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RESEARCH ARTICLE

Antimicrobial Effect of an Alcoholic Extract of an Ethnobotanical Mixture Against Clinical Isolates

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Abstract. Infectious diseases, exacerbated by antibiotic-resistant bacteria, continue to pose a significant global burden. Traditional Medicine offers interesting possibilities for combating drug resistance, with various plants producing secondary metabolites that exhibit biological activities. This study aims to evaluate the antimicrobial effects of an alcoholic extract of an ethnobotanical mixture (Hawan Samagri) against clinical isolates. The mixture consists of six herbs commonly used in the Hawan ritual in India i.e. *Giloy, Nagarmotha, Palash, Bakuchi, Bel, Kapoor kachari* and *Chid*. The study tested its extract against 11 clinical bacterial isolates using the microbroth dilution method. The results demonstrated that the alcoholic extract exhibited antimicrobial activity against all tested bacterial isolates, indicating potential broad-spectrum activity. The study also revealed varying levels of resistance among the isolates: *Acinetobacter baumannii, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* were most resistant, while *Branhamella catarrhalis* and *Proteus vulgaris* were relatively medium resistant, and the remaining five bacteria, i.e. Escherichia coli, Salmonella enterica, Coagulase-positive staphylococci, Citrobacter freundii and Coagulase-negative staphylococci, were relatively least resistant. Although the study provides valuable insights into the antimicrobial potential of the ethnobotanical mixture, further research is required to determine the specific concentrations, active compounds, and mechanisms of action along with their efficacy and optimal dosage. Nevertheless, these findings contribute to the use of indigenous resources for combating antimicrobial resistance and suggest the potential of incorporating such herbal mixtures into the daily practices of Hawan as a preventive measure.

Keywords. Antimicrobial activity, Hawan samagri, Clinical isolates, Multidrug-resistant bacteria, Antibiotic resistance.

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Introduction

Infectious diseases continue to pose a significant global burden, particularly affecting vulnerable populations [1]. The emergence of antibiotic-resistant bacteria has further escalated the concern, as some strains exhibit resistance to the most available antimicrobials [2]. While the development of new drugs and vaccines is crucial, effective delivery of these interventions is essential to reduce the burden of disease [3]. Drug-resistant infections result in increased morbidity, mortality, and healthcare expenses, particularly in cases like community-acquired pneumococcal infections [4]. Resistance is a global issue, with extended-spectrum beta-lactamases, quinolone resistance, and multi-drug-resistant sexually transmitted infections on the rise [5]. Traditional Medicine offers interesting possibilities for combating drug resistance, with various plants producing secondary metabolites that exhibit biological activities [6].

Therefore, the development of therapeutic agents from indigenous resources will be of great help. In this context, the present study aims to evaluate the in vitro antimicrobial effects of an alcoholic extract of an ethnobotanical mixture against clinical isolates. The ethnobotanical mixture, known as Hawan Samagri, is used in an ancient fumigation method (Yagya / Hawan) used in India and involves the use of clarified cow's butter and a mixture of odoriferous and medicinal plant parts. Various herbal mixtures are prepared for specific utility using the same method. One particular mixture used for oblation onto a consecrated fire, and its medicinal fumes produced have shown effectiveness in the treatment of tuberculosis [7]. Additionally, in another study, an extract of the other type of Hawan Samagri mixture was used as an oral medicine in patients [8].

In the present study, we obtained an herbal mixture made up of 6 herbs that are routinely used as Hawan Samagri in Hawan for spiritual and ritualistic purposes. The main objective of this study aimed to test its extract against various clinical bacterial isolates as a preliminary examination step for its potential as a fume for addressing multi-drug resistance bacteria. By leveraging the potential of indigenous resources, the study attempted to provide the base for a specific Hawan Samagri mixture in Hawan fumes to address the challenges posed by drug resistance, and also for discovering new therapeutic agents in it.

Materials and Methods

Sample collection

Dried Sample of *T. cordifolia* was collected from the Shantikunj Pharmacy, Dev Sanskriti University, Shantikunj-Haridwar, India.

Sample collection

Dried Sample of *T. cordifolia* was collected from the Shantikunj Pharmacy, Dev Sanskriti University, Shantikunj-Haridwar, India.

Results and Discussion

The study found relative resistance and sensitivity for 11 types of bacterial isolates of clinical samples (Figure 1). The original alcoholic extract of ethnobotanical (Hawan Samagri in the present study) extract without any solution was considered as 100% minimum inhibitory concentration percentage, while 2-fold dilution was considered 50% minimum inhibitory concentration percentage, and so on. The 12th fold solution was considered as 0.05% minimum inhibitory concentration percentage. The minimum inhibitory concentration percentage of alcoholic extract of ethnobotanical (Hawan Samagri) against 11 clinical isolates is given in Figure 1 and summarized in Table 1.

The study found that relatively among 11 bacterial isolates, 3 bacterial i.e. Acinetobacter baumannii, Klebsiella pneumoniae and Pseudomonas aeruginosa were most resistant, while Branhamella catarrhalis and Proteus vulgaris were relatively medium resistant, and remaining five bacteria, i.e. Escherichia coli, Salmonella enterica, Coagulase-positive staphylococci, Citrobacter freundii and Coagulase-negative staphylococci, were relatively least resistant towards the alcoholic extract of the ethnobacterial mixture used.

Age	Gender	Sample type	Organism	Concentration of Ethnobotnical mixture (%)											
				100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05
57	M	SPUTUM C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
81	M	PUS C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
53	F	ET SECRETION C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
18	M	ET SECRETION C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
27	M	ET SECRETION C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
72	M	FLUID FOR C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
61	F	PUS C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
22	M	BLOOD C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
50	M	PUS C/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
22	M	URINEC/S	Acinetobacter baumannii	N	N	N	N	P	P	P	P	P	P	P	P
27	F	PUS C/S	Branhamella catarrhalis	N	N	N	N	N	N	N	P	P	P	P	P
42	M	URINEC/S	Citrobacter freundii	N	N	N	N	N	N	N	N	N	N	P	P
52	M	PUS C/S	Citrobacter freundii	N	N	N	N	N	N	N	N	N	N	P	P
79	F	URINEC/S	Citrobacter freundii	N	N	N	N	N	N	N	N	N	N	P	P
41	M	PUS C/S	Citrobacter freundii	N	N	N	N	N	N	N	N	N	N	P	P
12	M	SPUTUM C/S	Citrobacter freundii	N	N	N	N	N	N	N	N	N	N	P	P
51	M	ET SECRETION C/S	Citrobacter freundii	N	N	N	N	N	N	N	N	N	N	P	P
52	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
42	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
27	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
29	F	VAGINAL SWAB C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
41	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
44	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
4	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
5	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
57	F	SPUTUM C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
68	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
60	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
75	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
65	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
25	F	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
27	F	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
70	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
58	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
82	M	WOUND SWAB C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
27	F	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
64	M	PUS C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
51	M	TT SECRETION C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
4	M	BLOOD C/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
78	M	URINEC/S	Escherichia coli	N	N	N	N	N	N	N	N	N	P	P	P
51	M	SPUTUM C/S	Enterococcus faecalis	N	N	N	N	N	N	N	N	P	P	P	P
46	F	PUS C/S	Enterococcus faecalis	N	N	N	N	N	N	N	N	P	P	P	P
56	F	PUS C/S	Klebsiella pneumoniae	N	N	N	N	P	P	P	P	P	P	P	P
76	M	URINEC/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
42	M	SPUTUM C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
44	F	SPUTUM C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
41	M	PUS C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
56	M	SPUTUM C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
24	F	SPUTUM C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
35	M	EAR SWAB C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
53	F	ET SECRETION C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
81	M	PUS C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
66	F	SPUTUM C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
60	M	PUS C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
81	M	PUS C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
61	M	PUS C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
20	M	ET SECRETION C/S	Klebsiella pneumoniae	N	N	N	N	N	P	P	P	P	P	P	P
33	M	EAR SWAB C/S	Proteus vulgaris	N	N	N	N	N	N	N	P	P	P	P	P
47	M	PUS C/S	Proteus vulgaris	N	N	N	N	N	N	N	P	P	P	P	P
41	M	PUS C/S	Proteus vulgaris	N	N	N	N	N	N	N	P	P	P	P	P
59	M	PUS C/S	Proteus vulgaris	N	N	N	N	N	N	N	P	P	P	P	P
42	F	SPUTUM C/S	Proteus vulgaris	N	N	N	N	N	N	N	P	P	P	P	P
18	M	EAR SWAB C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
7	M	PUS C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
67	M	SPUTUM C/S	Pseudomonas aeruginosa	N	N	N	N	N	N	P	P	P	P	P	P
35	M	EAR SWAB C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
48	F	URINEC/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
77	F	PUS C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
21	F	EAR SWAB C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
70	M	FLUID FOR C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
27	M	SPUTUM C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
2	F	EAR SWAB C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
79	F	URINEC/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
24	F	EAR SWAB C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
18	F	PUS C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
22	M	PUS C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
48	F	SPUTUM C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
32	M	PUS C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
52	F	SPUTUM C/S	Pseudomonas aeruginosa	N	N	N	N	N	P	P	P	P	P	P	P
52	M	STOOL C/S	Salmonella enterica	N	N	N	N	N	N	N	N	N	P	P	P
38	F	STOOL C/S	Salmonella enterica	N	N	N	N	N	N	N	N	N	P	P	P
61	F	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	P	P	P
27	F	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	N	N	N
77	F	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	N	N	N
35	M	SPUTUM C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	P	P	P
42	F	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	P	P	P
30	M	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	P	P	P
14	M	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	P	P	P
54	M	PUS C/S	Coagulase-positive staphylococci	N	N	N	N	N	N	N	N	N	P	P	P
63	F	ASCITIC FLUID C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
23	M	WOUND SWAB C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
67	M	SPUTUM C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
73	M	PUS C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
25	M	SWAB FOR C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
45	M	ET SECRETION C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
31	M	PUS C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P
61	M	PUS C/S	coagulase-negative staphylococci	N	N	N	N	N	N	N	N	N	N	P	P

Figure 1: Minimum inhibitory concentration percentage of alcoholic extract of the ethnobotanical mixture (Hawan Samagri) for 11 clinical bacterial isolates obtained from patients along with patients' demographic and their clinical source of bacterial isolates.

Bacterial Isolate	Number of Samples (n)	Minimum Inhibitory Concentration Percentage	Fold dilution
Acinetobacter baumannii	10	12.5	4
Branhamella catarrhalis	1	1.56	7
Citrobacter freundii	6	0.2	10
Escherichia coli	23	0.39	9
Enterococcus faecalis	2	0.78	8
Klebsiella pneumoniae	15	6.25	5
Proteus vulgaris	5	1.56	7
Pseudomonas aeruginosa	17	6.25	5
Salmonella enterica	2	0.39	9
Coagulase-positive staphylococci	8	0.39	9
Coagulase-negative staphylococci	8	0.2	10

Table 1: Minimum Inhibitory Concentration Percentage of alcoholic extract of ethnobotanical (Hawan Samagri) against 11 clinical isolates.

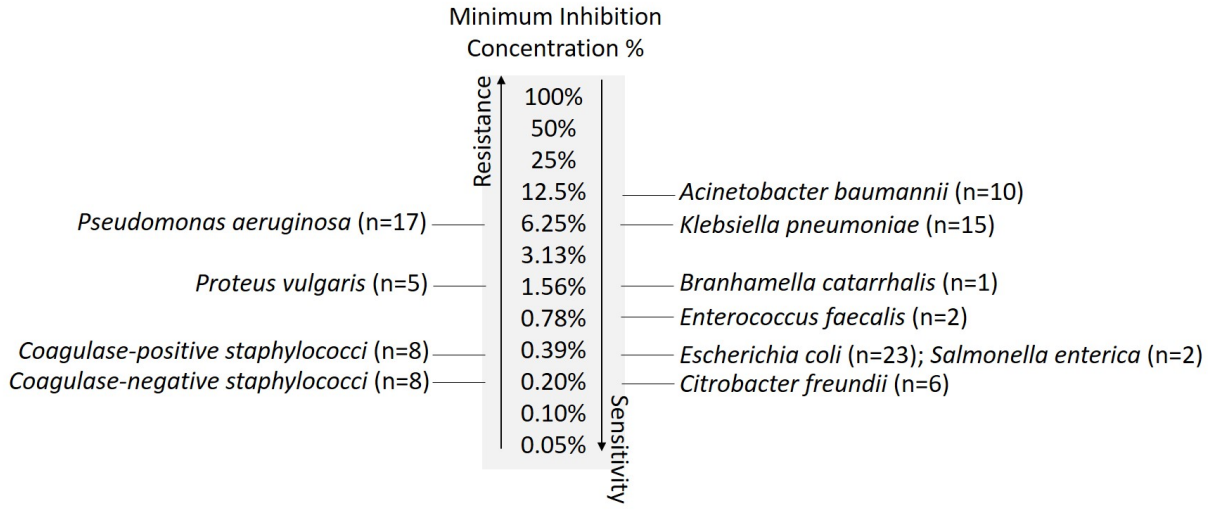

Figure 2: Relative resistance of 11 clinical bacterial isolates for alcoholic extract of the ethnobotanical mixture (Hawan Samagri).

The sequence of 11 isolates from high to low resistance towards alcoholic extract of the hawan samagri as follows (Figure 2): Acinetobacter baumannii (4-fold) > Klebsiella pneumoniae and Pseudomonas aeruginosa (5-fold) > Branhamella catarrhalis and Proteus vulgaris (7-fold) > Enterococcus faecalis (8-fold) > Escherichia coli and Salmonella enterica and Coagulase-positive staphylococci (9-fold) > Citrobacter freundii and Coagulase-negative staphylococci (10-fold)

Discussion

The present study aimed to evaluate the in vitro antimicrobial effects of an alcoholic extract of an ethnobotanical mixture (commonly used Hawan Samagri), against 11 clinical bacterial isolates (Figure 1, Table 1). The mixture consisted of six herbs frequently utilized in the Hawan ritual in India, namely Giloy (Tinospora cordifolia), Nagarmotha (Cyperus rotundus), Palash (Butea monosperma), Bakuchi (Psoralea corylifolia), Bel (Aegle marmelos), Kapoor kachari (Hedychium spicatum), and Chid (Convolvulus pluricaulis). It is worth mentioning that all of these herbs had previously shown an-

timicrobial activity against one or more of the bacterial strains tested, except for Branhamella catarrhalis (Table 2).

Herb (Botanical Name)	Prominent Ayurvedic Clinical Usage	A. baumannii	K. pneumoniae	P. aeruginosa	B. catarrhalis	P. vulgaris	E. faecalis	E. coli	S. enterica	C. freundii	C. +ve staphylococci	C -ve staphylococci
Giloy (Tinospora cordifolia)	Immunomodulatory properties; Used in the management of fever, respiratory disorders, and skin diseases.		[17–19]	[15, 17–19]		[14, 17, 18]	[14, 17]	[11–14, 16–19]	[14]		[11–14]	[11–14]
Nagarmotha (Cyperus rotundus)	Used for digestive disorders (diarrhea, dysentery, abdominal pain, etc). Also used in the treatment of menstrual disorders and joint pain.	[26]	[24–27, 30]	[22, 23, 26, 27]		[27]	[28–30]	[20, 24, 27]	[27, 28, 30]	[27]	[20, 23, 24]	[26, 28, 30]
Palash (Butea monosperma)	Used for its astringent properties, for like diarrhea, dysentery, uterine tonic and in skin diseases.	[31]	[31]	[31, 32]			31	[31, 32]	31	[31]	[31]	[31]
Bakuchi (Psoralea corylifolia)	Treatment of skin disorders, particularly vitiligo and leucoderma.	[36]	[34, 35]	[34]		[34]	[36]	[34, 35]	[33]		[34–36]	[34, 35]
Bel (Aegle marmelos)	Digestive properties; Used in diarrhea, dysentery, and irritable bowel syndrome. Also used for respiratory disorders and as a cardiac tonic.	[37]	[37]	[37]		[37]	[37]	[37, 39]	37	[37]	[38, 39]	[38]
Kapoor kachari (Hedychium spicatum)	Carminative and digestive stimulant - used in indigestion, flatulence, and abdominal pain; Anti-inflammatory properties - pain management		[43, 44]	[43, 44]		[44]		[43]			[43]	[43]
Chid (Convolvulus pluricaulis)	Used for its neuroprotective effects and in the treatment of neurological disorders such as epilepsy, insomnia, and anxiety.		[42]					[41]			[40–42]	[40–42]
Ethnobotanical Mixure consisting of all above herbs, used in the study	All of above											

Table 2: Known antimicrobial activity of each of herb and mixture of Ethnobotanical mixture (Hawan Samagri) against 11 clinical isolates. Full names of clinical isolates used in the table: Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Branhamella catarrhalis, Proteus vulgaris, Enterococcus faecalis Escherichia coli, Salmonella enterica, Citrobacter freundii Coagulase positive staphylococci, Coagulase negative staphylococci

The results of the study demonstrated that the alcoholic extract of the ethnobotanical mixture exhibited antimicrobial activity against all 11 tested bacterial isolates (Table 2). This finding suggests that the individual herbs used in the mixture possess antimicrobial properties, which collectively contribute to the observed broad-spectrum antimicrobial activity. This ability to exert antimicrobial effects against various bacterial strains is particularly significant in the context of combating multidrug-resistant bacteria.

However, it is important to note that the study provided information only on the relative resistance and sensitivity of the bacterial isolates (Figure 2), without quantifying specific concentrations or comparing them to other studies. This limitation makes it challenging to assess the potency of the alcoholic extract compared to other antimicrobial agents or herbal preparations containing the same herbs. Future studies should consider quantifying the concentrations of active compounds in the extract to enable comparisons and determine the potential clinical relevance of the findings.

Nevertheless, the study's findings support the idea that incorporating herbal mixtures such as Hawan Samagri into daily practices like the Hawan ritual may contribute to preventing antimicrobial resistance in both individuals and clinical settings. The observed antimicrobial ac-

tivity of the mixture against a wide range of bacterial isolates suggests its potential as an alternative or complementary approach to address the challenges posed by drug resistance.

Further research is warranted to elucidate the specific mechanisms of action and identify the active compounds responsible for the observed antimicrobial effects. Additionally, in vivo studies and clinical trials are necessary to assess the safety, efficacy, and optimal dosage of the ethnobotanical mixture for potential therapeutic use against infectious diseases caused by drug-resistant bacteria.

In conclusion, the present study highlights the potential of the Hawan Samagri mixture, consisting of six herbs, as a broad-spectrum antimicrobial agent against clinical bacterial isolates. These findings contribute to the growing body of evidence supporting the use of indigenous resources for developing therapeutic agents to combat antimicrobial resistance. Incorporating these herbal mixtures into daily rituals and practices of Hawan may serve as a preventive measure against antimicrobial resistance, although further research is required to validate their clinical applications.

Compliance with ethical standards Institutional ethical clearance was obtained.

Conflict of interest The authors declare that they have no conflict of interest.

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