et al*., 2023). Furthermore, the antifungal properties of *G. maderaspatana* have also been reported, with particular efficacy against fungal pathogens such as *Candida albicans* and *A. niger* (Kumar & Reddy, 2019). These findings highlight the versatility of the *G. maderaspatana*-derived phytochemicals against both bacterial and fungal infections. The antimicrobial activity of these phytochemicals is largely attributed to their ability to disrupt microbial cell walls, inhibit enzyme functions, and interfere with nucleic acid synthesis, thereby preventing microbial growth and proliferation (Singh *et al*., 2021; Thanuja *et al*., 2024). These mechanisms of action make *G. maderaspatana* a promising candidate for further development into antimicrobial agents or complementary therapies in managing infections. However, Chaudhary et al. (2023) has been emphasized additional medical applications viz., antifertility, analgesic, hepatoprotective, anti-inflammatory, cytotoxic, oestrogenicity, antiarthritic, antioxidant, and diuretic.

Research has shown that the alkaloid tolazoline, a key phytochemical found in *S. virginianum*, exhibits potent antimicrobial activity against both Gram-positive and Gram-negative bacteria. For instance, solasodine has been reported to effectively inhibit *E. coli*, *S. aureus*, and *P. aeruginosa*, which are common human pathogens associated with infections (Pandey *et al*., 2017). Sharma *et al*. (2020) have documented that the flavonoids isolated from the plant also demonstrate significant antibacterial and antifungal properties, particularly against fungal strains such as *C. albicans* and *A. niger*.

Table 3. Antifungal profile of different extracts of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* (L)

Plant Extracts & Standard antibiotics	Conc. (%)	Zone of inhibition (mm.) (Mean ± SD)					
		Aspergillus flavus ATCC 9643			*Alternaria solani* ATCC 6663		
		Methanol	Ethanol	Hot Water	Methanol	Ethanol	Hot water
Control	0	00	00	00	00	00	00
Grangea maderaspatana (L) Poir	25	8.3 ±0.21	7.9 ±0.10	5 ±0.11	7.7 ±0.13	5.3 ±0.08	6.1 ±0.14
	50	13.7 ±0.26	12.3±0.18	6.7 ±0.14	9.3 ±0.18	8.2 ±0.13	7.9 ±0.16
	75	17.3 ±0.32	14.8 ±0.24	10.6 ±0.26	13.5 ±0.24	13.6 ±0.17	10.6 ±0.25
	100	20.4 ±0.37	17.9 ±0.33	14.3 ±0.31	15.4 ±0.22	15.9 ±0.24	13.2 ±0.26
Solanum virginianum (L)	25	10.6 ±0.19	9.5 ±0.14	6.1 ±0.13	8.2 ±0.19	7.3 ±0.11	4.3 ±0.16
	50	13.8 ±0.28	13.6 ±0.21	9.3 ±0.16	13.6 ±0.24	9.5 ±0.18	6.1 ±0.19
	75	17.2 ±0.31	17.4 ±0.23	13.8 ±0.23	15.2 ±0.33	12.2 ±0.23	8.7 ±0.22
	100	22.5 ±0.36	21.3 ±0.38	16.4 ±0.31	16.1 ±0.35	14.9 ±0.29	11.1 ±0.28
Gentamycin (µg/ml)	10	21.3 ±0.42			23.8 ±0.41		
Kanamycin (µg/ml)	30	24.7 ±0.47			27.4 ±0.39		

We have observed significant antimicrobial activity with the methanolic extract of *S. virginianum*. Literature reported the saponins present in *S. virginianum* have been found to disrupt microbial cell membranes, leading to cell lysis and death. This mechanism underlies their efficacy against resistant bacterial strains making *S. virginianum*, a potential source for developing novel antimicrobial agents (Rohilla *et al*., 2023; Patel & Patel, 2019) and pharmaceutical applications (Mety *et al*., 2024). The ability of these phytochemicals to act synergistically enhances their overall antimicrobial potential, providing a broad-spectrum activity that can be harnessed for therapeutic applications.

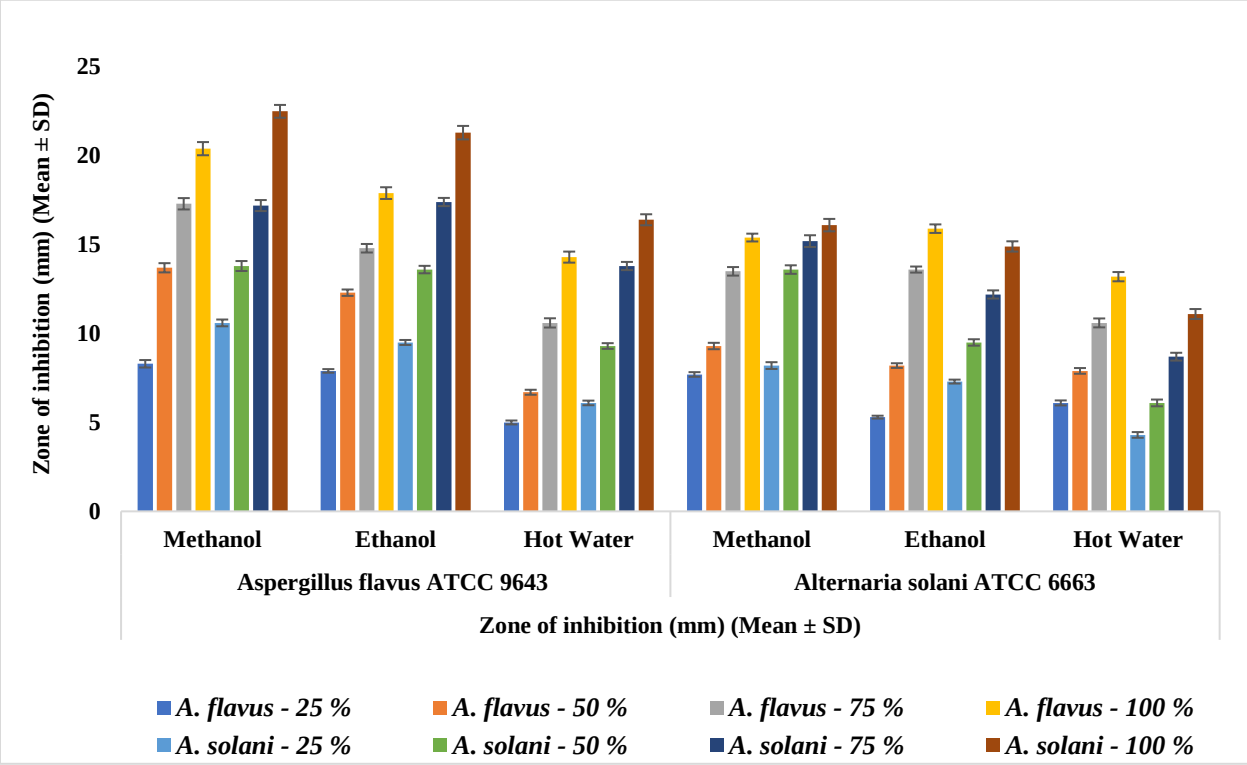

Fig. 2. Antifungal profile of different extracts of *Grangea maderaspatana* (L.) Poir and *Solanum virginianum* (L)

Henceforth, briefly our results showed that both plants exhibited dose-dependent antibacterial activity. Notably, at 100% concentration, *G. maderaspatana* was obareved with highest inhibition zone against *E. coli* with the methanolic extract (22.8 ± 0.41 mm) and against *S.aureus* with the methanolic extract (19.9 ± 0.23 mm). Similarly, *S. virginianum* was demonstrated strong antibacterial activity at 100% concentration, with the methanolic extract showing the largest inhibition zones against *E. coli* (21.3 ± 0.41 mm) and *S. aureus* (18.5 ± 0.54 mm). In general, methanol extracts significantly performed, followed by ethanol and hot water extracts, with hot water extracts showing the least antibacterial activity overall. These findings highlighted the potential of both plants, particularly in methanolic form, as sources of natural antibacterial agents.

CONCLUSION

Natural antimicrobial agents open up avenues for developing plant-based therapies to combat drug-resistant infections and promote sustainable healthcare solutions. Presently the interest in the antibacterial properties of certain compounds has been increasing for the last two decades which pushes up rapid growth in phytochemicals-based pharmaceutical products. Researchers explored plant phytochemicals as an alternative to cope with antibiotic resistance because they mitigate the rate of resistance development. The present results showed that the methanol extract of both *G. maderaspatana* (L.) Poir and *S. virginianum* (L) were effective against test bacterial and fungal strains, which could further be expanded for purification of bioactive agents and to evaluate their antimicrobial efficacy and toxicity aspects in animal cells.

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