

Article

Phytochemical Analysis of Bangladeshi Medicinal Plants Led to the Isolation of Anti-Staphylococcal Compounds

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Received: 20 June 2024; Revised: 26 August 2024; Accepted: 12 September 2024; Published: 24 September 2024

Abstract: Antibacterial resistance is a major threat to global health. Due to its new resistance mechanisms, it is spreading and emerging widely, thereby threatening the treatment of common infectious diseases. Ancient history and ethnopharmacological studies highlighted the importance of natural sources in treating resistance infections. This study involved bioassay-directed phytochemical investigation on Bangladeshi medicinal plants selected by an ethnopharmacological survey to explore antibacterial compounds against Methicillin resistance *Staphylococcus aureus* (MRSA). In 2016, an ethnopharmacological survey conducted in Bangladesh led to the recommendation of 71 medicinal plants by 127 respondents (71 Ayurvedic/Unani practitioners, 21 Ayurvedic patients and 35 local inhabitants) for the treatment of infectious diseases. Based on the literature review, data analysis of the ethnopharmacological survey and ease of availability of the plants, 18 plants were initially selected and collected from Bangladesh. After the initial antibacterial screening of 18 plants, five plants with Minimum Inhibitory Concentration (MIC) of 32–512 µg/mL were chosen based on potential antibacterial activity. These are (*Zingiber montanum*, *Uraria picta*, *Diospyros malabarica*, *Cynometra ramiflora*, *Swertia chirayita*). Extensive phytochemical work using different chromatographic and spectroscopic techniques on five Bangladeshi medicinal plants led to the isolation and identification of 24 compounds. Eight terpenes (zerumbol (3), zerumbone (4), buddledone A (5), germacrone (6), furanodienone (7), (–) borneol (1), camphor (2) and 8(17), 12-labdadiene-15, 16-dial (8) were isolated from *Zingiber montanum* with the MIC (32– >128 µg/mL). Eugenol (14) and steroids were isolated from *Uraria picta* (MIC 64– >128 µg/mL). Lupane-type triterpenoids (Lupeol (20), betulin (21), betulinaldehyde (23), betulone (24) and messagenin (22) were isolated and identified from *Diospyros malabarica* with the MIC (64– >128 µg/mL), while pentacyclic triterpene (glutinol (10), glutinone (11)), simple phenolic (ethyl 4-ethoxybenzoate (9)) and steroids were isolated from *Cynometra ramiflora* with MIC (64– >128 µg/mL). A series of xanthenes (swerchirin (16), swertiaperenin (17), bellidifolin (18) and decussatin (19)) were identified from *Swertia chirayita* with MIC (>128 µg/mL). 4-ethoxybenzoate (9) and messagenin (22) were identified as new natural compounds among these compounds. In terms of activity, 8(17), 12-labdadiene-15, 16-dial (8) (32 µg/mL against ATCC 5941) and zerumbol (3) (32 µg/mL against EMRSA 15) exhibited potential antibacterial activity. Phytochemical discoveries of Bangladeshi medicinal plants gave a new dimension to exploring anti-staphylococcal compounds.

Keywords: antibacterial resistance; MRSA clinical strains; ethnopharmacological survey; phytochemistry; isolation; column chromatography; identification of chemical structure; NMR; mass spectrometry; medicinal plant extract

1. Introduction

The incidence of the number of bacterial pathogens bestowing antimicrobial and multidrug resistance to antibiotics has remarkably accelerated over the past few decades. The mishandling, misuse and abuse of antimicrobial agents are the main reasons for the emergence of the resistant genes in microorganisms [1]. Apart from manifestation of the antimicrobial agent, antimicrobial resistance may occur due to the mutations in bacterial DNA or the acquisition of bacterial resistance gene through horizontal gene transfer [2]. Antimicrobial resistance is now a serious and complex problem for global health, requiring a multi-disciplinary approach involving partners from all health sectors, including public health authorities and the scientific community.

Resistant infection has become the third leading cause of mortality worldwide [3]. The statistics indicate that around 33,000 people die each year in Europe due to AMR (antimicrobial resistance), and more than 670,000 people are affected with antimicrobial resistance infections [4]. AMR has made cancer chemotherapy, organ transplantation, diabetes management and major surgery like caesarean sections more challenging [2]. Unless



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appropriate action is taken to tackle the threat, drug-resistant infections will kill an extra 10 million people annually worldwide by 2050, which is more than the predicted death of cancer, and it will cost 100 trillion USD of the world's economic output [5].

Medicines from natural sources have contributed to humankind's pharmacy since antiquity; hence, plants may be a source for developing new novel antimicrobial compounds followed by subsequent pharmacological, chemical and clinical studies [6]. For example, the most prominent anti-inflammatory agent, acetylsalicylic acid, commercially known as the drug aspirin, is isolated from the bark of the willow tree *Salix alba* L. [7]. Quinine isolated from the bark of *Cinchona succirubra* has been used traditionally to treat malaria, indigestion, fever, mouth and throat diseases for centuries [6].

An ethnopharmacological survey is a strategy to select medicinal plants for scientific exploration of biologically active agents. An ethnopharmacological survey was conducted in Bangladesh during the autumn of 2016 to document indigenous knowledge regarding the treatment of infections. The aim was to identify plants that could be selected for the phytochemical investigation to identify vital secondary metabolites responsible for their anti-infective properties.

Based on the outcome of the ethnopharmacological survey [8] and the availability of plants, 18 plants were initially chosen and collected from various parts of Bangladesh. Five plants were chosen for further phytochemical study based on the potential antibacterial activity of the plant extracts. The research involved finding potential anti-infective secondary metabolites from Bangladeshi medicinal plants using bioassay-directed chromatographic and spectroscopic methods. The author reported the isolation and identification of 24 pure compounds and their antibacterial activity against a panel of clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

2. Material and Methods

2.1. Ethnopharmacological Survey

The ethnopharmacological survey was carried out in Bangladesh during autumn 2016. A total of 127 respondents, including 71 practitioners, 21 Ayurvedic hospital patients, and 35 village inhabitants, participated in the survey [8].

2.2. Collection of Plant Materials

Eighteen plants (Section 3.1.) selected through the ethnopharmacological survey were collected from various parts of Bangladesh, including the Bangladesh National Botanical Garden (Mirpur, Dhaka), the Medicinal Plant Garden of Govt. Unani and Ayurvedic Hospital (Mirpur, Dhaka), and the Sundarbans mangrove forest (Khulna), Rajshahi University Botanical Garden (Rajshahi).

2.3. Drying and Grinding

All the samples were sun-dried (40 °C) for 2–3 days in Bangladesh. The selected plants' bark, stems, leaves, or fruits were the samples. After bringing the samples to the United Kingdom, they were sun-dried at the University of East London, United Kingdom, before being cut into pieces with a plant pruner and oven-dried at 30–40 °C for 30 min before grinding with a grinder. Subsequently, all the samples were ground into fine powders.

2.4. Extraction and Bioassay

Initially, small-scale (10–20 g) plant material was extracted sequentially with 80–100 mL solvents of increasing polarity (hexane, chloroform, and methanol), which offers efficient extractions for preliminary bioassay against MRSA clinical strains.

Each extract was subjected to antibacterial screening by microtiter assay using 96-well plates [9]. Five plants were selected for bioassay-guided phytochemical study to identify potential antibacterial secondary metabolites based on potential antibacterial activity against MRSA clinical strains (EMRSA-15, SA1199B, ATCC25941, XU212).

Large-scale extraction of 5 selected plants was completed with 230–300 g of plant material (Section 3.2.) with Soxhlet apparatus with solvents (approximately 800–900 mL) of increasing polarity Hexane < Chloroform < Methanol. Each extract was concentrated using a rotary evaporator under reduced pressure at a maximum temperature of 40 °C to afford crude extracts as semi-solid mass.

2.5. Fractionation of the Plant Extracts

Column chromatography with silica and Sephadex LH20, Vacuum liquid chromatography, SPE (Solid Phase Extraction) and preparative thin layer chromatography were used to isolate pure compounds. Thin layer chromatography and reagent 1% Vanillin in sulfuric acid were applied to monitor the fractions. The chromatography and combination of solvents used in isolating the compounds are listed in Tables S1–S6.

2.6. Identification of Pure Compounds

Isolated compounds were identified by 1D and 2D Nuclear Magnetic Resonance, High-Resolution Mass Spectrometry, and Infrared Spectroscopy.

2.7. Antibacterial Activity of the Pure Isolated Compounds

Antibacterial screening of the plant extracts and pure secondary metabolites was conducted using a minimum inhibitory concentration (MIC) assay against MRSA clinical strains.

Antibacterial screening was expressed in terms of $\mu\text{g/mL}$ [9]. The antibacterial activities were evaluated against a panel of clinical strains of MRSA, including XU212, EMRSA15, SA1199B, MRSA 340702 and MRSA 24821, along with MRSA standard strain ATCC 25941. These efflux strains are resistant to common antibiotics such as SA1199B, which is resistant to fluoroquinolones, and XU212, which is resistant to tetracycline. The antimicrobial activities of the active compounds were compared to the standard antibiotic, norfloxacin. The MIC of norfloxacin was found to be in the range of 16–128 $\mu\text{g/mL}$.

The starting concentration was 512 $\mu\text{g/mL}$ (four times more diluted than the stock concentration). The plant extracts with a MIC concentration $\leq 256 \mu\text{g/mL}$ were mainly chosen for further phytochemical investigation.

3. Results

3.1. List of Plants

Table 1 is the list of plants collected from various regions of Bangladesh. Plant parts such as leaf, fruit, bark, rhizome, or thorn of the selected plants were collected from 7 to 13 September 2016.

Table 1. List of the plants collected for the study.

Scientific Name	Plant Part(s) Collected	Place of Collection	Date of Collection
<i>Paedaria foetida</i>	Leaf	NBG	7 September 2016
<i>Aegle marmelos</i>	Fruit	NBG	8 September 2016
<i>Justicia adhatoda</i>	Leaf	NBG	8 September 2016
<i>Terminalia arjuna</i>	Bark	GUAH	9 September 2016
<i>Diospyros malabarica</i>	Leaf	NBG	9 September 2016
<i>Andrographis periculata</i>	Leaf	NBG	9 September 2016
<i>Tinospora cordata</i>	Leaf	NBG	10 September 2016
<i>Zingiber montanum</i>	Rhizome	NBG	10 September 2016
<i>Azadirachta indica</i>	Bark and Leaf	NBG	11 September 2016
<i>Tylophora indica</i>	Leaf	NBG	11 September 2016
<i>Tribulus terrestris</i>	Thorn	NBG	12 September 2016
<i>Uraria picta</i>	Leaf	RUBG	12 September 2016
<i>Cynometra ramiflora</i>	Leaf	SF	13 September 2016
<i>Swertia chirayita</i>	Leaf and thorn	NBG	13 September 2016
<i>Terminalia chebula</i>	Fruit	NBG	13 September 2016
<i>Glycyrrhiza glabra</i>	Fruit	NBG	13 September 2016
<i>Abroma augusta</i>	Leaf with fruit	GUAH	13 September 2016
<i>Feronia limonia</i>	Bark and Leaf	NBG	13 September 2016

3.2. Dry Plant Material

The chart of the powdered plant material indicated that the starting material of *Uraria picta* (300 g) was the highest among all other selected medicinal plants for phytochemical study (Figure 1).

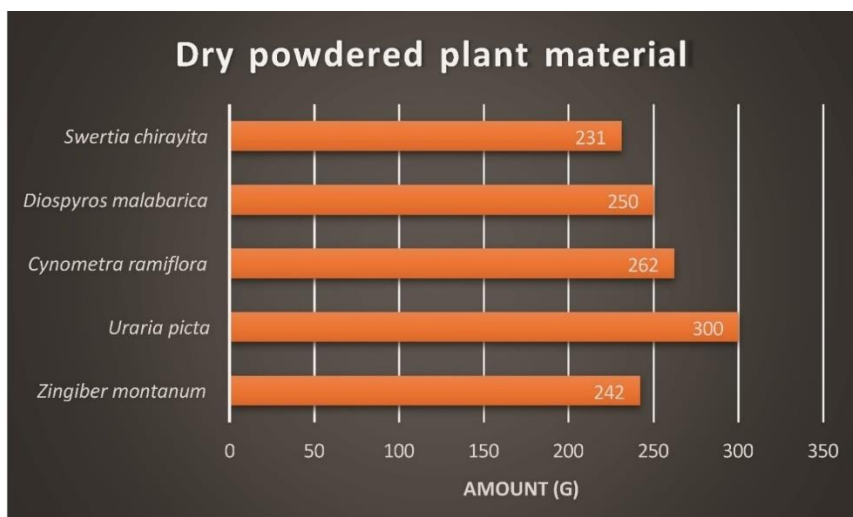


Figure 1. The starting amount of the plant material used for isolation and identification of pure compounds.

3.3. Percentage Yield of Extracted Plant Material

The Figure 2 shows the percentage yield of the plants' extraction with hexane, chloroform, and methanol of increasing polarity using the Soxhlet apparatus. Methanol extract of *Z. montanum* had the highest percentage yield (9.5%).

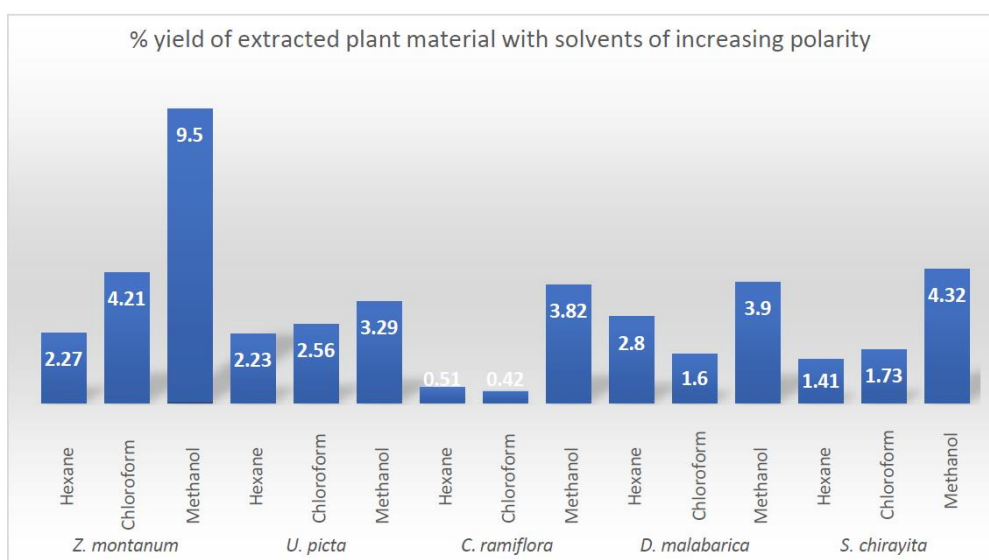


Figure 2. the percentage yields of the plant extracts with solvents of increasing polarity.

3.4. The Chemical Structures of the Isolated Compounds Are Given Below

3.4.1. Compounds isolated from *Z. montanum*

Eight compounds were isolated from *Z. Montanum*. Their chemical structures were listed in Figure 3.

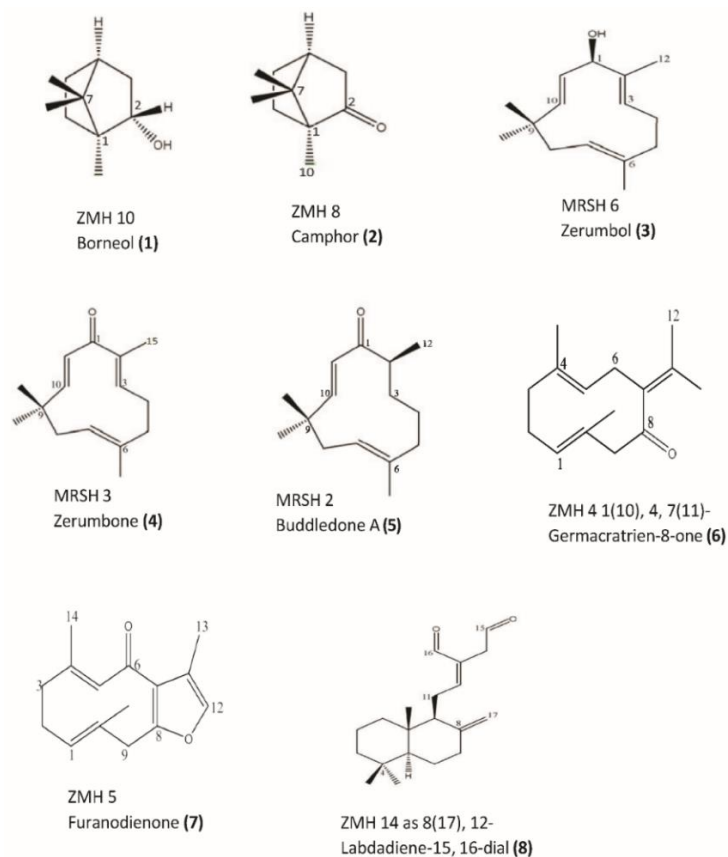


Figure 3. Chemical structures of compounds isolated from *Z. montanum*.

3.4.2. Compounds isolated from *C. ramiflora*

Five compounds were isolated from *C. ramiflora*. Their chemical structures were listed in Figure 4.

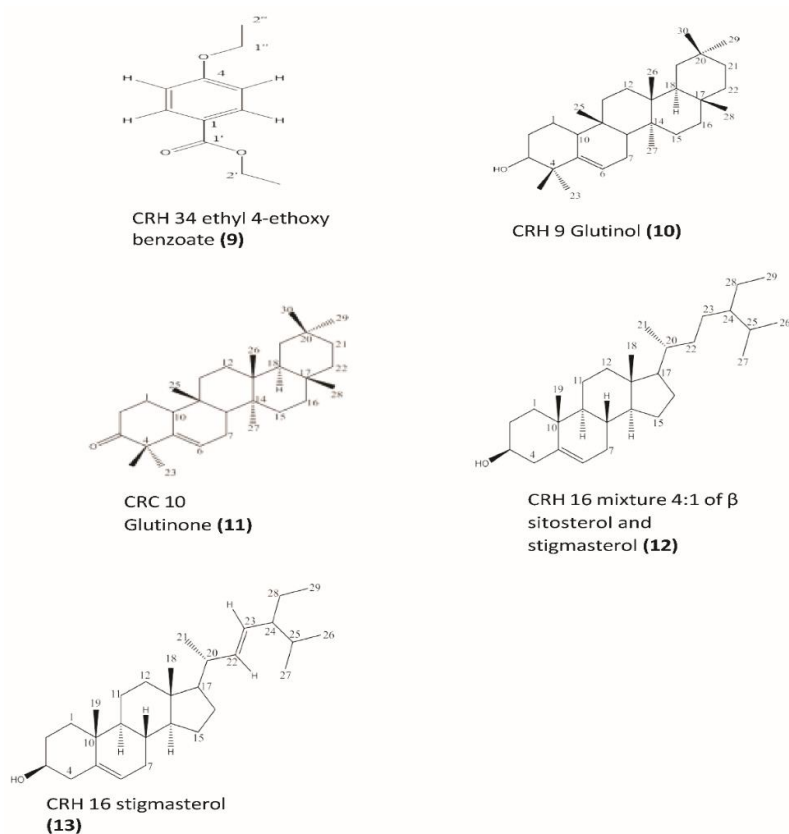


Figure 4. Chemical structures of compounds isolated from *C. ramiflora*.

3.4.3. Isolation of compounds from methanol extract of *U. picta*

Two compounds were isolated from *C. ramiflora*. Their chemical structures were listed in Figure 5.

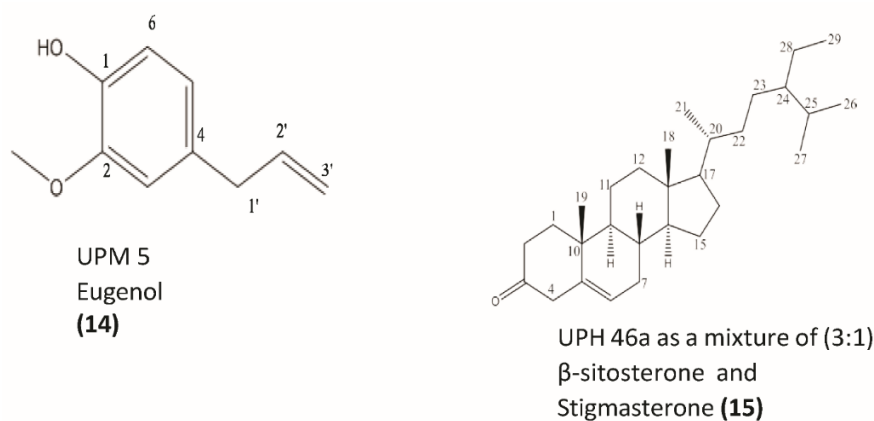


Figure 5. Chemical structures of compounds isolated from *U. picta*.

3.4.4. Compounds Isolated from *S. chirayita*

Four compounds were isolated from *C. ramiflora*. Their chemical structures were listed in Figure 6.

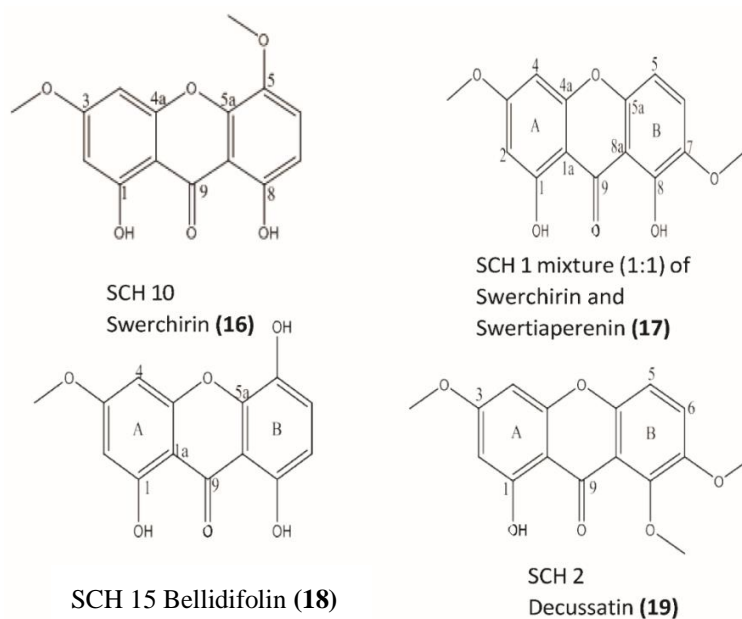


Figure 6. Chemical structures of compounds isolated from *S. chirayita*.

3.4.5. Compounds isolated from *D. malabarica*

Five compounds were isolated from *C. ramiflora*. Their chemical structures were listed in Figure 7.

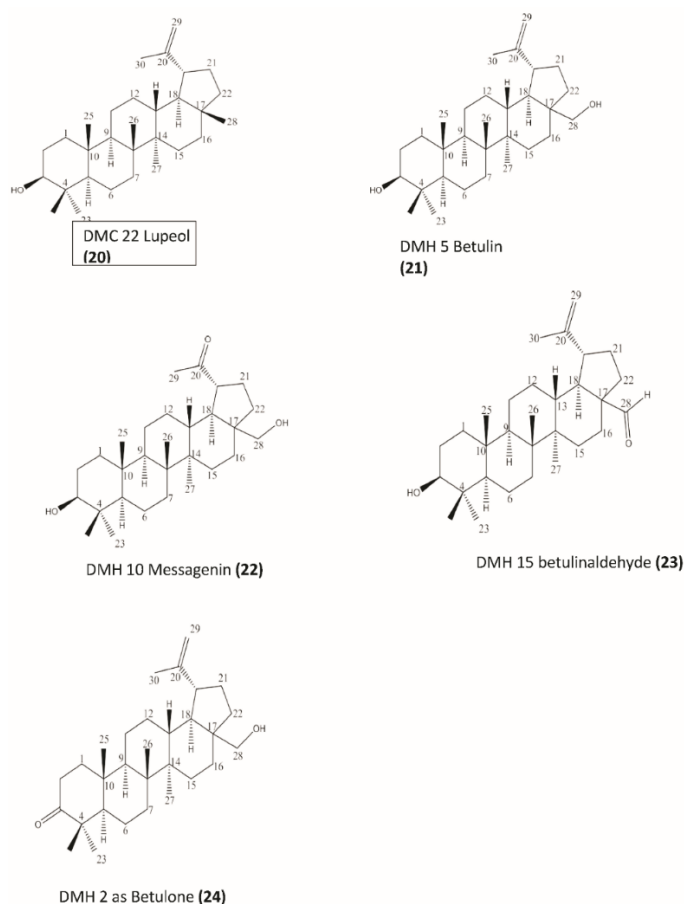


Figure 7. Chemical structures of compounds isolated from *D. malabarica*.

3.5. Antibacterial Screening of Eighteen Plant Extracts

Antibacterial screening of the eighteen medicinal plants resulted in the selection of five plants for further phytochemical investigation based on their potent antibacterial activity (MIC concentration $\leq 256 \mu\text{g/mL}$) (Table 2).

Table 2. The antibacterial screening result of 18 selected plants against clinical strains of Methicillin-resistant *Staphylococcus aureus* in $\mu\text{g/mL}$.

Plant Extract/Standard	MRSA Strains in $\mu\text{g/mL}$			
	EMRSA 15	ATCC 25941	SA1199B	XU212
		<i>Paedaria foetida</i>		
Hexane	512	512	512	512
Chloroform	512	512	512	512
Methanol	>512	>512	>512	>512
		<i>Justicia adhatoda</i>		
Hexane	128	512	128	512
Chloroform	128	512	128	512
Methanol	256	512	512	512
		<i>Aegle marmelos</i>		
Hexane	128	64	128	512
Chloroform	128	64	128	512
Methanol	256	512	512	512
		<i>Abroma augusta</i>		
Hexane	>512	>512	>512	>512
Chloroform	>512	>512	>512	>512
Methanol	>512	>512	>512	>512
		<i>Terminalia arjuna</i>		
Hexane	>512	>512	>512	>512
Chloroform	>512	>512	>512	>512
Methanol	>512	>512	512	128
		<i>Diospyros malabarica</i>		

Hexane	64	128	>512	512
Chloroform	64	128	>512	512
Methanol	>512	>512	>512	>512
<i>Andrographis periculata</i>				
Hexane	256	512	512	512
Chloroform	256	512	512	512
Methanol	256	512	512	>512
<i>Tinospora cordata</i>				
Hexane	>512	>512	>512	>512
Chloroform	>512	>512	>512	>512
Methanol	>512	>512	>512	>512
<i>Azadirachta indica</i>				
Hexane	>512	>512	>512	>512
Chloroform	>512	>512	>512	>512
Methanol	>512	>512	>512	>512
<i>Tylophora indica</i>				
Hexane	512	512	>512	>512
Chloroform	512	512	>512	>512
Methanol	>512	>512	>512	>512
<i>Tribulus terrestris</i>				
Hexane	64	>512	32	64
Chloroform	64	>512	32	64
Methanol	>512	>512	512	>512
<i>Uraria picta</i>				
Hexane	128	64	32	64
Chloroform	128	64	32	64
Methanol	64	64	128	128
<i>Cynometra ramiflora</i>				
Hexane	128	64	128	64
Chloroform	128	64	128	64
Methanol	256	>512	512	256
<i>Swertia chirayita</i>				
Hexane	256	512	>512	>512
Chloroform	256	512	>512	>512
Methanol	256	512	512	>512
<i>Terminalia chebula</i>				
Hexane	128	256	512	512
Chloroform	128	256	512	512
Methanol	>512	>512	>512	>512
<i>Glycyrrhiza glabra</i>				
Hexane	512	512	128	256
Chloroform	512	512	128	256
Methanol	>512	>512	>512	>512
<i>Feronia limonia</i>				
Hexane	512	>512	>512	>512
Chloroform	512	>512	>512	>512
Methanol	128	>512	512	>512
<i>Zingiber montanum</i>				
Hexane	256	128	128	128
Chloroform	128	256	64	128
Methanol	>512	>512	>512	>512
Norfloxacin	16	16	32	64

3.5.2. Antibacterial Screening of Isolated Compounds

Potential antibacterial activity of isolated compounds against Methicillin-resistant *Staphylococcus aureus* clinical isolates listed in Table 3.

Table 3. Showed the results of antibacterial screening of the pure compounds.

Compounds (Number)	MRSA Strains (MIC Values in µg/mL)					
	XU212	ATCC 25941	EMRSA 15	MRSA 340702	MRSA 24821	SA1199B
<i>Z. montanum</i>						
ZMH 10 (1)	>128	128	>128	>128	>128	>128
ZMH 8 (2)	128	128	>128	>128	>128	>128
MRSH 6 (3)	128	64	32	128	128	128
MRSH 3 (4)	>128	128	64	>128	>128	64

MRSH 2 (5)	128	128	128	128	128	128
ZMH 4 (6)	>128	64	>128	>128	>128	>128
ZMH 5 (7)	>128	>128	>128	>128	>128	>128
ZMH 14 (8)	64	32	64	64	128	64
			<i>C. ramiflora</i>			
CRH 34 (9)	>128	64	128	>128	>128	64
CRH 9 (10)	128	>128	128	>128	>128	64
CRC 10 (11)	>128	64	>128	>128	>128	128
			<i>U. picta</i>			
UPM 5 (14)	64	128	128	128	>128	>128
			<i>S. chirayita</i>			
SCH 10 (16)	>128	>128	>128	>128	>128	>128
SCH 1 (17)	>128	>128	>128	>128	>128	>128
SCH 15 (18)	>128	>128	>128	>128	>128	>128
SCH 2 (19)	>128	>128	>128	>128	>128	>128
			<i>D. malabarica</i>			
DMC 22 (20)	64	128	>128	>128	>128	128
DMH 5 (21)	>128	64	>128	>128	>128	>128
DMH 10 (22)	64	>128	64	>128	>128	>128
DMH 15 (23)	>128	64	>128	>128	>128	64
DMH 2 (24)	>128	128	>128	>128	>128	>128
Control (Norfloxacin)	64	32	16	64	64	32

4. Discussion

Z. montanum (Fam. Zingiberaceae) is one of the five chosen plants. It is indigenous to Bangladesh, where it is widely used in the northern part of the country. The ethnopharmacological survey's outcome indicated that *Z. montanum* was being used by traditional practitioners and local village people for the treatment of gastrointestinal infections [8].

The phytochemical analysis led to the isolating of eight terpenes/terpenoids isolated from hexane and chloroform extracts of *Z. montanum*. These compounds were characterized as zerumbone (4), zerumbol (3), buddledone A (5), camphor (2), borneol (1), furanodienone (7), germacrone (6), (*E*)-8(17),12-labdadiene-15,16-dial (8) (Figure 3) using 1D and 2D NMR spectroscopy and mass spectrometry [10]. (*E*)-8(17),12-labdadiene-15,16-dial (8) and zerumbol (3) showed potential antibacterial activity (MIC) values 32–128 µg/mL against a series of clinical isolates of multi-drug resistant (MDR) and MRSA [10] (Table 3). Terpenes are a potential molecule to exert antibacterial activity via a membrane disruption mechanism [11]. Zerumbol (3) was isolated for the first time in this study. Previously, Takashi and his colleagues [12] synthesised optically active zerumbol from zerumbone as the starting material for conversion to useful chiral products such as paclitaxel.

Sesquiterpene zerumbol (3) showed moderate antibacterial activity with an MIC result of 32–128 µg/mL (Table 3). The presence of a hydroxyl group in zerumbol (3) instead of the carbonyl group might make zerumbol (MRSH 6) more active than zerumbone (4) [13]. The antibacterial activity against SA1199B MRSA strains was the same as the crude hexane extract of *Z. montanum*. Still, the compound showed 3-fold stronger antibacterial activity against XU212 and 2-fold stronger antibacterial activity against ATCC clinical strain of MRSA compared to the crude extract. Isolated fraction zerumbol (3) was proved to be bioactive compared to crude extract because the fraction contained a concentrated active principle but might be diluted in other fractions [14].

(*E*)-8(17),12-Labdadiene-15,16-dial (8) (Table 3) exhibited the most potent antibacterial activity with the MIC value 32–128 µg/mL. The compound (MIC value 64 µg/mL) showed the same activity as the control (Norfloxacin, MIC 128 µg/mL) against XU212 and MRSA 340702 bacterial strains. The compound is a labdadiene diterpene with exomethylene, olefin and two aldehyde groups. The presence of these groups and unsaturation could account for the significant antibacterial activity against MRSA strains. Other isolated compounds ZMH 4 (6) and ZMH-5 (7), ZMH 8 (2) and ZMH 10 (1) exhibited moderate activity against MRSA clinical strains. Two isolated fractions of *Z. montanum* exhibited antibacterial activity, which supports its traditional use to treat infectious diseases.

C. ramiflora belongs to the family Fabaceae and is used in herbal medicine [15]. It is Indigenous to India and Bangladesh, predominantly in the mangrove forest Sundarbans, Sri Lanka, and tropical areas of Africa and Australia [16]. The therapeutic activity, such as antihyperglycemic [17], anti-ulcer [18], anti-oxidant [19], antibacterial [20], and cytotoxicity [21] of the leaf and bark of the plant were investigated in-vitro, but the secondary metabolites responsible for the activity was still under research. Therefore, three triterpenoids β sitosterol (12), glutinol (10), glutinone (11), and ester ethyl 4- ethoxy benzoate (9) (Figure 4), were isolated for the first time from *C.*

ramiflora. Among these compounds, ethyl 4-ethoxybenzoate (**9**) appeared to be a new natural product. The isolated compounds exerted moderate antibacterial activity against MRSA clinical strains.

During this study, eugenol (**14**), and a mixture of (3:1) β -sitosterol and stigmasterol (**15**) (Figure 5) were isolated from *U. picta*. Eugenol (**14**) is the major chemical constituent in clove (*Syzygium aromaticum*) oil and manifests a versatile pharmacological action. It was reported for the first time from *U. picta*. Eugenol (**14**) exerted moderate antimicrobial activity with a MIC of 64–128 $\mu\text{g/mL}$ against XU212, ATCC 5941, EMRSA 15, and MRSA 340702 (Table 3). In 2010, Qiu and his colleagues investigated the effect of eugenol on MRSA clinical strains; they suggested eugenol as the base of the new antibacterial drug to combat infectious diseases associated with *S. aureus* [22].

S. chirayita, the popular ethnomedicinal herb indigenous to the Himalayas, has been well-documented in Ayurveda, Unani, Siddha, and other conventional medical systems for its wide spectrum of pharmacological properties [23]. Xanthones are abundant in *S. chirayita*. The current study has led to the isolation of xanthones, including swerchirin (**16**), swertiaperenin (**17**), decussatin (**19**), and bellidifolin (**18**), from the hexane extract of *S. chirayita*. Bellidifolin (**18**) and decussatin (**19**) (Figure 6) were first isolated in 1973 from *S. Chirayita* [24]. The hypoglycemic and antimalarial activity of swerchirin were investigated previously [25,26]. *S. chirayita* contains 40 xanthone derivatives [23]. Ethanolic, aqueous and methanolic extracts of *S. chirayita* documented as exhibiting antimicrobial activity against both Gram-positive and Gram-negative bacteria [27–31]. However, in the current study, xanthone derivatives did not exert imperative antibacterial activity against MRSA clinical strains. Therefore, the author concluded that the crude extract of *S. chirayita* may exert antibacterial activity synergistically compared to individual compounds.

D. malabarica, indigenous to India, Pakistan and Bangladesh, produces edible seasonal fruit [32]. The genus *Diospyros* has been widely researched in terms of phytochemistry. The genus *Diospyros* is dominated by the production of lupane series triterpenoids and naphthoquinones. [33]. In this current study, the lupane series triterpenoids- lupeol (**20**), betulone (**24**), betulin (**21**), betulinaldehyde (**23**) and messagenin (**22**) (Figure 7) were isolated and reported for the first time from the hexane leaf extract of *D. malabarica* species. Among the Lupane series, Messagenin (**22**) has been synthesised in previous studies, but there is no literature on isolating this compound from natural sources [34]. Therefore, the natural compound messagenine (**22**) was reported for the first time in this study. The lupane series are common natural compounds mostly abundant in fruit and vegetables like green pepper, mangoes, grapes, white cabbage and in medicinal plants such as *Celastrus paniculatus*, *Tamarindus indica*, *Himatanthus sucuuba*, *Zanthoxylum riedelianum*, *Leptadenia hastata*, *Sebastiania adenophora* and *Bombax ceiba* [35].

The lupine series was isolated from *D. malabarica* and was tested against MRSA strains. Betulone (**24**) was inactive against all the MRSA strains, while lupeol (**20**) exerted moderate antimicrobial activity against XU212 (64 $\mu\text{g/mL}$) and ATCC 5941 (128 $\mu\text{g/mL}$). Lupeol (**20**) is a triterpene with an exomethylene and hydroxyl group, conferring better antimicrobial activity than Betulone (**24**). Another compound such as messagenin (**22**), displayed activity against XU212 and EMRSA 15 with MIC 64 $\mu\text{g/mL}$, betulinaldehyde (**23**) exerted activity against ATCC 5941 and SA1199B with the MIC of 64–128 $\mu\text{g/mL}$, while betulin (**21**) exerted activity against ATCC 5941 (64 $\mu\text{g/mL}$) only (Table 3).

5. Conclusion

The ethnopharmacological knowledge of Bangladeshi medicinal plants and the demonstration of their anti-infective activity contributed to preserving medicinal plants in decline. In this study, the combination of traditional knowledge and extensive scientific work using a wide range of chromatographic and spectroscopic techniques led to the identification of a total of 24 compounds, including terpenes and simple phenolic compounds with potential antibacterial activity against clinical isolates of a panel of MRSA. This study gives an insightful approach to the scientific explanation of using medicinal plants as primary health care to tackle infectious diseases. In the future, investigating the activity of the plant extracts and their active compounds to understand virulence or pathogenesis will provide useful knowledge of alternative bacterial targets for natural compounds.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.sciltp.com/journals/jmnp/2024/1/405/s1>, Table S1: Isolation of compounds from hexane extract of *Zingiber montanum*.; Table S2: Isolation of compounds from chloroform extract of *Zingiber montanum*. (The starting amount was 242 g); Table S3: Isolation of compounds from methanol extract of *Uraria picta*.; Table S4: Isolation of compounds from Hexane extract of *Cynometra ramiflora*.; Table S5: Isolation of compounds from Hexane extract of *Diosphyros malabarica*.; Table S6: Isolation of compounds from Hexane extract of *Swertia chirayita*.

Author Contributions: The ethnopharmacological survey and phytochemical work were conducted by HS. The final draft of the manuscript was written and edited by HS and MR. MR supervised and elucidated the chemical structures. All authors have read and agreed to the published version of the manuscript.

Funding: The University of East London supported the project.

Institutional Review Board Statement: In this study, participants participated in an ethnopharmacological survey, which was ethically approved by the University of East London's research ethics committee (UREC) (Reference number: UREC—1516 154).

Informed Consent Statement: Not applicable.

Data Availability Statement: All experimental data of this study are stored in the hard disk drive of a laboratory computer and will be attainable upon request.

Acknowledgments: The author is thankful to the supervisors Mukhlesur Rahman and Barbara Pendry for their guidance during the work. The author is thankful to Mukhlesur Rahman for helping in elucidating the structures of the isolated pure compounds, and thanks to the University of East London for a three-year PhD studentship.

Conflicts of Interest: There is no conflict of interest to declare.

Abbreviations

AMR	Antimicrobial Resistance
NBG	National Botanical Garden
GUAH	Govt. Unani and Ayurvedic Hospital
RUBG	Rajshahi University Botanical Garden
SF	Sundarbans mangrove forest
UEL	University of East London
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MIC	Minimum inhibitory concentration
NMR	Nuclear Magnetic Resonance

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