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Traditional Knowledge Driven Screening of 30 Medicinal Plants from Gandhmardhan, Odisha, That are Effective Against Human Multidrug Resistant Pathogenic Bacteria

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Abstract

The medico folklore use of medicinal plants is the mainstay healthcare system of the tribals in and around the Gandhamardan, Western Odisha. These people depend on the forest and traditional Vaidyas to treat various health ailments, including bacterial infections. While documenting folklore claims, we found the acute dependency of traditional diarrhoea on 30 medicinal plants from the Gandhamardan for treating respiratory tract infections, skin diseases, wound infections, diarrhoea, and dysentery. The plant parts of these plants were collected from the wild, and their phytochemical composition was determined using aqueous and ethanol extracts. The crude extracts at different concentrations were used to elucidate their antibacterial activity based on the zone of inhibition using the agar well diffusion method using eight pathogenic bacteria. The plant extracts revealed the presence of eight secondary metabolites, viz. alkaloids, glycosides, terpenoids, reducing sugar, saponins, tannins, flavonoids, and steroids. All 30 plants were very effective against all the MDR bacteria. The study would provide a scientific basis for clandestine ethnobotanical knowledge that would benefit the antimicrobial stewardship program.

Keywords: Ethnobotanical information, Gandhamardan, MDR pathogens, Medicinal plants, Antibacterial activity, Phytochemical analysis.

Introduction

Throughout history, the indigenous medicinal plants of the Gnadhamardan hill range have provided health treatment for marginalized tribal residents. However, using unprocessed medicinal plants was intended to be a preventative measure against health issues. Two opposing schools of thought regarding the mechanisms underlying the herbal products' purported therapeutic value exist. In one perspective, these benefits are explained as placebo effects, whereas the synergy of multiple bioactive components is attributed in the other (1, 2). Herbal medications have been extensively studied as supplements in folk and traditional medicine, even if the precise mechanism of action for most of them is still unknown. But to effectively explore native medicinal plants, scientific research is needed to clarify biological activity in addition to chemical research, which allows for the quick identification of recognized active chemicals and false positives. Due to this, several new chemical entities from ethnomedicine will be developed (3). This conventional knowledge-driven active chemical identification method will be an effective search engine. Most critical, though, is that it will make it much easier to conduct targeted and secure natural product research to discover the drug development process (4, 5). Countless antibiotics have been created over time, saving millions of lives and easing their pain. However, overuse of antibiotics, urbanization, pollution, the AIDS epidemic, and other concurrent factors have significantly accelerated the creation of antibiotic-resistant microorganisms over the past few decades. Multidrug-resistant (MDR) forms of bacterial pathogens have emerged, making infections the world's leading cause of mortality. This is a primary global health concern (5, 6).

Gandhamardhan is a hill range in the state of Odisha, India, known for its rich biodiversity and

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the presence of various medicinal plants. The region is famous for Gandhamardan Parvat, which is associated with the mythological story of Lord Hanuman carrying the Sanjeevani herb to heal Lakshmana in the Ramayana. Traditional medicine is often an integral part of the daily life of the tribal communities of Gandhamardhan. The remedies are not only used for treating illnesses but also for preventive healthcare. Certain plants and herbs may be included in dietary practices or used in daily rituals. Tribal communities of this region have a profound understanding of the local flora, including various plants, herbs, and trees. They rely on this knowledge to identify medicinal plants and their uses for treating different ailments. Different parts of plants, such as leaves, roots, bark, and seeds, are often used to prepare traditional remedies. These remedies may be administered orally, topically, or through other methods. Their medicinal practices are often intertwined with rituals and cultural ceremonies. Healing is seen as a holistic process that involves not only the physical aspects but also spiritual and emotional well-being. Rituals may include the use of specific plants or herbs, along with chants or prayers. Some commonly used plants are neem, turmeric, amla (Indian gooseberry), tulsi (holy basil), ashwagandha, and others. These plants are chosen based on their perceived medicinal properties.

Our need to create innovative, potent antimicrobial agents resistant to established resistance mechanisms stemmed from the growing prevalence of antibiotic resistance. These medicines are based on principles derived from native medicinal plants and have unique modes of action (7). antimicrobials derived from plants are less expensive than synthetic medications, rarely cause adverse effects, and offer a vast therapeutic potential for treating various infectious disorders. Given the potential of plant medicines as antifungal and antibacterial agents, a systematic search is beneficial to find novel ones that work as additional or alternative control agents against multidrug-resistant infections (7–10).

Materials & Methods

Collection and authentication of plant materials

Ethnobotanical surveys were conducted in the Gandhamardan hill ranges situated in between Bargarh and Bolangir districts of western Odisha, India, which lies between 20°42' a 21°00' N latitude and 82°41' a 83°05' E longitude (Figure 1). Frequent visits were made to collect forest plant samples in November and December 2019. The village dwellers, the herbal medicine practitioners commonly known as Kabirajs or Vaidyas, and other traditional healers were contacted and interviewed during the visits to record their ethnomedicinal uses, dosages, and mode of administration. Information on 30 ethnomedicinal plants has been recorded in this manuscript as used by the tribals and inhabitants for curing bacterial disorders (Table 1). This information on medicinal uses was also crosschecked with the earlier published literature. The identification of gathered plant species was aided by the local flora. Samples on vouchers are kept in the herbarium of Sambalpur University's Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics. The bark, leaves, and roots of the plant were removed; they were then washed, dried in a tray drier, ground into a powder with a pulverizer, sieved, and sealed in containers. The materials were kept for future research in a dry, cool environment (11-13).

Preparation of plant extracts

About 20 g of powder samples were solvent extracted using a sufficient quantity of 80% ethanol and sterile double-distilled water using the Soxhlet apparatus for 18 hours at 60-80 ^{o}C. The extracts were concentrated, dissolved in 2.0 mL aliquots of 10% dimethyl sulfoxide (DMSO), and stored at four °C until further use.

Qualitative analysis of phytochemicals

The extracts were subjected to phytochemical screening tests to detect various constituents using the conventional protocol (14-16).

Test for alkaloids: Dragendroff's test, Hager's reagent test, Mayer's reagent test, and Wagner's reagent test.

Test for flavonoids: Aqueous sodium hydroxide test, Ammonia test, The concentrated nitric acid test

Test for sterols: Moleschott's test, Hess's test, Salkowski's test

Test for triterpenoids: Liebermann Burchard's test; Tin and thionyl chloride test

Test for tannins and phenolic compounds: Ferric chloride test; Reaction with lead acetate; Reaction with gelatin solution:

Test for saponins: Foam test

Test for reducing sugars: Fehling's test

Test for glycosides: Legal's test

Isolation and Identification of Microorganisms

Test Microorganisms: Antibacterial activity was tested against eight strains of antibiotic-resistant bacteria, of which 3 were Gram-positive *E. faecalis*, *S. aureus*, *S. pyogenes*, *A. baumannii*, *C. freundii*; *P. mirabilis*; *P. vulgaris*; *P. aeruginosa*. The bacterial strains were obtained from the NICU and PICU of a private medical college, ODISHA, and they were antibiotic-resistant strains (17-19).

Detection of MRSA and ESBL producers

MRSA was detected using a chromogenic agar media test. In contrast, the double-disc synergy test (DDST) detected the extended-spectrum beta-lactamases (ESBL) producing gram-negative bacterial strains (17-19).

Antibacterial activity test by agar well diffusion method

The antibacterial potentiality of plant extracts against the eight bacterial pathogens was tested by the "agar well diffusion method", where piperacillin-tazobactam 30 mg/mL was the standard positive control and 10% DMSO as the negative control, as previously detailed (17-19).

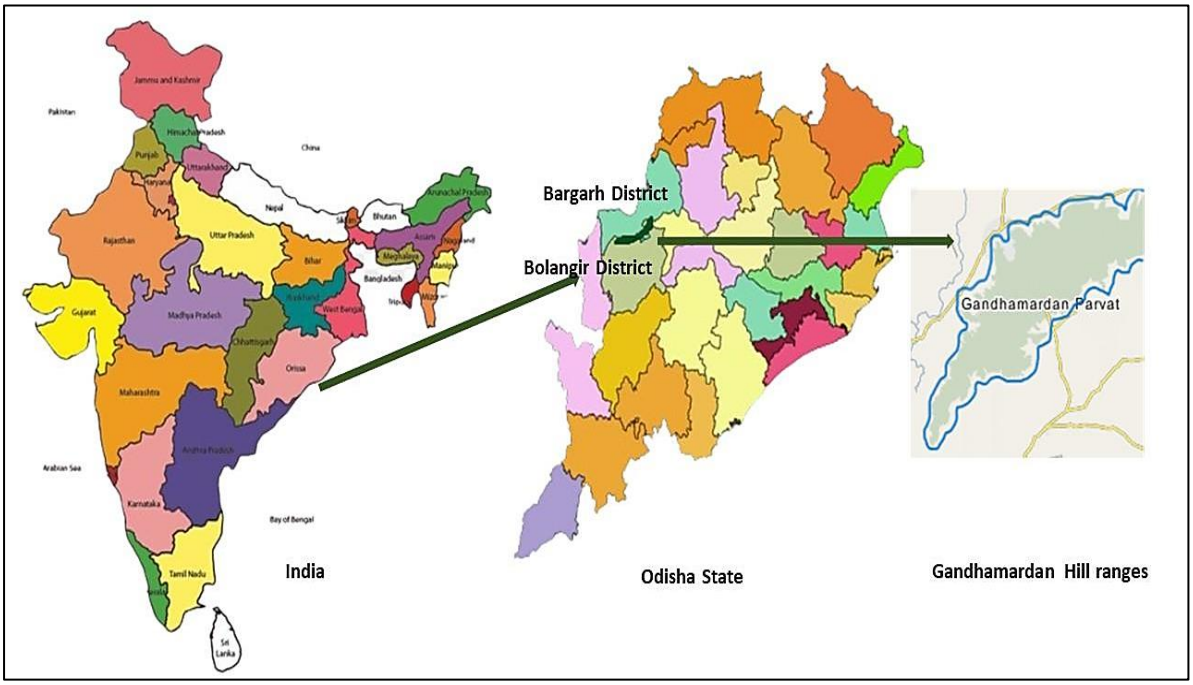

Figure 1: Map of Gandhmardhan hills range

Results

Screening of ethnomedicinal plants

Based on the information collected from the traditional healers and other herbal medicine practitioners on the ethnomedicinal uses of medicinal plants from the Gandhamardan hills, we screened 30 plant species for this study (Table 1). These plants are in use for treating diseases such as allergies, asthma, bronchitis, dyspepsia, leprosy, jaundice, cholera, malaria, rheumatism, nausea, fever, colitis, skin, stomach, etc., by the tribals and other ethnic communities in and around the Gandhamardan.

Table 1: Ethnomedicinal uses of 30 plants by locals in and around the Gandhamardan hills range, Western Odisha, India

S. No.	Plants and Family name	Local Name, Parts used	Ethnomedicinal uses
1	*Andrographis paniculata* (Burm. f) Wall, Acanthaceae	Bhuinimba Leaf	Treatment of respiratory infection, cough, cold, joint pain, mosquito repellant.
2	*Anthocephalus cadamba* (Roxb.) Rubiaceae	Kadamba Leaf and bark	UTI, diarrhoea, fever, cough, inflammation, vomiting, wounds, and ulcers.
3	*Arisaema murrayi* Araceae	Sarpa Kanda Leaf, seeds	Eye troubles, wound infections, cold sores, skin diseases, and jaundice.
4	*Aspidopterys tomentosa* (Blume) Malpighiaceae	Alatilaha Roots	Roots are used against eczema and skin rashes.
5	*Bacopa monnieri* L. *Pennell* Scrophulariaceae	Brahmi Whole plants	Ulcer, diarrhoea and fevers, and asthma.
6	*Bupleurum marginatum L.* Apiaceae	Bana jeera Leaf	Diarrhea, UTI, skin diseases
7	*Calamus guruba* Buchaham Arecaceae	Kanta beta Fruits	Skin diseases and chest pain.
8	*Calotropis procera* (Aiton), Asclepiadaceae	Arakha Leaf, bark	Asthma, fever, respiratory, skin and intestinal infections
9	*Cissus quadrangularis* L. Vitaceae	Hadajoda Leaf	Colic, leprosy, ulcers, tumours and skin diseases.
10	*Cassytha filiformis* L.Lauraceae	Akashi bela Leaf	Skin diseases, inflammations, syphilis, boils, leprosy, dry cough, bronchitis and dysentery.
11	*Diospyrous malabarica* Desr. Ebenaceae	Dhusara kendu Fruit, leaf	Wounds and ulcers, dysentery and diarrhoea.
12	*Diospyrous melanoxylon* Roxb Ebenaceae	Kendu Leaf, Bark	UTI, skin infections, diarrhoea, and dyspepsia.
13	*Elephantopus scaber* L. Asteraceae	Mayurachulia Leaf, root	Loose motions, inflammations, oral and skin infections
14	*Erythroxylum monogynous* Roxb. Erythroxylaceae	Debadaru Fruit, leaf, bark	Stomachic, diaphoretic and diuretic.
15	*Ficus glomerata* Roxb. Moraceae	Dumer Leaf	Diarrhoea, diabetes, piles and skin infections
16	*Ficus racemosa* L. Moraceae	Bidimiri Fruit, leaf, bark	Antiseptic, wound healing, piles, diarrhoea.
17	*Glycyrrhiza glabra* L. Fabaceae	Yasthimadhu Leaf	Fever, oral ulcer, throat infections and
18	*Gardenia gummifera* L.Rubeaceae	Bhurudu koli Fruits, leaf gum	Antiseptic, diarrhoea, and nervous disorder.
19	*Lantana camara* L. Verbenaceae	Nagaoiri Leaf	Common cold, fever, skin infections, malaria and tuberculosis
20	*Madhuca indica* Gmel. Sapotaceae	Mahula Leaves, flowers, oil	Joint pains, itches, tonsillitis, diabetes, constipation, piles.
21	*Ocimum sanctum* L.Lamiaceae	Banatulasi Leaf	Cold, cough, skin diseases.
22	*Opilia amentaceae* Roxb. Opiliaceae	Dureikoli Fruits, leaves	Dropsy, swellings, gout and kidney troubles, and leprosy.
23	*Oroxylum indicum* (L.) Kurz. Bignoniaceae	Phanphana Leaf, Bark	Skin infections, diarrhoea, and oral infections

24	*Passiflora foetida* L. Passifloraceae	Gandhatamala fruits, leaves, barks	Wound healing, hysteria, itches.
25	*Rauvolfia serpentina* L. Benth. Apocynaceae	Sarpagandha Leaf	Snake and reptile bites, fever, constipation, feverish intestinal diseases, achy joint pain, epilepsy.
26	*Shorea robusta* Roth. Dipterocarpaceae	Shala Leaf	Wound healing and diarrhea.
27	*Urgenia indica* Kunth. Liliaceae	Bagomundi pyaz Bulb	Cold, cough, diarrhoea and dysentery.
28	*Uraria picta* (Jacq.) DC Fabaceae	Root or whole plant	Anti-inflammatory, expectorant, and diuretic properties; also used in healing bone fractures
29	*Withamnia somnifera* L. Dunal.Solanaceae	Ashwagandha Leaf	Arthritis, skin infections.
30	*Ziziphus xylopyrus* Retz. Rhamnaceae	Gotha koli Fruit, leaves	Skin diseases wound healing.

Preliminary phytochemical screening

Qualitative phytochemical analysis of the aqueous and ethanol extracts of all 30 medicinal plants was done to determine the presence or absence of secondary metabolites. Both aqueous and ethanol extracts of *A. venenata* and *B. monnieri* revealed the presence of eight secondary metabolites, *viz*. alkaloids, glycosides, triterpenoids, reducing sugar, saponins, tannins, flavonoids, and sterols. In contrast, the aqueous extract of *E. monogynum* also contains all eight secondary metabolites. It was evident that all plants' ethanol leaf extracts invariably possess most of the secondary metabolites.

Antibacterial activity of plant extracts

The agar well diffusion method tested the antibacterial efficacy of aqueous and ethanol leaf extracts of all 30 medicinal plants against isolated 8 MDR pathogenic bacteria. It was discernible that the ethanolic extracts had better antibacterial efficacy than their corresponding aqueous extracts (Table 2). The plant species, such as *A. paniculata, A. tomentosa, F. glomerata,* and *G. gummifera,* had minor antibacterial activity, as there was no or tiny zone of inhibitions evident in the agar well diffusion method towards most of the bacteria. Similarly, plant species such as *A. murraya, C. guru, C. procera, C. filiformis, E. scaber, O. sanctum,* and *S. robusta* showed moderate antibacterial activities (zone of inhibition < 20 mm) against MDR bacteria. In contrast, the plant species *A. cadamba, B. monnieri, B. marginatum, C. quadrangularis, D. malabarica, E. monogynum, F. racemosa, L. camera, M. indica, O. amentaceae, O. indicum, P. foetida, R. serpentine, U. indica, U. picta, W. somnifera* and *Z. xylopyrus*

Table 2: Antibacterial activity of 30 medicinal plants against the isolated 8 MDR pathogenic bacteria by agar well diffusion method. Zone of inhibition in mm

Plant species	Ef	Sa	Sp	Ab	Cf	Pm	Pv	Pa
A. paniculata	12 (a)	14(11)	a (a)	a(a)	a(a)	a(a)	12(a)	a(a)
A. cadamba	26(24)	27(26)	19(17)	24(23)	22(20)	24(23)	20(18)	22(19)
A. murrayi	15(13)	16(14)	14(13)	12(09)	12(09)	12(09)	a(a)	14(12)
A.tomentosa	14(12)	16(13)	a(a)	11(09)	a	13(11)	11(10)	15(13)
B.monnieri	22(19)	25(23)	25(23)	21(19)	18(15)	16(15)	20(18)	17(14)
B.marginatum	23(21)	25(23)	22(19)	18(16)	20(18)	19(17)	16(15)	22(20)
C. guruba	21(19)	23(21)	15(12)	17(15)	11(a)	11(a)	12(11)	14(13)
C.procera	16(15)	19(17)	17(15)	15(14)	12(11)	10(a)	12(10)	18(17)

C. quadrangularis	24(23)	26(25)	19(18)	18(17)	14(13)	17(16)	12(11)	20(19)
C. filiformis	16(14)	20(18)	15(13)	16(15)	13(11)	15(13)	14(13)	17(15)
D. malabarica	24(23)	27(25)	19(17)	18(17)	15(13)	16(14)	14(13)	19(18)
E. melanoxylon	21(19)	24(22)	17(15)	15(13)	12(11)	13(12)	18(16)	18(17)
E. scaber	20(18)	22(19)	19(17)	13(12)	10(a)	a	14(12)	13(11)
E. monogynum	26(24)	29(28)	21(20)	17(15)	19(18)	16(15)	22(21)	18(16)
F. glomerata	22(21)	23(22)	12(11)	13(12)	a	a	a	14(13)
F. racemosa	19(17)	21(19)	11(10)	14(13)	10(a)	14(12)	15(13)	17(16)
G. glabra	17(15)	19(18)	14(13)	15(14)	12(11)	13(12)	12(11)	16(14)
G. gummifera	10(a)	11(10)	a	a	a	12(11)	a	a
L. camara	23(20)	26(25)	20(19)	20(18)	24(22)	20(18)	21(19)	22(20)
M. indica	22(21)	25(24)	14(13)	18(17)	15(13)	13(12)	16(14)	15(14)
O. sanctum	19(17)	22(21)	11(10)	a	13(12)	14(13)	16(15)	13(12)
O. amentaceae	21(20)	24(23)	13(12)	16(15)	15(14)	18(17)	19(18)	20(19)
O. indicum	24(23)	28(26)	19(18)	17(16)	15(14)	23(22)	25(24)	23(22)
P. foetida	22(21)	25(24)	20(19)	13(12)	14(13)	21(20)	18(17)	21(20)
R. serpentina	25(24)	29(27)	21(20)	24(23)	22(21)	28(27)	23(22)	29(27)
S. robusta	18(17)	20(18)	13(12)	11(10)	a	14(13)	12(11)	16(15)
U. indica	22(19)	24(23)	17(15)	13(11)	15(13)	16(14)	14(13)	13(12)
U. picta	26(25)	25(24)	25(23)	21(20)	22(21)	26(24)	27(26)	24(23)
W. somnifera	19(18)	22(20)	14(12)	15(14)	16(15)	17(15)	15(13)	11(a)
Z. xylopyrus	17(15)	23(21)	16(15)	21(20)	14(13)	11(10)	16(15)	14(13)
Linezolid (30µg/mL)	29	29	33	-	-	-	-	-
Imipenem (10µg/mL)	a	a	a	31	29	29	26	34

Table 3: Screening of bark extracts of 5 medicinal plants against the isolated 8 MDR pathogenic bacteria by agar well diffusion method. Zone of inhibition in mm

Plants	Ef	Sa	Sp	Ab	Cf	Pm	Pv	Pa
A. paniculata	13(a)	15(12)	a	a	11(a)	a	13(10)	a
E. monogynum	27(25)	20(19)	16(14)	23(22)	20(18)	16(15)	24(23)	17(16)
F. racemosa	30(29)	16(15)	15(13)	17(14)	22(20)	15(14)	27(26)	16(14)
O. indicum	25(24)	29(27)	24(23)	20(19)	19(17)	24(21)	26(25)	29(28)
P. foetida	23(22)	26(24)	22(21)	22(20)	14(13)	22(21)	19(18)	28(26)

recorded high antibacterial activity against all the 8 MDR bacteria. Aqueous and ethanol extract of *C. paniculatus, L. camara, O. indicum, P. santalinus* and *W. fruticosa* recorded excellent antibacterial activity against most of the isolated MDR bacteria (zone of inhibition > 20 mm). Similarly, the zone of inhibitions of all extracts was recorded in Tables 2 and 3.

Discussion

It is discernable from the study that, *A. cadamba, B. monnieri, B. marginatum, C. quadrangularis, D. malabarica, E. monogynum, F. racemosa, L. camera, M. indica, O. amentaceae, O. indicum, P. foetida, R. serpentine, U. indica, U. picta, W. somnifera,* and *Z. xylopyrus* recorded high antibacterial activity against all the 8 MDR bacteria. Aqueous and ethanol extracts of *C. paniculatus, L. camara, O. indicum, P. santalinus* and *W. fruticosa* recorded excellent antibacterial activity against most of the isolated MDR bacteria and most of them contained the maximum secondary metabolites.

An impenetrable barrier is the exquisite stress of phytodrugs, a naturally occurring mixture of many chemicals in a crude plant extract. Consequently, the crude extract of no medicinal plant could overcome MDR bacteria, even though

they were well armed with the arsenal of multidrug resistance. From this angle, the lack of commitment to using crude phytodrugs as antimicrobials would offer a quick and workable remedy in the fight against rapidly developing multidrug-resistant infections (20–22). However, to achieve the ultimate goal of comprehensive disease control, the hunt for pure compounds from crude extract should continue. Crude extracts consistently control bacterial strains in vitro, as evidenced by the countless publications on the antibacterial activity of medicinal plants against drug-sensitive/standard strains of bacteria. Therefore, discrediting crude extracts as medications would erode the legitimacy of therapeutic plants and create a frenzy of opposition to the drug-targeting initiative (23–25).

Antibiotic-sensitive pathogens have a limited ability of virulence as standard antibiotics can control *in vivo*. At a particular stage, the host's resistant framework additionally permits dealing with the pathogens, while they don't last much. Without a doubt, for the immune system, anti-infection-creating life forms possess antibiotic-safe qualities in plasmids and chromosomes, just as the related switch components. In this manner, such qualities or potential transposon ought to be taken up, a cloister, on a level plane by utilizing the powerless association of microscopic organisms through bacterial change and conjugation (26, 27). Additionally, microbes having basic/plastic genomes experience characteristic (changes) or secure hereditary (conjugations and change) adjustments inside the nearness of an anti-infection as a weight factor from a medication-safe strain. Subsequently, gathering antimicrobial opposition components is the logical determinant of the pathogenesis. The direct transfer of hereditary materials from one life form to another appears to be speedier than mutational changes, a marvel prevalently alluded to as the 'development of quantum jumps'. Gradually, using an expanding number of antitoxins to control irresistible disorders has prompted various protections (28). As an impact, such a large number of anti-infection agents are inadequate in forestalling the bit-by-bit developing safe hints of pathogens—with the progression of time, transformation, and procurement of qualities from related/random microscopic organisms end in incredibly repellant to multidrug opposition (28). A medication-safe small-scale living gains the use of enduring and duplicating underneath antitoxin pressure circumstances, affirming the natural standard. Any constraining circumstance for practically all may be a top-notch open door for the minority. Within the sight of a medication in a body *in vivo,* the descendants of a medication delicate strain are wiped out, and the safe pressure endures, increases as though created from a doppelgänger, and prevails over the long haul in causing capacity pathogenesis (29). If unrefined/mostly refined plant concentrate were available in corresponding with the utilized anti-infection, there would be the pined for the blithesome outcome (30, 31).

The major ramification of the use of crude extracts is its standardization and quality control. Ensuring the consistent quality and potency of antibacterial plant products can be challenging due to variations in plant growth conditions and extraction methods. Further, the regulation of herbal remedies and plant-based medicines varies globally. Establishing standardized protocols for testing and quality control is important for ensuring safety and efficacy. To address this issue, more research is essential to identify new antibacterial plants, understand their mechanisms of action, and optimize their use in various applications. Advances in biotechnology may enable the development of genetically modified plants with enhanced antibacterial properties.

Conclusions

While there is promising evidence for the antimicrobial properties of plant extracts, their effectiveness can vary, and more research is needed to establish their clinical use. Additionally, the use of crude plant extracts raises challenges related to standardization, quality control, and potential side effects. Moreover, the ethnic information on plants is a boon to scientific researchers during their work on drug targeting. This work would help the apothecaries locate phytodrugs as complementary /supplementary drugs to treat these MDR bacteria.

Abbreviations

Nil

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Author's contribution

DD and SR conceptualized, conducted the study, and drafted the manuscript. SKT helped in phytochemical analysis. SKS and SI helped in the literature survey. DD and SR finalized the manuscript. All authors critically verified the manuscript.

Conflicts of interests

The authors declare they do not have any conflicts of interests

Ethical approval

Approved by Institute Ethical Committee vide letter no IMS SH/IEC/2018/37 dated 15/03/2018.

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