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ORIGINAL ARTICLE

Phytochemical Analysis and Demonstration of Antioxidant, Antibacterial, and Antibiofilm Activities of Ethnomedicinal Plants of North East India

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Abstract

Ethnomedicinal plants are a rich reservoir of active compounds with potent pharmacological properties. Therefore, plants could serve as a source for the discovery of active antimicrobial and antioxidant agents and are focused because of their low toxicity, economic viability, easy availability, etc. In this regard, phytochemical analyses, viz. β-carotene, total sugar, reducing sugar, vitamin C, total carotenoids, protein, total phenolic content (TPC), and total flavonoid content (TFC) of 20 ethnomedicinal plants of North East India (NEI) were evaluated in this study. The antibacterial activity against human pathogens and antioxidant potential of plant extracts was also demonstrated. The minimum inhibitory concentration (MIC_{80}), minimum bactericidal concentration (MBC), and total antibacterial activity (TAA) of the active extracts were evaluated against *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. The active extracts were also examined for antibiofilm as well as anti-pyocyanin activities against *P. aeruginosa* and anti-QS activity against *C. violaceum* at sub-MICs. The study demonstrated variable concentration of phytochemicals of the extracts, viz. β-carotene (0.29–8.91 mg g^{-1}), total sugar (2.92–30.6 mM), reducing sugar (0.44–14.5 mM), vitamin C (8.41–31.3 mg g^{-1}), total carotenoids (14.9–267.0 mg g^{-1}), protein (5.65–283 mg g^{-1}), TPC (5.32–31.0 mg GAE/g DW), and TFC (1.74–68.2 mg QE/g DW). The plant extracts also exhibited potent antioxidant and antibacterial activities against both Gram-positive and Gram-negative bacteria. Some of the extracts also demonstrated significant biofilm inhibition and eradication, anti-pyocyanin, and anti-QS activities at sub-MICs. The selected ethnomedicinal plants are rich in phytochemicals and demonstrated potent antioxidant, antibacterial, and antibiofilm activities, thus could serve as the important source of novel antioxidant and antimicrobial agents.

Keywords Ethnomedicinal plant · Pathogenic bacteria · Antioxidant · Antibacterial · Antibiofilm · North East India

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Abbreviations

TPC	Total phenolic content
TFC	Total flavonoid content
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
TAA	Total antibacterial activity
ROS	Reactive oxygen species
RSS	Reactive sulphur species
RNS	Reactive nitrogen species
ZOI	Zone of inhibition
QS	Quorum sensing
BSI	Botanical Survey of India
DNS	3,5-Dinitrosalicylic acid
DPPH	1,1-Diphenyl-2-picryl-hydrazyl assay
TCA	Trichloroacetic acid
C_6-AHL	Hexonyl homoserine lactone
AHL	Acyl homoserine lactone
EPS	Extracellular polymeric substances

Introduction

Plants have been widely used as a source of food, clothing, shelter, fibre, and fodder as well as a remedy to treat chronic diseases since ancient times. The use of ethnomedicinal plants to cure various human and animal diseases has been a part of human tradition and civilization. Traditional plants have potential pharmacological properties because of the abundance of various bioactive compounds [1]. The people living in rural and remote areas are dependent on traditional medicine systems to cure various ailments. About 60% and 80% of the populations of the world and developing countries, respectively, are dependent on ethnomedicines as per the reports of the World Health Organization (WHO) [2]. The interest in the development of medicines from ethnomedicinal plants has increased among researchers due to their large-scale availability, accessibility, and applicability. The traditional medicinal plants are explored for their pharmaceutical properties and measures are taken to integrate them into basic healthcare systems. A course for traditional and complementary medicine (T&CM) released in a publication of the *WHO Traditional Medicine Strategy 2014–2023* highlights the requirement for the incorporation of traditional medicine into public health systems [3]. The rise of ineffectiveness, soaring prices, and vast side effects associated with allopathic medicines have shifted the paradigm from the allopathic medicinal system to the traditional medicinal system. It is documented that about 25% of allopathic medicines are obtained from active plant compounds [4]. Furthermore, it is estimated that about 7000 plant-derived medicinal compounds are employed in Western pharmacopoeia and more attention is paid to the preparation of different plant extracts, isolation of bioactive compounds, and evaluation of their different pharmacological properties [5]. The medicinal plants are rich in various secondary metabolites including polyphenols, saponins, amides, alkaloids, carotenoids, flavonoids, terpenoids, glycosides, and tannins with potential biological properties. Therefore, ethnomedicinal plants have been vastly studied for various

pharmacological properties including antibacterial, antifungal, antiviral, antimycobacterial, antiprotozoal, antibiofilm, ant-quorum sensing, antioxidant, anticancer, anti-inflammatory, hepatoprotective, anti-antirheumatic, antiulcer, anti-thrombotic, antiatherosclerotic, antisyphilitic, anthelmintic, antiscorbutic, astringent, antiperiodic purgative, antidiarrheal, mosquitocidal, insecticidal, antinociceptive, larvicidal, acaricidal, diuretic and demulcent, antihyperglycemic, antipyretic, emollient, expectorant, analgesic, aphrodisiac, carminative, laxative, and diaphoretic [5, 6].

The plant-derived antioxidants are reported to show significant scavenging of free radicals, reducing cellular damage, age delaying, and preventing aging-associated diseases including cardiovascular, cancer, hepatic, and neurodegenerative [7]. The major categories of free radicals include (a) reactive oxygen species (ROS), (b) reactive sulphur species (RSS), and (c) reactive nitrogen species (RNS), e.g. oxygen singlets, hydroxyl radical, superoxides, hypochlorite, hydrogen peroxide, peroxynitrite radicals, and nitric oxide–induced oxidative stress. The free radicals are reported to give rise to diseases like acute renal failure, cancer, Alzheimer's disease (AD), Parkinson's disease, and diabetes. Different traditional medicine systems including Chinese, Unani, and Ayurveda are providing substantial proof regarding the availability and extraction of biologically active and therapeutically important antioxidant compounds from plants [8].

The plant secondary metabolites synthesized in response to environmental stresses and microbial infections possess potent antimicrobial activity against a wide range of microorganisms affecting human health, the food industry, animal husbandry, etc. Besides antimicrobial potential, plant extracts are reported to have antibiofilm and anti-quorum sensing activities against pathogenic microorganisms. Conventional antibiotics are reported less effective against biofilm-forming microbes (> 1000 times more resistant than planktonic form) resulting in enhanced multi-drug resistance and subsequent recurrent infections [9]. Biofilm-forming microbes, their chronic pathogenicity, and enhanced antibiotic resistance are the biomedical concerns demanding efficient antibiofilm alternatives. Biofilm formation is a quorum sensing (QS)–dependent multistep process and plant-derived antibiofilm agents work solely or synergistically against them either by inhibiting their adhesion to surfaces, attenuating the maturation of biofilms, inhibiting QS signalling cascades, etc. [10].

It has been reported that about 65% of people in India use ethnomedicines [11] and out of 17,000 enlisted species of plants in India 7500 have reported medicinal values [4]. In India, plants have been used as a basic medicinal system since Vedic times as the use of plants to cure diseases is mentioned in Rig Veda and Athar Veda [12]. North East India (NEI) includes the states of Assam, Arunachal Pradesh, Mizoram, Nagaland, Sikkim, Meghalaya, Tripura, and Manipur. It encompasses different biomes of the world since it is part of both the Himalayas and Indo-Burma biodiversity hotspots. It constitutes about 50% of India's biodiversity and is regarded as the richest and most diverse reservoir of plant diversity in India [13].

In this regard, 20 ethnomedicinal plants from different regions of NEI were evaluated for their antioxidant, antibacterial, antibiofilm, and anti-quorum sensing activities. Additionally, the plants were also evaluated for sugars, proteins, β-carotene, vitamin C, total carotenoids, total phenolic content, and total flavonoid content. The plants enlisted in Table 1 were selected based on available traditional knowledge, wide usage, reported biological activities, and availability.

Table 1 Traditional importance, reported biological activities, distribution, habit and habitat, and family of selected ethnomedicinal plants

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Acmella paniculate (Wall. Ex DC) R.K.Jansen Family: Asteraceae	Flowers and leaves are used in folklore remedy for toothache; treat gum and throat infections, rheumatism, purgation, urinary tract infection (UTIs), cold and fever, pulverization of kidney, gall stones; and remedy for stammering in children	Antioxidant, antibacterial, anthelmintic, antipyretic, antiemetic, anti-plasmodial, antinociceptive, anti-inflammatory	Native to Bangladesh, Colombia, Ecuador, Brazil, China, India, Nepal, Papua, New Guinea, Indonesia, Peru, Islands, Sri Lanka, Solomon Taiwan, Province of China, Thailand, and Vietnam	Annual, hairy herb, 32–60 cm high, multi-stem; grows along fresh-water habitats (rivers, streams, lakes, ponds, marshes, swamps), waste wetlands, paddy and sugarcane fields, etc.	[14]
Allium hookeri Thwaites Family: Liliaceae	In folk medicinal used to cure cough, cold, wounds, heal burns, skin diseases, applied externally to cure rashes, etc.	Antioxidant, anti-inflammatory, anti-microbial, immune-enhancing property, improvement of bone health, anti-obesity, anti-diabetic effects, hypolipidemic, anticoagulant, antihypertensive, anticancer, and anti-asthmatic	Widely distributed in Southeast Asia, Eastern Himalayas and China, Sri Lanka, Bhutan	A bulbous herb with fleshy roots; grows in forest margins, moist places, meadows, etc.	[15]
Allium sativum L Family: Liliaceae	It is used in traditional Chinese, homoeopathic, ayurvedic, Islamic, and folklore medicine systems. It is used is an effective remedy for various diseases including heart problems, worms, tumours, headache, bites, intestinal and lung disorders, an antiseptic lotion, fever, cholera, dysentery, cold, influenza, and renoprotective	Antimicrobial (antibacterial; antiviral, antifungal), wound healing, antioxidant, antiparasitic, antiprotozoal, anti-diabetic, antihypertensive, antitumour, liver protective, detoxifying, radioprotective, anticancer, diuretic, cardioprotective, anticancer, and Alzheimer's disease protective activity	Native to Iran, Kazakhstan, Kirgizstan, Tadzhikistan, Turkmenistan, Uzbekistan Introduced to Albania, Algeria, Amur, Austria, Germany, Greece, India, Iraq, Italy, Turkey, Ukraine, Pakistan, Egypt, etc.	Perennial herb; Cosmopolitan	[16]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Alternanthera sessilis (L.) R. Br. Ex DC Family: Amaranthaceae	Traditionally used to treat skin and eye diseases, rheumatism, wound healing, and as an antidote for snake bites, treatment of malaria, diarrhoea, night blindness, and helminthiasis. Also reported as a febrifuge, galactagogue, abortifacient, etc.	Diuretic, hematinic, antioxidant, anti-inflammatory, cytotoxic, antipyretic, hepatoprotective, antiulcer, antimicrobial, and wound healing	Widespread in the tropics and subtropics of the world	Spreading herb; found in moist areas, damps, ditches, puddles, roadsides, and can be found from sea level to over 2000 m	[17]
Camellia sinensis var. *assamica* Family: Theaceae	Prevents heart diseases; abundance of natural fluoride prevents tooth decay, used internally in the treatment of gastroenteritis, hepatitis diarrhoea, amoebic as well as bacterial dysentery; leaves are used as cardiotonic, stimulant, astringent, diuretic, and expectorant; as a poultice to insect bites, cuts, ophthalmia, treat burns, swellings, bruises, etc.	Antiatherosclerotic, antimutagenic, antioxidant, antibacterial, prevent diabetic nephropathy	India, Burma, China, Indochina, SE Asia, Laos, Myanmar, Thailand	Hardy evergreen shrub or small tree; Cool, humid, tropical highlands, evergreen broad-leaved forests at elevations of 100–1500 m (up to 1900 m in Southern China)	[18]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Crotalaria pallida Aiton (rattle or rattlesnake) Family: Fabaceae	Traditionally leaf extract is used as vermifuge, root extract to cure stomachache and indigestion; root poultice to reduce joint inflammation; seeds to treat neurasthenia, tumours, leukorrhea, and dizziness; leaf juice to treat mumps; leaf and bark paste used topically to treat skin infections; decoction of aerial parts is taken for internal heat; boiled roots taken orally to treat kidney stones and other urinary problems	Antioxidant, antimicrobial, anti-inflammatory, anthelmintic, anti-HIV, antidiabetic, cytotoxic and apoptotic, anti-proliferative, analgesic and antipyretic, anti-nociceptive, estrogenic, and mutagenic activities	Grows widely in tropical and subtropical areas including India and Bangladesh, Nepal, China, Thailand, Cambodia, and Vietnam	Annual erect herb; very common near roadsides, paths and waste places	[19]
Dillenia indica L Family: Dilleniaceae	In the folk medicinal system, oral or topical preparations are used to cure abdominal and joint pain, constipation, fever, cough, diarrhoea, malaria, tumours, laxative, diabetes, diabetes mellitus, jaundice, reducing fatigue, preventing dandruff, etc.	Anti-inflammatory, antidiabetic, antinociception hypolipidemic, anti-leukaemic, antimicrobial, antioxidant, anti-diarrheal effects, etc.	Originated from tropical Asia and distributed in India, Indonesia, Thailand, Vietnam, Sri Lanka, and Malaysia	Evergreen tree; grows well in moist, sunny, well-drained slightly acidic sandy loam	[20]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Diplazium esculentum (Retz.) Sw Family: Athyriaceae	Traditionally used for the treatment of dysentery, glandular swellings, cough, indigestion, diarrhoea and various skin infections; as a decoction to treat haemoptysis, rheumatism, headache, fever, wounds, pain, measles, oligospermia, bone fracture, etc.	Mast cell stabilizer and can prevent anaphylactic shock, antioxidant, antidiabetic, immunomodulatory, CNS stimulant, antibacterial, and hepatoprotective activity	Cambodia, Indonesia, Japan, Malaysia, Vietnam, China, India, Papua New Guinea, Singapore, Taiwan, Pakistan, Philippines, Thailand, and Bangladesh	Herbaceous fern; grows in hills with an altitudinal range up to 2300 m, banks of rivers and streams, canals, and marshy areas	[21]
Erigeron karvinskianus DC Family: Asteraceae	Used in folk medicine to treat skin infections	Antioxidant	Distributed widely in temperate, tropical, and subtropical areas of the world including India Africa, Australia, Japan, northwest Himalayas, and Europe	Herb; mostly found in moist, disturbed sites, shaded rock walls, archaeological sites, wastelands, cement cracks, roadsides, cultivated fields, etc.	[22]
Eupatorium riparium Regel Family: Asteraceae	In folk medicine, the leaves of the plant have been used to treat infections, swelling, etc.	Allelopathic, antiproliferative, antifungal	Humid tropical or subtropical rainforests, warm temperate regions	Creeping spreading subshrub; grows in south-facing hillsides, river banks, and moist places; also grows as an invasive weed	[23]
Garcinia acuminata Planch. & Triana (Mahi thekera) Family: Clusiaceae	To treat stomach and skin problems, dry fruits used to treat blood dysentery		S. America to central, eastern, and northern Brazil, Bangladesh, India, Thailand, Malaysia, Myanmar	Evergreen tree, about 15 m tall; grows in a fringing forest in the savannah regions, evergreen forests (600–1200 m)	[24]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Garcinia lanceifolia Roxb. var. *oxyphylla* (Planch. & Triana) Laness (Rupohi thekera) Family: Clusiaceae	Ripe fruits are used to treat diarrhoea, gum resins and fruits are used to cure fever, urinary problems, jaundice, dyspepsia, biliousness, and diabetes; leaves are used as vegetables and pickles and used as stomachic and diuretic	Analgesic, antioxidant, antifungal, antibacterial, anti-inflammatory	Found in India, Bangladesh, Myanmar; Endemic to Assam, India	Middle-sized evergreen tree; common in evergreen forests	[25]
Houttuynia cordata Thunb Family: Saururaceae	In folk medicine, leaf juice is used for heat-clearing, detoxifying, treating furuncle, and inducing menstruation, curing blood deficiency and purification of blood, treating dysentery, gonorrhoea, measles, haemorrhoids, and eye trouble, cholera, curing rheumatism, pneumonia, cough arrest by enhancing lung moisture, and strengthening bones and muscles, hypertension, constipation, and hyperglycaemia; tropically to treat snake bites	Anti-leukaemic effect and virucidal effects on the human immunodeficiency virus (HIV), antimutagenic effect, anti-inflammatory action, hepatoprotective, and antianaphylaxis effect; antibacterial, antioxidant, antiallergic, anticancer; hair promoting effect, the extracts cure respiratory problems, surgical, gynaecological, infectious diseases, malignant pleural effusion, and nephrotic syndrome	Widely grown in Southeast Asia (in Korea, China, Japan, Taiwan, and India-North Eastern Region)	Erect to ascending, herbaceous perennial plant; mostly found in moist habitats with higher organic carbon	[26, 27]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Ipomoea aquatica Forssk Family: Convolvulaceae	The plant is used in the treatment of liver diseases, constipation, diabetes, abscesses, mental illness, and intestinal problems nose bleeds, and high blood pressure	Anthelmintic, antibacterial, antiepileptic, antioxidant, anti-inflammatory, anxiolytic activity; potent CNS depressant, hypolipidemic effects, potent purgative property, as an antidote against scorpion venom	Native to China, Bangladesh, Indonesia, Malaysia, Philippines, Cambodia, India, Nepal, Laos, Myanmar, Thailand, Pakistan, Sri Lanka, Vietnam, Papua New Guinea, most of Africa, and northern Australia	Perennial herbaceous creeping vine; grows in moist soils, side-lines of fresh water, lakes, ditches, ponds, wet rice fields, and marshes	[28]
Justicia gendarussa Burm. f Family: Acanthaceae	Traditionally used to cure chronic rheumatism, fever, headache, earache, muscle, asthma pain, inflammations, bronchitis respiratory disorders, eye diseases, digestion problems, earache, vaginal discharges, dyspepsia, eczema; internally given for hemiplegia, cephalalgia, and facial paralysis	Antipyretic, antinociceptive, antiproliferative, antiangiogenic, antimicrobial, antioxidant, antiarthritic, anthelmintic, hepatoprotective, hyperuricemia activities	Native to China and distributed across India, Sri Lanka, Malaysia	Erect deciduous shrub; grows in shady areas, forest margins, along the streams	[29]
Mikania micrantha Kunth Family: Asteraceae	To treat insect bites or scorpion stings, rashes, and skin itches, as a decoction to treat diabetes, stroke, hypercholesterolemia and hypertension, stomach ache, jaundice, fever, rheumatism, cold and respiratory diseases, wound dressing, and healing of sores	Anti-diabetic, antimicrobial, antioxidant, antidermatophytic, antistress, antiviral, anti-inflammatory, anti-proliferative, anti-spasmodic, antiprotozoal, anthelmintic, anticancer	India, Malaysia, Thailand, Indonesia, Nepal, Papua New Guinea, Philippines, and also Australia	Perennial climber; grows along the roadside, swampy woods, bushes of moist places, forest borders, and also along streams and rivers	[30]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Oxalis corniculata L Family: Oxalidaceae	Good appetizer, cure anaemia, dyspepsia, convulsion, piles, and dementia; plant boiled with buttermilk is used to cure indigestion and diarrhoea; fresh leaf paste is used to stop bleeding and headache, scorpion stings are cured with the extract; home remedy for datura intoxication; treat skin diseases like warts	Antimicrobial, anticancer, cardio relaxant, nematocidal, antiamoebic, antioxidant, allelopathic, steroidogenic properties	Distributed in tropical and subtropical regions of the world; native to India	Delicate-appearing, low-growing, herbaceous plant abundantly distributed in damp shady places, road-sides, plantations, lawns	[31]
Oxalis debilis var. *corymbosa* (DC.) Lourteig	Leaf decoction is used by tribals of North-Eastern India to treat diabetes, dysentery, etc.	Antioxidant, antimicrobial, anticancer, anti-inflammatory, analgesic, antiamoebic, astringent, febrifuge, and diuretic activities	Native to South America-distributed worldwide	Perennial herbaceous plant; grows in wet moist places, forests, grasslands, and irrigation channels	[32]
Piper peepuloides Wall Family: Piperaceae	Folk medicine: a mixture of seed powder, honey, and egg yolk is used to treat cough; paste of dried leaves is consumed to get relief from fever	Insecticide, larvicide, weak antioxidant, antibacterial activities	Tropical and sub-tropical evergreen forests of NEI (Assam, etc.), China, Myanmar, Bangladesh, Nepal, Thailand	Evergreen limber-slender bush; grows in areas with elevations 100 to 800 m above sea level with high rainfall	[33]

Table 1 (continued)

Name and family	Traditional uses	Reported activities	Distribution	Habit and habitat	References
Spondias dulcis Forst Family: Anacardiaceae	Bark as a remedy for diarrhoea; used in eye infections, inflammation, and eyesight enhancement; treat anaemia and stomach problems; to cure wounds, sores, and burns; leaf infusions to cure mouth infections and sore throats; fruit is diuretic, young fruit aids women in labour; etc.	Antimicrobial, cytotoxic, antioxidant, antidiabetic, peroxidase, and thrombolytic properties	Native to Melanesia through Polynesia, widely distributed to many tropical, sub-tropical, and equatorial regions of the world (Bangladesh, India, Sri Lanka, Vietnam, Indonesia, Malaysia)	Fast-growing deciduous tree (about 18 m); dry or secondary forests from sea level to 500 m	[34]

Material and Methods

Chemicals and Media

Growth media and other chemicals were purchased from Hi-media. Crystal violet, gentamicin, and other solvents were purchased from Sigma-Aldrich.

Bacterial Strains and Growth Conditions

Gram-positive bacteria, viz. *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Mycobacterium smegmatis*, and *Staphylococcus aureus* with MTCC 430, MTCC 121, MTCC 839, MTCC 14,468, and MTCC 3160, respectively; Gram-negative bacteria, viz. *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Pseudomonas diminuta*, *Salmonella typhimurium*, and *Yersinia enterocolitica* with MTCC 40, MTCC 618, MTCC 2297, MTCC 3361, MTCC 3232, and MTCC 859, respectively; and a fungal strain *Candida albicans* MTCC 183 were purchased from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. An indicator strain of QS *Chromobacterium violaceum* ATCC12472 and a mutant strain of *C. violaceum* (CV026) were purchased from American Type Culture Collection (ATCC). Luria–Bertani broth (LB), nutrient broth (NB), and tryptone soya broth (TSB) were used to culture the bacterial strains while potato dextrose broth (PDB) was used for the growth of fungal strain.

Collection of Plants and Preparation of Extracts

Different parts (root, leaf, branch, bark, seed, fruit) of 20 ethnomedicinal plants were collected from different locations in NEI. The plants were identified by the Botanical Survey of India (BSI), Eastern Regional Centre Shillong (reference nos. BSI/ERC/2018/Tech/469 and BSI/ERC/Tech/2020–2021/1491). The collected parts were cleaned, washed, and surface sterilized with 70% alcohol and 5% aqueous sodium hypochlorite solution. After proper washing, 10% (w/v) methanolic extracts were prepared, followed by lyophilization, and storage at − 20 °C [35]. The lyophilized extracts were dissolved in water for performing experiments. The name of the collected plants, extract code name, part used, place of collection, and date of collection are described in the Supplementary Information.

Phytochemical Analysis

Total and Reducing Sugar

Total sugar in the plant powder was estimated as per the method of Dubois et al. [36] The plant extracts (2 mL) were pipetted into a test tube, followed by the addition of 0.5 mL of 80% phenol and 0.5 mL of concentrated sulphuric acid. The intensity of the solution was measured after 30 min of rest at 485 nm using a UV–vis spectrophotometer against distilled water as a blank. Glucose was used as a standard to quantify the content in the unknown sample. Reducing sugar was estimated by the 3,5-dinitrosalicylic acid (DNS) method of Miller et al. [37] where 1 mL of extract was mixed with 80% ethanol and 3 mL of DNS followed by boiling at 90 ℃ for 15 min. The solution was then allowed to cool at

room temperature followed by adding 1 mL of 2% sodium potassium tartrate tetrahydrate solution. The intensity of the solution was then measured at 575 nm using a UV–vis spectrophotometer (Cary 60, Agilent UNICO Products and Instruments Inc., Shanghai, China). Fructose was used as a standard.

Vitamin C Content

The AOAC official methods (21st ed.) (2019) (AOAC 967.21) [38] were used for the determination of vitamin C content. The redox titration method using 2,6-dichlorophenol indophenol dye was adopted to assess the vitamin C content of the plant materials. First, indophenol dye (50 mg) was prepared in the distilled water (250 mL) followed by the addition of sodium carbonate (42 mg). Briefly, 2 mL of extract was mixed with 5 mL of metaphosphoric acid followed by titration against indophenol dye until rose pink colour.

β-carotene Content

β-carotene content in plant material was estimated as per the procedure outlined by Biswas et al. [39], where plant powder (1 g) was mixed with 5 mL of chilled methanol followed by centrifugation at 1370 rcf for 10 min. The absorbance of the extract was determined at 449 nm, and concentration was calculated using β-carotene as standard in the range of 0–32 mg mL^{-1}.

Total Carotenoids

Total carotenoid content in the plant material was determined using a spectrophotometer method as described by de Carvalho et al. [40]. One gram of powder was taken and extracted by mixing it in 14 mL hexane and acetone (3:2 $v.v^{-1}$) solution and using an Eppendorf centrifuge at 10,000 relative centrifugal speed for 10 min at 4 ℃. The obtained supernatants were collected and absorbance was measured at 502 nm using a UV–vis spectrophotometer. The following equation was used to calculate the total carotenoid content in plant powder:

$$\text{Total Carotenoids}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{\text{OD}_{502} \times 3.12}{\text{mass of the sample (g)}} \times 1000$$

Protein Content

Protein content in the plant material was determined according to the procedure outlined by Lowry et al. [41].

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The methods described by Kara et al. [42] were used for the determination of TPC and TFC of 20 plant extracts. Briefly, a mixture of 25 µL of plant extract (1 mg/mL), 100 µL of 0.075 g/mL sodium carbonate, and 125 µL of 10% v/v Folin–Ciocalteu reagent was incubated for 30 min and absorbance was taken at 765 nm. Total phenolic content was expressed as milligrams of gallic acid equivalent per gram dry weight (mg GAE/g DW)

and was measured from the calibration curve of gallic acid (Sigma-Aldrich). For the estimation of TFC, a mixture of 25 µL of plant extract (1 mg/mL), 75 µL of 95% ethanol, 10 µL of 10% w/v potassium acetate, 10 µL of 10% w/v $AlCl_3$, and 140 µL of deionized water was incubated for 40 min and absorbance was measured at 415 nm. Total flavonoid content was expressed as milligrams of quercetin equivalent per gram dry weight (mg QE/g DW) and was calculated from the calibration curve of quercetin (Sigma-Aldrich).

Antioxidant Activity of Plant Extracts

Antioxidant activity of plant extracts was determined by 1,1-diphenyl-2-picryl-hydrazyl assay (DPPH) and reducing power assay mentioned by González-Palma et al. [43].

1-Diphenyl-2-picryl-hydrazyl Assay

Briefly, a mixture of 190 µL of 60 µM DPPH solution and 10 µL of plant extracts (1 mg/mL) was placed in the dark for 30 min. After incubation, absorbance was measured at 517 nm and % DPPH scavenging activity was calculated by using the following formula. Ascorbic acid and quercetin were used as positive controls while methane served as carrier control.

$$\%\ \text{DPPH Scavenging activity} = \left[\left(\frac{OD_{control} - OD_{test}}{OD_{control}}\right)\right] \times 100$$

where $OD_{control}$ and OD_{test} are the absorbances of blank and extracts, respectively.

Reducing Power Assay

Briefly, mixtures of 50 µL plant extract (1 mg/mL), 0.2 mL each of 1% potassium ferricyanide, and 0.2 M phosphate buffer (pH 6.6) were incubated in a water bath at 55 °C for 20 min. After incubation, 250 µL trichloroacetic acid (TCA) was added to the mixture and centrifuged at 1000 rpm for 10 min. Five hundred microliters of supernatant was mixed with 500 µL deionized water and 100 µL of 0.1% $FeCl_3$. The absorbance (OD_{700}) was measured after 10 min which is directly proportional to the reducing power.

Antibacterial Activity

Agar Well Diffusion Assay

To demonstrate the antibacterial activity of different crude extracts, agar well diffusion assay was performed. Briefly, 100 µL of 0.4 OD_{600} of *B. cereus*, *B. subtilis*, *L. monocytogenes*, *M. smegmatis*, *S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. diminuta*, *S. typhimurium*, *Y. enterocolitica*, and *C. albicans* was spread on growth medium agar plates. The plant extract (4 mg/mL), gentamicin (2.5 mg/mL-positive control), nystatin (5 mg/mL-positive control for *C. albicans*), and methanol (carrier control) were placed into separate wells prepared by metallic borer (6 mm). After an incubation of 24 h at 37 °C, the plates

were evaluated for inhibition zones around the wells and diameters of zone of inhibition (ZOI) (mm) were measured [44].

Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were defined as the minimum concentration of plant extracts where no visible growth was demonstrated and which inhibited the total growth of the bacteria, respectively. Briefly, MIC values were determined by microdilution assay and MBC values were evaluated by placing culture on agar plates from the wells with higher concentrations than MIC values. Subsequently, the plates were incubated at 37 °C for 24 h and bacterial growth was observed after incubation. The concentration at which no growth was observed on the plate was considered the MBC value. The minimum concentration of plant extracts that could inhibit 80% of the bacterial growth was considered to be the MIC_{80}, which was calculated as follows:

$$\text{Inhibition } \% = \left[\left(OD_{control} - OD_{test}\right)/OD_{control}\right] \times 100$$

where $OD_{control}$ and OD_{test} are OD_{600} nm to demonstrate bacterial growth in the positive control (media + culture) and treated samples (media + culture + extract).

All the subsequent experiments were carried out at sub-MICs and the MIC_{80} and MBC values were estimated only against *P. aeruginosa* and *C. violaceum*. Furthermore, the total antibacterial activity (TAA) (mL/g) of plant extracts was calculated from the ratio of the mass of crude extract (mg) obtained from 1 g of plant sample and MIC (mg/mL) [45].

Total antibacterial activity (mL/g) = mass of crude extract per gram of plant/MIC.

Determination of Antibiofilm Activity by Crystal Violet Staining Assay

Effect of Plant Extracts on the Initial Attachment of *P. aeruginosa*

The antibiofilm activity of the extracts was evaluated against *P. aeruginosa* by the method described by Famuyide et al. [45] with slight modifications.

Scanning Electron Microscopy

To demonstrate the biofilm inhibitory effect of plant extracts at a microscopic level, SEM was performed. Briefly, *P. aeruginosa* was grown on coverslips in the presence (treated) and absence (control) of plant extracts. After incubation, the cells were fixed by 2.5% (v/v) glutaraldehyde followed by dehydration using 50, 70, 75, 80, 85, 90, and 95% ethanol and were analysed under SEM (JEOL JSM-6390LV, Tokyo, Japan) [46].

Effect of Plant Extracts on Preformed Biofilm of *P. aeruginosa* — Biofilm Eradication Assay

To demonstrate the effect of plant extracts on preformed biofilm, the crystal violet staining method mentioned by Famuyide et al. [45] was used. Briefly, biofilm eradication activity of those plant extracts was estimated which exhibited potential biofilm inhibition activity

(inhibition of initial attachment) against *P. aeruginosa*. Biofilms were allowed to form in a 96-well tissue culture plate by incubating 10 µL aliquot of 0.4 OD_{600} of *P. aeruginosa* and 190 µL of TSB for 24 h and 48 h at 37 °C in a static condition. After respective incubation periods, potential plant extracts were added at a final concentration of 1 mg/mL (sub-MIC) and were incubated for 24 h at 37 °C. % biofilm eradication was determined by the crystal violet method as mentioned earlier. Methanol and furanone (1 mM) served as the negative and positive control, respectively. The following formula was used to calculate the percentage of biofilm eradication

$$\%\ \text{Biofilm eradication} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

where $OD_{control}$ and OD_{test} are the absorbances of untreated control and treated cultures, respectively.

Effect of Plant Extracts on Pyocyanin Production

The method described by Das et al. [47] was used for the quantification of pyocyanin production. The following formula was used to calculate the percentage of pyocyanin inhibition;

$$\%\ \text{Pyocyanin inhibition} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

where $OD_{control}$ and OD_{test} are the absorbances of untreated control and treated cultures, respectively.

Additionally, to validate the inhibition of pyocyanin by plant extracts was not due to their bactericidal nature, the bacterial growth of the treated and untreated samples was recorded at 600 nm after 24 h of incubation.

Effect of Plant Extracts on Quorum Sensing of *C. violaceum*

Violacein Quantification Assay

The violacein quantification method described by Moradi et al. [48] was used for the estimation of violacein inhibition. The following formula was used to calculate the percentage of violacein inhibition;

$$\%\ \text{Violacein inhibition} = \left[\left(\frac{OD_{control} - OD_{test}}{OD_{control}}\right)\right] \times 100$$

where $OD_{control}$ and OD_{test} are the absorbances of untreated and treated cultures, respectively.

Additionally, to demonstrate that violacein inhibition was due to anti-QS effects and not due to the bactericidal effect of extracts, $\log_{10}$CFU/mL value of all the treated and untreated samples was counted after 24 h of incubation.

Evaluation of Anti-quorum Sensing Activity Through Quorum Sensing Signal Inhibition

To determine the violacein inhibition of plant extracts via interruption of QS signalling, a biosensor strain *C. violaceum* CV026 was used in addition to *C. violaceum* CV12472. CV026 is incapable of producing violacein due to a mutation in the acyl-homoserine lactone (AHL)-producing gene. To prove that violacein inhibition activity of plant extracts is due to their interruption in QS signalling, CV12472 was grown in the presence (1 mg/mL) of plant extracts supplemented with and without external AHL, viz. hexonyl homoserine lactone (C_6-AHL) at a concentration of 0.225 µg/mL. Similarly, CV026 was allowed to grow in the presence and absence of C_6-AHL. The experiment was set up as discussed above and % violacein production was calculated [48].

Statistical Analysis

All experiments were performed in triplicate, and the obtained results are expressed as the mean values and standard deviations. Statistical difference was evaluated by using Student's *t*-test (two-tailed) for unpaired samples. Statistics were calculated in GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA), in which a *p*-value of ≤ 0.01 was considered statistically significant.

Results

Phytochemical Analysis

Total and Reducing Sugar

Sugar functions as a food as well as a central signalling or regulatory molecule in plants, modulating gene expression in areas such as growth, development, metabolism, stress response, and disease resistance. The total sugar content ranged from 2.92 to 30.6 mM with the highest amount in *D. indica* at 30.6 mM, followed by *A. hookeri* at 28.7 mM, and the lowest was in *D. esculentum* at 2.92 mM (Table 2).

The reducing sugar of the studied plant samples ranged from 0.44 to 14.5 mM (Table 2). Among the twenty plant samples studied, reducing sugar content was found highest in *A. sativum* at 14.5 mM, followed by *D. indica* at 12.5 mM, and lowest in *D. esculentum* at 0.44 mM, followed by *O. corniculata* at 0.45 mM. In similar studies, Preetha et al. [49], Ayam et al. [50], and Choudhury et al. [51] reported that the reducing sugar of *A. sessilis*, *A. hookeri*, and *D. esculentum* was 56.1 ± 0.20 (mg g^{-1}), 0.18 ± 0.01 mg 100 g^{-1}, and 0.13 ± 0.01 mg g^{-1} dwt, respectively.

Vitamin C

Vitamin C is an important redox buffer and cofactor for enzymes that regulate photosynthesis, hormone production, and the regeneration of other antioxidants. From Table 2, it is evident that *P. peepuloides* has the highest amount of vitamin C content of 31.3 mg g^{-1}, followed by *A. sessilis* (29.9 mg g^{-1}), while the lowest vitamin C content was found in *A.*

Table 2 Phytochemical analysis of the selected ethnomedicinal plants

Sample	Total sugar (mM)	Reducing sugar (mM)	Vitamin C (mg/g)	Beta carotene (mg/g)	Total carotenoids (mg/g)	Protein (mg/g)	TFC (mg QE/g DW)	TPC (mg GAE/g DW)
C. sinensis var. assamica	7.12 ± 0.29	2.53 ± 0.11	16.5 ± 1.55	5.78 ± 0.74	267 ± 15.6	161 ± 6.28	68.2 ± 0.25	31.0 ± 0.89
A. sessilis	14.5 ± 0.62	5.57 ± 0.52	29.9 ± 1.43	5.31 ± 0.35	166 ± 21.3	75.4 ± 4.55	26.9 ± 0.11	9.66 ± 0.17
M. micrantha	11.7 ± 0.49	8.54 ± 0.96	23.6 ± 1.58	5.03 ± 0.29	229 ± 8.19	60.0 ± 6.32	36.3 ± 0.24	19.2 ± 0.28
A. hookeri	28.7 ± 1.37	5.10 ± 0.32	28.2 ± 1.32	8.09 ± 0.80	211 ± 10.2	170 ± 9.15	54.6 ± 0.29	28.6 ± 0.99
D. esculentum	2.92 ± 0.51	0.44 ± 0.01	19.6 ± 1.62	1.91 ± 0.21	103 ± 2.36	40.3 ± 3.06	2.40 ± 0.77	6.61 ± 0.16
H. cordata	17.2 ± 1.36	1.98 ± 0.15	10.0 ± 1.08	0.81 ± 0.04	39.6 ± 1.68	6.02 ± 0.32	41.9 ± 0.12	7.74 ± 1.72
D. indica	30.6 ± 1.14	12.5 ± 1.65	18.2 ± 1.29	8.91 ± 0.96	136 ± 2.26	283 ± 10.15	1.74 ± 0.54	19.2 ± 1.22
O. debilis var. corymbosa	15.3 ± 0.18	6.73 ± 0.28	14.7 ± 1.62	2.64 ± 0.14	94.7 ± 2.85	44.6 ± 2.15	18.6 ± 0.48	5.55 ± 0.83
A. paniculate	10.5 ± 0.95	2.02 ± 0.32	20.9 ± 2.03	3.60 ± 0.84	144 ± 15.3	5.65 ± 0.85	52.8 ± 0.28	30.6 ± 0.68
A. sativum	21.2 ± 1.45	14.5 ± 0.80	8.41 ± 0.39	0.29 ± 0.80	14.9 ± 1.19	35.1 ± 3.65	3.05 ± 0.22	5.97 ± 0.22
J. gendarussa	6.85 ± 0.32	0.69 ± 0.00	16.1 ± 1.22	3.96 ± 0.66	89.9 ± 8.95	19.5 ± 1.55	55.0 ± 0.19	13.9 ± 0.69
P. peepuloides	13.8 ± 1.62	4.60 ± 0.85	31.3 ± 1.49	6.86 ± 0.39	209 ± 14.3	141 ± 9.22	3.15 ± 0.46	22.7 ± 0.92
O. corniculata	7.08 ± 0.65	0.45 ± 0.02	29.9 ± 1.40	3.92 ± 0.29	218 ± 15.3	51.5 ± 8.16	13.9 ± 0.78	5.32 ± 0.07
I. aquatica	6.83 ± 0.32	1.78 ± 0.09	22.6 ± 2.85	4.77 ± 0.75	212 ± 20.3	41.6 ± 2.63	24.2 ± 0.81	5.97 ± 0.55
S. dulcis	12.4 ± 0.81	3.18 ± 0.08	24.9 ± 2.33	4.03 ± 0.68	170 ± 10.2	62.1 ± 8.26	14.0 ± 0.11	5.97 ± 0.69
E. karvinskianus	8.16 ± 0.84	1.42 ± 0.04	21.8 ± 1.95	4.68 ± 0.52	141 ± 8.25	92.2 ± 3.69	36.3 ± 0.41	13.9 ± 0.83
E. riparium	9.74 ± 0.39	2.62 ± 0.55	24.4 ± 1.76	4.31 ± 0.62	191 ± 20.1	45.2 ± 4.25	26.9 ± 0.17	6.32 ± 0.58
C. pallida	8.60 ± 0.51	7.69 ± 0.68	17.5 ± 1.23	4.81 ± 0.33	96.9 ± 2.63	5.69 ± 0.26	38.8 ± 0.14	10.1 ± 0.48
G. acuminata	13.1 ± 1.35	4.80 ± 0.33	14.8 ± 1.63	1.30 ± 0.19	59.1 ± 5.69	28.3 ± 2.85	63.9 ± 0.91	9.37 ± 0.22
G. lanceifolia	8.00 ± 0.52	3.93 ± 0.48	27.1 ± 2.35	0.77 ± 0.00	117 ± 10.7	52.9 ± 3.95	57.6 ± 0.31	6.74 ± 0.52

sativum 8.41 mg g^{-1}. In a similar study, Preetha et al. [49] found 1.25 µg g^{-1} vitamin C content in *A. sessilis*. The vitamin C content of *A. hookeri* in our study was found to be comparable to that obtained in an investigation by Ayam et al. [50]. Our study demonstrated the vitamin C concentration of *D. esculentum* is 19.6 mg g^{-1}, which is lower than the 46.0 mg g^{-1} reported in research by Choudhury et al. (2017). The vitamin C content of most of the plants examined in this study has never been studied before to the best of our knowledge.

β-carotene

β-carotene aids in normal vision, morphogenesis, cell proliferation, and differentiation and is important for iron utilization and humoral immunity. Plants contain β-carotene, a pigment that gives them their colour. The β-carotene concentration in Table 2 was found to be between 0.29 and 8.91 mg g^{-1}. The highest concentration of β-carotene was found in *D. indica* (8.91 mg g^{-1}), followed *by A. hookeri* (8.09 mg g^{-1}). *A. sativum* has the lowest β-carotene concentration (0.29 mg g^{-1}), followed by *G. lanceifolia* (0.77 mg g^{-1}). In a study carried out by Devi et al. [52], the β-carotene content of *A. hookeri* was found to be 0.60 mg 100 g^{-1}.

Total Carotenoids

Carotenoids are required for photosynthesis and photoprotection in plants. They are important light-harvesting pigments and photosystem structural components. Total carotenoids in plant materials ranged from 14.9 to 267 mg g^{-1} in the current study (Table 2). *C. sinensis* has the highest carotenoid concentration (267 mg g^{-1}), followed by *M. micrantha* with 229 mg g^{-1}. *A. sativum*, on the other hand, had the lowest concentration of 14.9 mg g^{-1}. Carotenoids have the antioxidant feature of being very powerful physical and chemical quenchers of singlet oxygen ($^1O_2$) as well as potent scavengers of other reactive oxygen species (ROS). The presence of carotenoids in plant extracts has been shown to protect against a variety of ROS-mediated diseases, including cardiovascular disease, cancer, neurological disorders, and photosensitive or eye-related ailments [53].

Protein Content

Proteins are essential biomolecules required for the normal function, structural maintenance, and regulation of the body of all living organisms. Protein concentrations among the tested extracts ranged from 5.65 to 283 mg g^{-1} (Table 2). *D. indica* has the highest protein content at 283 mg g^{-1}, followed by *A. hookeri* at 170 mg g^{-1}. *A. paniculate* had the lowest concentration of 5.65 mg g^{-1}, followed by *C. pallida Aiton* with 5.69 mg g^{-1}. *C. pallida* seeds were found to have about 22% protein as reported by Ukil et al. [54]. Antimicrobial, antioxidant, ribosome-inactivating, and neuro-modulatory properties have been found in proteins isolated from plants.

TPC and TFC

The Folin–Ciocalteu method was employed for the determination of TPC in the plant extracts. The values were evaluated from the gallic acid standard curve with $R^2 = 0.95$ and were expressed as mg GAE/g DW. The amount of the TPC in the plant extracts

was found as CSMD (31.0), APMF (30.6), AHMF (28.6), PPMF (22.7), MMMF (19.2), DIMD (19.2), EKMD (13.9), JGMD (13.9), CPMF (10.1), ASMD (9.66), GAMD (9.37), HCMF (7.74), GLMD (6.74), DEMD (6.61), ERMD (6.32), ASMF (5.97), IAMF (5.97), SDMF (5.97), ODMF (5.55), and OCMF (5.32) mg GAE/g DW (Table 2).

Similarly, the TFC values were evaluated from the quercetin standard curve with $R^2 = 0.99$ and were expressed as mg QE/g DW. The values ranged between 1.74 and 68.2 mg QE/g DW with highest the TFC of CSMD (68.2) followed by GAMD (63.9) while the lowest TFC was reported in DIMD (1.74) mg QE/g DW (Table 2).

Antioxidant Activity of Plant Extracts

The scavenging effect of plant extracts on DPPH radical shown in figure demonstrates the following order: CSMD (87.5%) > APMF (83.9%) > HCMF (83.0%) > MMMF (82.2%) > AHMF (81.2%) > PPMF (80.2%) > GAMD (77.5%) > GLMD (75.7%) > ASMD

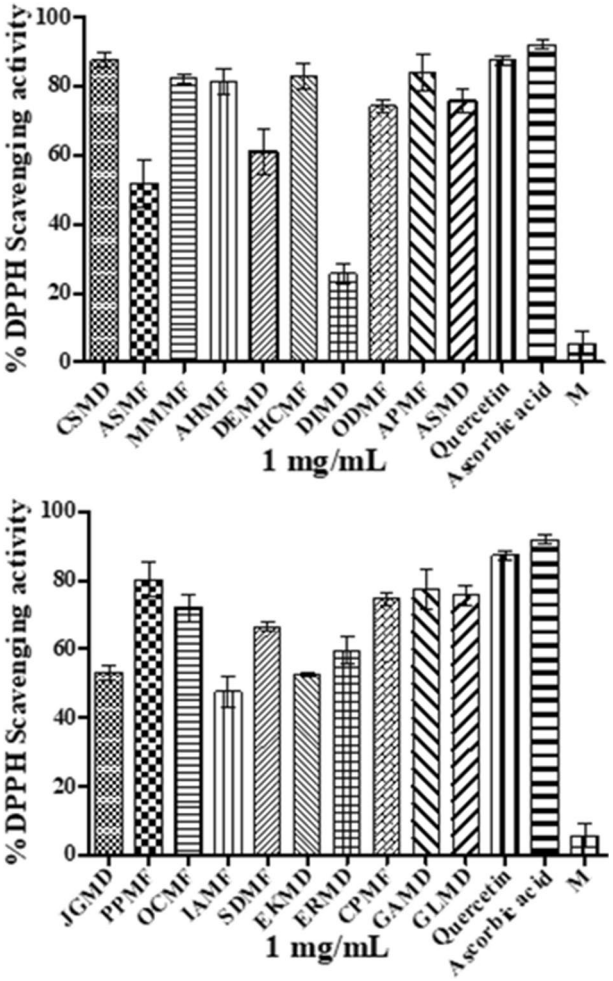


Fig. 1 Evaluation of the antioxidant activity of the extracts by DPPH assay. The tested plant extracts at a concentration of 1 mg/mL demonstrated significant % DPPH scavenging activity in comparison to positive controls quercetin and ascorbic acid. The highest and lowest % DPPH scavenging activity of 87.59 and 25.78% was exhibited by *C. sinensis* (CSMD) and *D. indica* (DIMD), respectively. Methane (carrier control) demonstrated insignificant (5.5%) % DPPH scavenging activity. One milligram per millilitre in the *X*-axis depicts the tested concentration of plant extracts as well as the positive controls. Error bars represent the standard deviations of three measurements

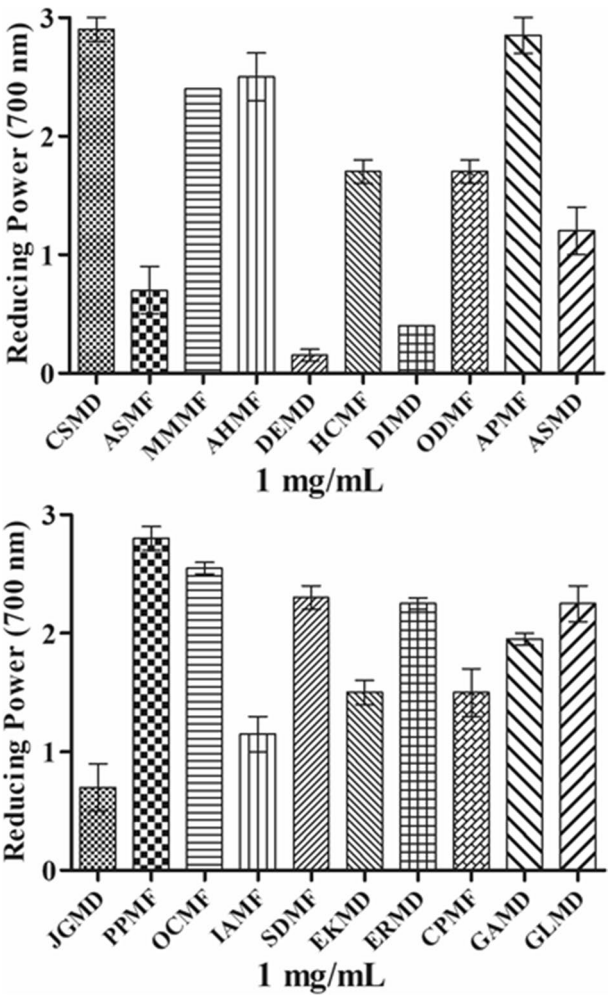


Fig. 2 Evaluation of the antioxidant activity of the extracts by reducing power assay. The extracts showed variable absorbance at 700 nm in the range of 2.9–0.15 which is directly proportional to the reducing powers. The highest and lowest absorbances (reducing powers) of 2.9 and 0.15 were exhibited by *C. sinensis* (CSMD) and *D. esculentum* (DEMD), respectively. One milligram per millilitre in the *X*-axis depicts the tested concentration of plant extracts. Error bars represent the standard deviations of three measurements

(75.7%) > CPMF (74.8%) > ODMF (74.2%) > OCMF (72.0%) > SDMF (66.6%) > DEMD (61.0%) > ERMD (52.6%) > JGMD (53.3%) > EKMD (52.6%) > ASMF (51.8%) > IAMF (47.7%) > DIMD (25.8%). The positive controls quercetin and ascorbic acid demonstrated % DPPH scavenging of 87.4 and 92.0, respectively (Fig. 1).

Figure 2 depicts the reducing powers of tested plant extracts at a concentration of 1 mg/mL. The decreasing order of the reducing power was CSMD > APMF > PPMF > OCMF > AHMF > MMMF > SDMF > ERMD = GLMD > GAMD > HCMF = ODMF > EKMD = CPMF > ASMD > IAMF > ASMF = JGMD > DIMD > DEMD. CSMD demonstrated a maximum reducing power of 2.9, followed by APMF (2.85), PPMF (2.80), and OCMF (2.55) while the least reducing power was exhibited by DEMD (0.15).

Table 3 Diameter of zone of inhibition (mm) of plant extracts against pathogenic microorganisms

Plant extract	BC	BS	EC	KP	LM	MS	PA	PD	ST	SA	YE	CA	M
APMF	**18 ± 1**	**12 ± 1**	ND	ND	ND	ND	**16 ± 1**	**12 ± 1**	ND	ND	**19 ± 1**	ND	ND
AHMF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ASMF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ASMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CSMD	**18 ± 1**	**17 ± 1**	**16 ± 1**	**16 ± 1**	**15 ± 1**	**15 ± 1**	**16 ± 1**	**18 ± 1**	**18 ± 1**	**18 ± 1**	**18 ± 1**	ND	ND
CPMF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DIMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DEMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
EKMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ERMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
GAMD	**16 ± 1**	**16 ± 1**	**17 ± 1**	**17 ± 1**	**17 ± 1**	**14 ± 1**	**18 ± 1**	**19 ± 1**	**16 ± 1**	**14 ± 1**	**15 ± 1**	ND	ND
GLMD	**13 ± 1**	**14 ± 1**	**15 ± 1**	**13 ± 1**	**12 ± 1**	**14 ± 1**	**15 ± 1**	**12 ± 1**	**10 ± 1**	**13 ± 1**	**15 ± 1**	ND	ND
HCMF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IAMF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
JGMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MMMD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCMF	**19 ± 1**	**17 ± 1**	**18 ± 1**	**20 ± 1**	**15 ± 1**	**20 ± 1**	**22 ± 1**	**21 ± 1**	**20 ± 1**	**23 ± 1**	**18 ± 1**	ND	ND
ODMF	**17 ± 1**	**18 ± 1**	**16 ± 1**	**15 ± 1**	**14 ± 1**	**14 ± 1**	**17 ± 1**	**17 ± 1**	**15 ± 1**	**18 ± 1**	**15 ± 1**	ND	ND
PPMF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SDMF	**12 ± 1**	**18 ± 1**	**12 ± 1**	**12 ± 1**	**12 ± 1**	**11 ± 1**	**13 ± 1**	**13 ± 1**	**ND**	**16 ± 1**	**12 ± 1**	ND	ND

The values in bold signify zone of inhibition (mm)

Evaluation of Antibacterial Activity and MIC, MBC Values of Plant Extracts

Of the twenty plants, seven demonstrated antibacterial activity against the tested pathogens (Supplementary Information). The ZOI against Gram-positive bacteria ranged between 11 and 23 mm with a maximum ZOI of 23 mm by OCMF against *S. aureus* and a minimum ZOI of 11 mm by SDMF against *M. smegmatis*. Similarly, ZOI against Gram-negative bacteria ranged between 10 and 22 mm with maximum and minimum ZOIs of 22 and 11 mm by OCMF and GLMD against *P. aeruginosa* and *S. typhimurium*, respectively. The antibacterial results (ZOIs) demonstrated that BC, BS, EC, KP, LM, MS, PA, PD, ST, SA, and YE are highly susceptible to OCMF, ODMF and SDMF, OCMF, OCMF, GAMD, OCMF, OCMF, OCMF, OCMF, OCMF, and APMF (larger ZOI) and least susceptible to SDMF, APMF, SDMF, SDMF, GLMD and SDMF, SDMF, SDMF, APMF and GLMD, GLMD, GLMD, and SDMF, respectively (smaller ZOI). Furthermore, no antibacterial activity was exhibited by AHMF, ASMF, ASMD, CPMF, DIMD, DEMD, EKMD, ERMD, HCMF, IAMF, JGMD, MMMD, and PPMF against any of the tested strains. Also, methanol (M) does not show any antibacterial activity while gentamicin showed prominent activity against all the tested bacteria. Furthermore, none of the extracts inhibited *C. albicans* while nystatin demonstrated potential antifungal activity (Table 3).

The MIC_{80} and MBC values of the active extracts respectively ranged between 1.2–1.9 mg/mL and 1.6–2.3 mg/mL against *P. aeruginosa* and 1.1–1.7 mg/mL and 1.3–2.1 mg/mL against *C. violaceum*. The MIC values also demonstrated that *P. aeruginosa* and *C. violaceum* are more susceptible to OCMF and ODMF, respectively, and least susceptible to SDMF. Furthermore, TAA values were also evaluated as described earlier which demonstrated that GLMD and SDMF have the highest (274.61 mL/g) and lowest (150.0 mL/g) TAA against *P. aeruginosa*, respectively, while GLMD and GAMD demonstrated the highest (274.61 mL/g) and lowest (153.57 mL/g) TAA against *C. violaceum*, respectively (Table 4).

Table 4 MIC_{80} (mg/mL), MBC (mg/mL), and total antibacterial activity(mL/g) of active plant extracts against *P. aeruginosa* and *C. violaceum*

Plant extract	Yield (mg)	*P. aeruginosa*			*C. violaceum*		
		MIC_{80} (mg/mL)	MBC (mg/mL)	TAA (mL/g)	MIC_{80} (mg/mL)	MBC (mg/mL)	TAA (mL/g)
APMF	302	1.9	2.1	158.54	1.6	1.9	188.75
CSMD	260	1.4	1.8	185.71	1.3	1.8	200.0
GAMD	215	1.3	1.8	165.38	1.4	1.8	153.57
GLMD	357	1.3	1.7	274.61	1.3	1.5	274.61
OCMF	227	1.2	1.6	189.17	1.2	1.3	189.17
ODMF	241	1.5	1.9	160.67	1.1	1.4	219.09
SDMF	315	2.1	2.3	150.0	1.7	2.1	185.30

Fig. 3 Demonstration of the antibiofilm effect of the active extracts against *P. aeruginosa*. The tissue culture plate method demonstrated significant inhibition (≥50%) of initial attachment of *P. aeruginosa* to TCP by the extracts (CSMD, ODMF, GAMD, APMF, MMMF, SDMF, GLMD, OCMF, JGMD) at sub-MIC in comparison to untreated control. Furanone (F) and methanol (M) are positive and negative controls, respectively. One milligram per millilitre in the *X*-axis depicts the tested concentration of plant extracts while furanone (F) and methanol (M) were used at 1 mM and 10% v/v, respectively. Error bars represent the standard deviations of three measurements. ***, $p < 0.01$ compared with the control

Demonstration of Antibiofilm Activity of active plant extracts Against *P. aeruginosa*

Effect of Plant Extracts on Inhibition of Biofilm Formation (Initial Attachment)

The result demonstrated the inhibition of biofilm formation against *P. aeruginosa* by plant extracts in the range of 8.99–88.3%. In reference to the established criteria, the percentage of biofilm inhibition greater than 50% is considered good antibiofilm activity while % inhibition in the range of 0–49% is considered poor or weak. In this context, 9 extracts demonstrated good antibiofilm activity, viz. CSMD (88.3%) > ODMF (77.1%) > GAMD (76.6%) > APMF (75.9%) > MMMF (74.9%) > SDMF (67.3%) > GLMD (67.1%) > OCMF (63.8%) > JGMD (56.3%). The extracts significantly inhibited the initial attachment or adherence of cells to the surfaces in comparison to the untreated control. However, no inhibition was seen in methanol while furanone (positive control) showed 94.85% inhibition (Fig. 3).

Scanning Electron Microscopy

The SEM images also confirmed the inhibition of biofilm by the plant extracts. It is evident from Fig. 4 that a matrix of densely packed *P. aeruginosa* cells along with extracellular polymeric substances (EPS) network could be seen in absence of extract (control); however, a significant reduction in cell density attached to the surfaces as well as EPS production could be seen in treated samples.

Effect of Plant Extracts on Preformed Biofilm

The plant extracts that attenuated the initial attachment of bacteria were evaluated for their activity on preformed biofilm (24 h and 48 h). Six plant extracts, viz. CSMD, ODMF, GAMD, APMF, SDMF, GLMD, and JGMD reduced biofilm biomass of *P. aeruginosa* at 24 h post-development with percentage inhibition of 84.2%, 66.1%, 72.0%, 58.9%, 60.6%, 61.4%, and 54.2%, respectively. Similarly, the effect of plant extracts on biofilm eradication or biofilm maturation on 48-h preformed biofilm demonstrated significant results in CSMD (73.0%), GAMD (61.9%), SDMF (53.9%), and GLMD (60.9%) (Fig. 5).

Pyocyanin Activity

The quantification of the pyocyanin assay demonstrated significant pyocyanin reduction against *P. aeruginosa* by some plant extracts. Of the 20 plant extracts, 10 demonstrated significant (≥50%) pyocyanin inhibition, viz. GAMD (83.9%), APMF (83.6%), SDMF (83.5%), ODMF (75.8%), DIMD (74.1%), GLMD (73.7%), CSMD (65.9%), MMMF (65.0%), OCMF (60.0%), and CPMF (53.5%). The lowest pyocyanin inhibition was reported in AHMF (0.72%) followed by IAMF (4.16%) and DEMD (7.00%).

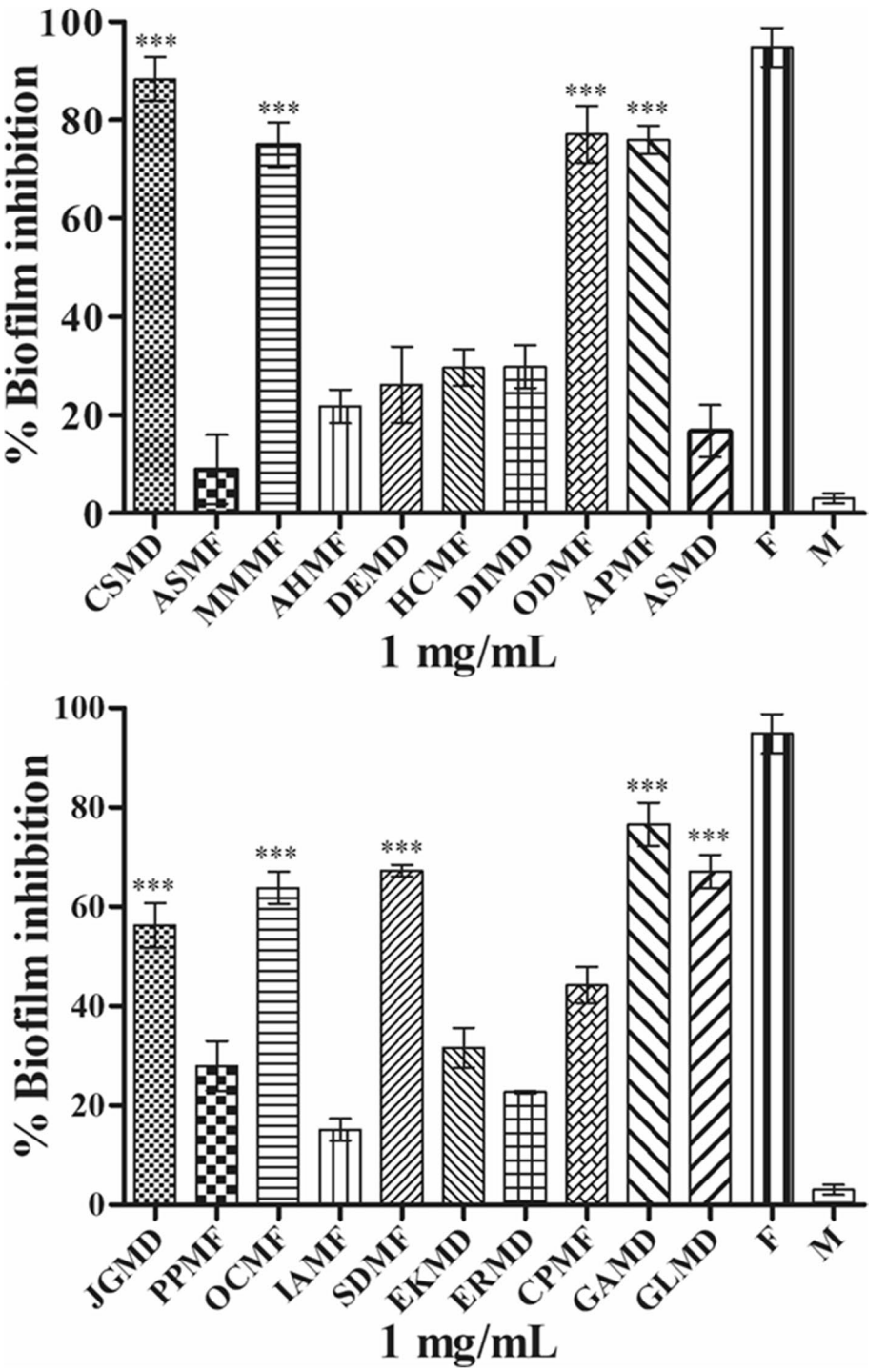

% Biofilm inhibition
100
80
60
40
20
0
***
CSMD
ASMF
MMMF
AHMF
DEMD
HCMF
DIMD
ODMF
APMF
ASMD
F
M
1 mg/mL
% Biofilm inhibition
JGMD
PPMF
OCMF
IAMF
SDMF
EKMD
ERMD
CPMF
GAMD
GLMD
F
M
1 mg/mL

Fig. 4 Scanning electron microscopy. The figure demonstrates inhibition of bacterial attachment to the surface in presence of different plant extracts, viz. CSMD, ODMF, GAMD, APMF, MMMF, SDMF, GLMD, OCMF, and JGMD; a network of cells embedded within matrix could be seen in control; scale bar of 5 µm and magnification 3500×

Methanol showed insignificant pyocyanin inhibition of 3% while furanone demonstrated 88.0% inhibition. Furthermore, a uniform bacterial growth was observed in all the treated (OD_{600} = 1.95–2.10) and untreated samples (OD_{600} = 2.15) validating that pyocyanin inhibition was due to the inhibitory effect of the extracts on virulence production and not due to their toxicity (Fig. 6).

Anti-quorum Sensing Activity

Violacein Inhibition Assay

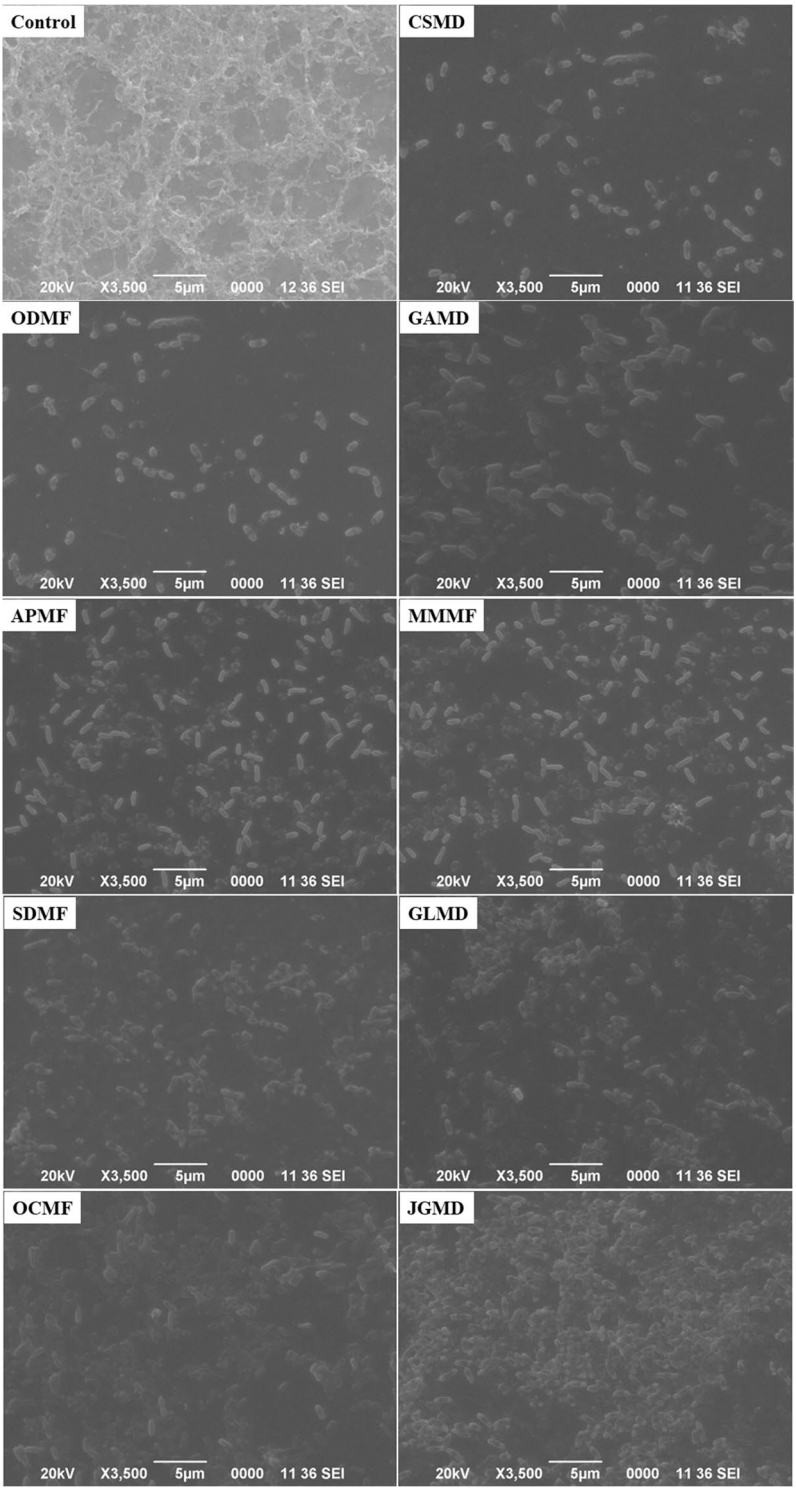
Figure 7 demonstrates the inhibition of violacein production by the tested plant extracts. The result demonstrated the inhibition of violacein production in the range of 88.8–2.93%. The maximum violacein inhibition was reported in CSMD (88.8%) followed by APMF (81.8%) while the lowest activity was reported in IAMF (2.93%) followed by PPMF (12.9%). Methanol and furanone demonstrated violacein inhibition of 1.0% and 94.8%, respectively. Furthermore, almost similar $\log_{10}$CFU/mL values of treated (6.5–6.9$\log_{10}$CFU/mL) and untreated (6.9$\log_{10}$CFU/mL) samples after 24 h incubation demonstrated that the violacein inhibition was due to anti-QS effects of the extracts and not because of their bactericidal activity.

Evaluation of Anti-quorum Sensing Activity Through Quorum Sensing Signal Inhibition

We proposed that the antibiofilm activity of plant extracts could be because of the interruption of QS. The plant extracts that exhibited both antibiofilm and ant-QS activities were evaluated for violacein production by CV12472 in presence of external C_6-AHL. It was found that violacein production was restored in CV12472 treated with plant samples after adding C_6-AHL signifying that the antibiofilm activity of the plant extracts could be due to their interruption of QS signalling. CV026 used as a positive control also produced purple pigment after the addition of C_6-AHL while no violacein production was observed in C_6-AHL-deficient CV026. The violacein production was restored up to 90.8% (Fig. 8).

Discussion

Plants are significantly contributing to basic healthcare despite the advancements in conventional modern medicines. Natural products, predominantly from plant sources, are referred to as the important source of novel and potent therapeutics. Plants are a reservoir of different bioactive compounds like phenolics, polyphenolics, and flavonoids. Therefore, they are being explored for the discovery of novel compounds with different biological activities including antimicrobial, antibiofilm, and antioxidant properties [46]. The plant-derived antioxidants including ascorbic acid, polyphenols, gallic acid, and carotenoids were reported to decrease oxidative stress in humans through scavenging, reduction of lipid peroxidation, and nitrosation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GAMD
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APMF
20kV X3,500 5μm 0000 11 36 SEI
MMMF
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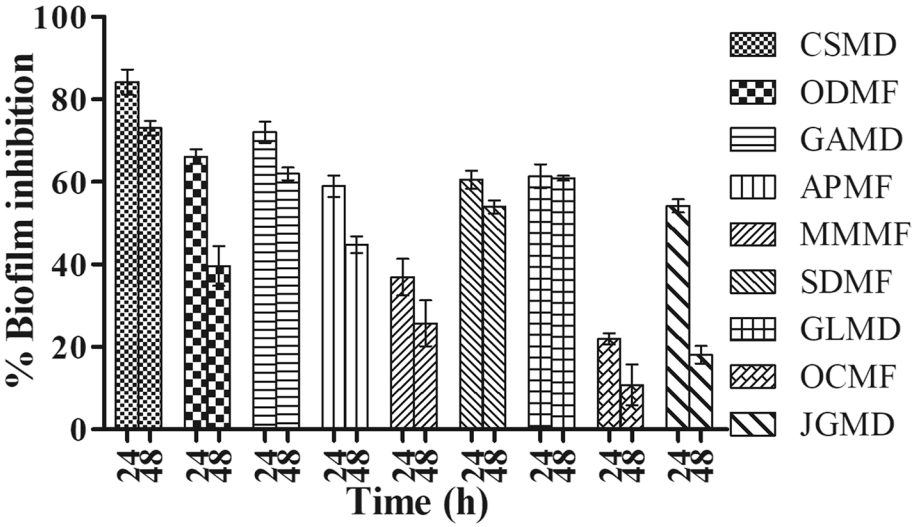

Fig. 5 Evaluation of the antibiofilm effect on preformed biofilm of *P. aeruginosa*. The graph demonstrates the eradication and inhibition of biofilm maturation of biofilm (≥ 50%) by the extracts CSMD, ODMF GAMD, APMF, SDMF, GLMD, and JGMD in 24-h period of preformed biofilm. Similarly, CSMD, GAMD, SDMF, and GLMD eradicated or inhibited biofilm maturation (≥ 50%) in 48-h period of preformed biofilm. Error bars represent the standard deviations of three measurements

reactions [8]. The ethnomedicinal plants from NEI are being used in the folk medicinal system to treat diseases including fever, asthma, skin allergies, diabetes, menstrual cramps, diarrhoea, cough, and hypertension [55]. In this context, the current study was carried out to evaluate the antioxidant properties of different plant extracts from NEI by DPPH and reducing power assays. The purple-coloured DPPH solutions turn colourless due to the scavenging of DPPH by antioxidants or free radicals. The higher the change in colour intensity, the higher is the free radical scavenging activity and thus, the higher the antioxidant potential of the tested extract. Various tested plant extracts demonstrated significant antioxidant activity via DPPH assay. Among all *C. sinensis* var. *assamica*, *A. paniculate*, *A. hookeri*, *P. peepuloides*, *M. micrantha*, and *H. cordata* demonstrated the highest DPPH scavenging activity as a high degree of colour change of the DPPH solution (decrease in absorbance) was observed in them. Similarly, reducing power assay also demonstrated the significant antioxidant of the tested extracts particularly *C. sinensis* var. *assamica*, *A. paniculate*, *O. corniculate*, *P. peepuloides*, *A. hookeri*, and *M. micrantha* since the highest colour shift (yellow to green) was observed among them. The assay is based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form in presence of reductants. It has been reported that the reducing properties demonstrated antioxidant potential by breaking the free radical chain by the donation of hydrogen atoms [56].

Food and nutrition science has developed an interest in phenolic and flavonoid-rich diets owing to their different biological activities. Furthermore, phenolic-rich plant materials are widely used in the food industry since they enhance the nutritional value and quality of food by reducing the oxidative degradation of lipids. Phenolics act as strong antioxidants either by donating electrons or by enhancing the endogenous synthesis of antioxidant molecules within the cell [57]. In the current study, the TPC values ranged between 31.0 and 5.32 mg GAE/g DW. The highest TPC values of CSMD (31.0), APMF (30.6), AHMF (28.6), PPMF (22.7), and MMMF (19.2) could be attributed to their strongest antioxidant

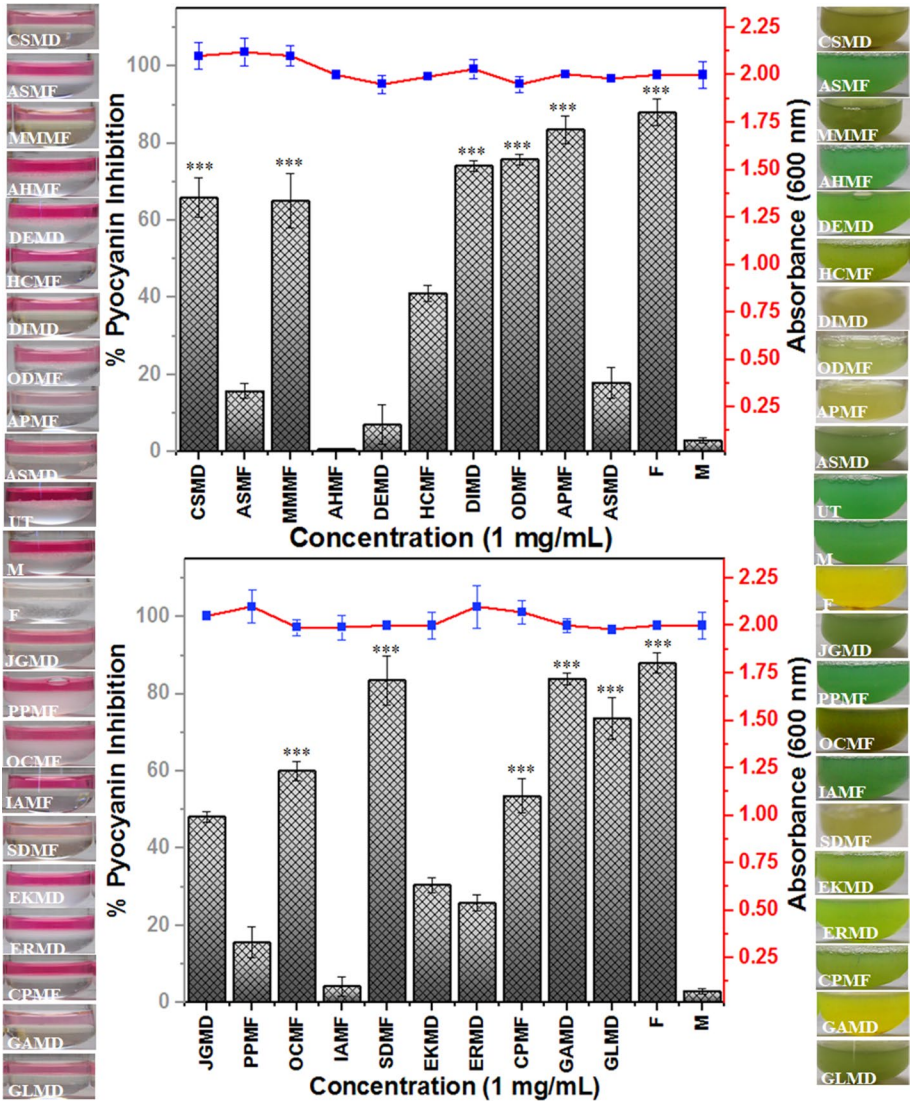

Fig. 6 Antipyocyanin activity of the extracts. The figure depicts significant (≥ 50%) pyocyanin inhibition in *P. aeruginosa* by some plant extracts, viz. GAMD, APMF, SDMF, ODMF, DIMD, GLMD, CSMD, MMMF, OCMF, and CPMF. The figure also demonstrated no significant growth change (absorbance 600) of *P. aeruginosa* in treated samples in comparison to untreated control. Furanone (F) and methanol (M) are positive and negative controls, respectively. One milligram per millilitre in the *X*-axis depicts the tested concentration of plant extracts while furanone (F) and methanol (M) were used at 1 mM and 10% v/v, respectively. Error bars represent the standard deviations of three measurements. ***, $p < 0.01$ compared with the control

potential values discussed earlier. Similarly, TFC values ranged between 68.2 and 1.74 mg QE/g DW and the high TFC of CSMD, GAMD, APMF, AHMF, and MMMF could be responsible for their strong antioxidant activity. A similar study carried out by Chavan et al. [58] demonstrated TFC and TPC values in the range of 0.1–15.3 mg of rutin equivalents/

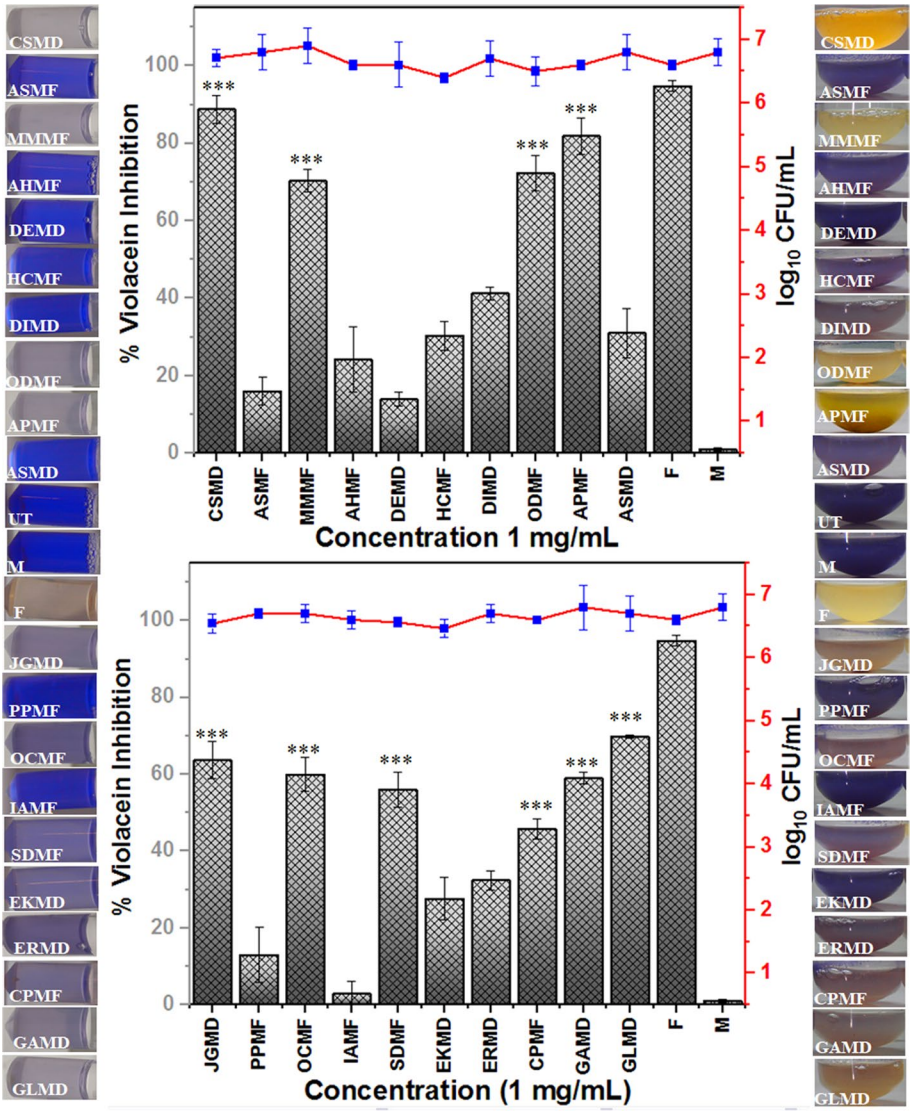

Fig. 7 Effect of the extracts on violacein production. A strong violacein inhibitory activity was shown by some plant extracts (≥50%) against *C. violaceum*. The figure also illustrates no significant change in log10CFU/mL of *C. violaceum* in treated and untreated samples. Furanone (F) and methanol (M) are positive and negative controls, respectively. One milligram per millilitre in the *X*-axis depicts the tested concentration of plant extracts while furanone (F) and methanol (M) were used at 1 mM and 10% v/v, respectively. Error bars represent the standard deviations of three measurements. ***, $p < 0.01$ compared with the control

gram fresh weight and 0.3–28.5 mg of tannic acid equivalents/gram fresh weight, respectively, for different *Ceropegia* sp. The other phytochemical analyses including the determination of vitamin C, β-carotene, total carotenoids, total sugars, reducing sugars, and protein content of all the plants were carried out. The analysis demonstrated their abundance in the plant samples signifying the dietary and pharmaceutical values of the extracts.

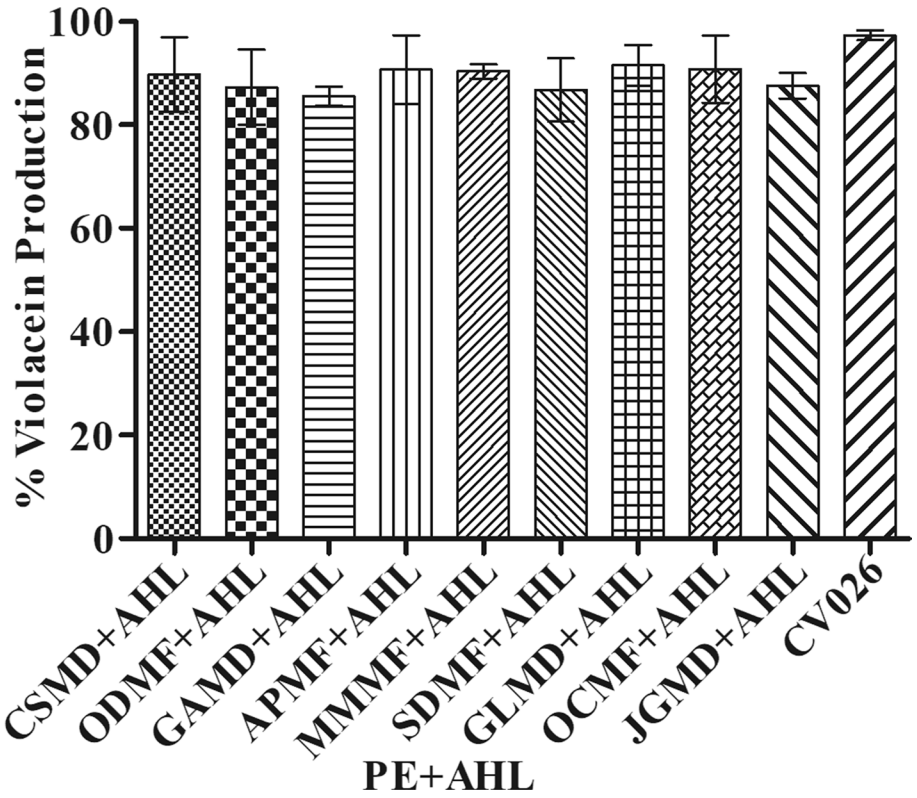

Fig. 8 Anti-violacein activity of the extracts via quorum sensing inhibition. The figure shows restoration of violacein production in active extract-treated *C. violaceum* (CV12472) supplemented with exogenous acyl homoserine lactone, viz. C_6-AHL. AHL-deficient strain CV026 also restored violacein synthesis in presence of C_6-AHL. PE + AHL: plant extract supplemented with AHL. Error bars represent the standard deviations of three measurements

Plants continue to be one of the leading sources of therapeutic compounds including antimicrobials against pathogenic microorganisms, viz. bacteria, fungi, viruses, etc. to minimize the spread and recurrence of infectious diseases. The use of ethnomedicinal plants to impede the spread of infectious diseases offers the most emergent non-toxic and eco-friendly antimicrobial approach [59]. Our results demonstrated that *C. sinensis* var. *assamica*, *A. paniculate*, *G. acuminata*, *G. lanceifolia*, *O. corniculate*, *O. debilis*, and *S. dulcis* exhibited potent antibacterial activity against the tested pathogenic strains. The extracts exhibited inhibitory effects against both Gram-positive and Gram-negative bacteria. The tested strains are important in the human healthcare system, food industry, and animal husbandry since they are associated with clinical infections, food spoilage, and food-borne diseases. Subsequently, the MIC and MBC values of active extracts against *P. aeruginosa* and *C. violaceum* showed their variable susceptibility towards them. It was found that *P. aeruginosa* was more susceptible to *O. corniculate* and least susceptible to *S. dulcis* while *C. violaceum* was most susceptible to *O. debilis* and least susceptible to *S. dulcis*. The TAA value gives the volume of solvent to which 1 g of the extract is added and still shows antimicrobial activity. For example, the highest TAA 274.61 (mL/g) of *G. lanceifolia* against

P. aeruginosa reported in the current study signifies that the extract will show antibacterial activity against it even if 1 g of the extract is dissolved in about 0.27 L of the solvent. Furthermore, MIC and TAA values are important pharmacological tools for determining the potency (mg/mL) for isolating bioactive compound and efficacy (mL/g) of the extract (plant selection). A similar study carried out by Elisha et al. [59] demonstrated the antibacterial activity, MIC, MBC, and TAA values of nine plant extracts, viz. *Cremaspora trifloral*, *Maesa lanceolata*, *Maesa lanceolata*, *Hypericum roeperianum*, *Elaeodendron croceum*, *Calpurnia aurea*, *Hypericum roeperianum*, *Heteromorpha arborescens*, and *Pittosporum viridiflorum* against both Gram-positive and Gram-negative bacteria.

Microbial antibiotic drug resistance and biofilm induction are the biggest challenges the healthcare sector is prevailing through. The microbes develop biofilms in response to environmental cues to develop resistance against conventional chemotherapies (antibiotics) [60]. *P. aeruginosa*, a ubiquitous opportunistic pathogen infecting animals, humans, insects, and plants, forms strong biofilms and is responsible for 57% of nosocomial infections [46]. The plant extracts are reported to exhibit antibiofilm activity against a wide range of microbes. The current study demonstrated a significant antibiofilm activity of some extracts against *P. aeruginosa* by inhibiting initial attachment to the surface. Furthermore, the plant extracts that inhibited ≥ 50% biofilm were checked for their effect on preformed biofilm. Of all the active extracts, *C. sinensis* var. *assamica*, *G. acuminata*, *S. dulcis*, and *G. lanceifolia* significantly eradicated the biofilm of 24- and 48-h periods. The antibiofilm activity of the extracts could be due to the effect on cellular hydrophobicity, inhibition of EPS secretion, cell-associated virulence factors, overall interruption of the biofilm-related signalling cascade, etc. *P. aeruginosa* is associated with the production of virulence factors, viz. pyocyanin, alginate, rhamnolipid, elastase, and protease which induce pathogenicity to it and help invasion to host tissues. The secretion of these virulence factors is regulated by cell-to-cell communication called QS. Pyocyanin is a blue-coloured phenazine compound that produces ROS and induces cellular damage by attacking host biological membranes and exhausting intracellular c-AMP as well as ATP levels, thus impairing cellular respiration as well [61]. The study demonstrated significant pyocyanin inhibition by some plant extracts at sub-MIC. *G. acuminata*, *S. dulcis*, *A. paniculate*, etc. demonstrated the highest reduction in green colour intensity, thus indicating the highest pyocyanin reduction.

The abundance of different phytochemicals in traditional plants including phenolics, flavonoids, terpenoids, polystyrenes, quinones, and alkaloids has been reported involved in QS inhibition and attenuation of microbial pathogenesis associated with their biofilms [62]. In this regard, some of our tested extracts demonstrated significant violacein inhibition against CV12472 at sub-MIC. The results indicate the presence of quorum sensing inhibitors (QSIs) in the plant extracts. Furthermore, plant secondary metabolites especially flavanones, quinones, terpenoids, and phenolics are reported to interrupt QS signalling or damage QS receptors owing to their similar chemical structure to that of AHLs (QS signals). In an attempt to justify that our active plant extracts inhibited biofilm and QS by inhibiting AHL production via interruption of QS signalling, external AHL was added to extract-treated CV12472. The results demonstrated resumption of violacein synthesis in extract-treated CV12472 which indicated that the extracts inhibited AHL synthesis by interrupting QS. To validate our results that the reduction of pyocyanin and violacein production was due to interruption of QS and not due to cytotoxicity or bactericidal nature of plant extracts, $\log_{10}$CFU/mL and growth pattern (OD_{600}) of all the treated and untreated samples remained the same after 24 h of incubation. The results demonstrated consistency with the findings of Moradi et al. [48].

Various QSIs have been discovered from different sources including microorganisms, plants, and chemical synthesis which play a vital role in impeding QS-regulated biofilm formation and production of virulence factors. Different mechanisms have been established regarding the inhibition of microbial communication by QSIs, viz. competitive binding of QSIs at QS signal molecule binding sites, inhibition of synthesis of autoinducers (AIs), degradation of signal molecules, immunological scavenging of signal molecules, interfering QS signalling cascades and impeding QS-regulated genes, etc. [63–66].

Conclusion and Future Perspectives

The discovery of plant-derived novel antioxidants and antimicrobial agents offers an effective, non-toxic, and sustainable alternative to conventional drugs. In this regard, the ethnomedicinal plants of NEI were evaluated for their phytochemical composition, antioxidant, antibacterial, antibiofilm, and anti-QS activities to validate their traditional importance. Our study demonstrated the presence of variable concentrations of different phytochemicals like phenolics, flavonoids, total carotenoids, β-carotene, vitamin C, sugars, and proteins in the selected extracts. The abundance of these phytochemicals in the extracts attributed significant antioxidant potential to them. Some of the plant extracts examined in the study demonstrated inhibitory potential against both sessile and planktonic forms of bacteria, therefore could be potential candidates for isolation of antimicrobial and antibiofilm compounds. In conclusion, this study provides comprehensive detail of the biological activities of the plants of NEI used in traditional medicine systems and provides validation for their inclusion in the basic healthcare system. The active plant extracts in this study could be developed as strong antioxidant and antimicrobial agents subjected to further investigation and validation. Furthermore, it is imperative to determine the mechanism of action of the plant extracts on different pathogenic microorganisms.

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Author Contribution Muzamil Ahmad Rather: conceptualization, methodology, conducted experiments, writing original draft, preparation, investigation, data analysis. Kuldeep Gupta, Arun Kumar Gupta: investigation. Poonam Mishra, Asifa Qureshi, Tapan Kumar Dutta, Siddhartha Narayan Joardar: review and editing. Manabendra Mandal: supervision, fund acquisition, investigation, project administration, review and editing. All authors contributed to the article and approved the submitted version.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate No human participant and/or animal were used in the study.

Competing Interests The authors declare no competing interests.

References

1. Raj, A. J., Biswakarma, S., Pala, N. A., Shukla, G., Kumar, M., Chakravarty, S., & Bussmann, R. W. (2018). Indigenous uses of ethnomedicinal plants among forest-dependent communities of Northern Bengal. *Journal of Ethnobiology and Ethnomedicine, 14*(1), 1–28. https://doi.org/10.1186/s13002-018-0208-9
2. Faruque, M. O., Uddin, S. B., Barlow, J. W., Hu, S., Dong, S., Cai, Q., Li, X., & Hu, X. (2018). Quantitative ethnobotany of medicinal plants used by indigenous communities in the Bandarban District of Bangladesh. *Frontiers in Pharmacology, 9*, 40. https://doi.org/10.3389/fphar.2018.00040
3. Quílez, A. M., Fernández-Arche, M. A., García-Giménez, M. D., & De la Puerta, R. (2018). Potential therapeutic applications of the genus Annona: Local and traditional uses and pharmacology. *Journal of Ethnopharmacology, 225*, 244–270. https://doi.org/10.1016/j.jep.2018.06.014
4. Bhat, J. A., Kumar, M., & Bussmann, R. W. (2013). Ecological status and traditional knowledge of medicinal plants in Kedarnath Wildlife Sanctuary of Garhwal Himalaya, India. *Journal of Ethnobiology and Ethnomedicine, 9*(1), 1–18. https://doi.org/10.1186/1746-4269-9-1
5. Tlili, H., Hanen, N., Ben Arfa, A., Neffati, M., Boubakri, A., Buonocore, D., Dossena, M., Verri, M., & Doria, E. (2019). Biochemical profile and in vitro biological activities of extracts from seven folk medicinal plants growing wild in southern Tunisia. *PLoS ONE, 14*(9), e0213049. https://doi.org/10.1371/journal.pone.0213049
6. Ullah, R., Alqahtani, A. S., Noman, O. M., Alqahtani, A. M., Ibenmoussa, S., & Bourhia, M. (2020). A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. *Saudi Journal of Biological Sciences, 27*(10), 2706–2718. https://doi.org/10.1016/j.sjbs.2020.06.020
7. Cui, X., Lin, Q., & Liang, Y. (2020). Plant-derived antioxidants protect the nervous system from aging by inhibiting oxidative stress. *Frontiers in Aging Neuroscience, 12*, 209. https://doi.org/10.3389/fnagi.2020.00209
8. Kumar, M., Pratap, V., Nigam, A. K., Sinha, B. K., Kumar, M., & Singh, J. K. G. (2021). Plants as a source of potential antioxidants and their effective nanoformulations. *Journal of Scientific Research, 65*(3), 57–72. https://doi.org/10.37398/JSR.2021.650308
9. Rather, M. A., Gupta, K., & Mandal, M. (2021). Microbial biofilm: Formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology, 52*(4), 1701–1718. https://doi.org/10.1007/s42770-021-00624-x
10. Mishra, R., Panda, A.K., De Mandal, S., Shakeel, M., Bisht, S.S. and Khan, J. (2020). Natural anti-biofilm agents: Strategies to control biofilm-forming pathogens. *Frontiers in Microbiology 2640*. https://doi.org/10.3389/fmicb.2020.566325
11. Singh, A., Nautiyal, M. C., Kunwar, R. M., & Bussmann, R. W. (2017). Ethnomedicinal plants used by local inhabitants of Jakholi block, Rudraprayag district, western Himalaya, India. *Journal of Ethnobiology and Ethnomedicine, 13*(1), 1–29. https://doi.org/10.1186/s13002-017-0178-3
12. Kumar, M., Devi, H., Prakash, S., Rathore, S., Thakur, M., Puri, S., Pundir, A., Bangar, S. P., Changan, S., Ilakiya, T., & Samota, M. K. (2021). Ethnomedicinal plants used in the health care system: Survey of the mid hills of solan district, Himachal Pradesh, India. *Plants, 10*(9), 1842. https://doi.org/10.3390/plants10091842
13. Mao, A. A., Hynniewta, T. M., & Sanjappa, M. (2009). Plant wealth of Northeast India with reference to ethnobotany. *Indian j. tradit. knowl., 8*(1), 96–103.
14. Patel, S., Gamit, S., Qureshimatva, U., & Solanki, H. (2019). Distribution patterns of *Acmella paniculata* (Wall. Ex DC.) RK Jansen in Gujarat, India. *International Journal of Research in Advent Technology, 7*, 186–191.
15 Jang, J. Y., Lee, M. J., You, B. R., Jin, J. S., Lee, S. H., Yun, Y. R., & Kim, H. J. (2017). Allium hookeri root extract exerts anti-inflammatory effects by nuclear factor-κB down-regulation in lipopolysaccharide-induced RAW264. 7 cells. *BMC Complementary and Alternative Medicine, 17*(1), 1–9. https://doi.org/10.1186/s12906-017-1633-3
16. Alam, K., Hoq, O., & Uddin, S. (2016). Medicinal plant *Allium sativum*. A review. *Journal of Medicinal Plants Studies, 4*(6), 72–79.
17. Mondal, H., Saha, S., Awang, K., Hossain, H., Ablat, A., Islam, M. K., Jahan, I. A., Sadhu, S. K., Hossain, M. G., Shilpi, J. A., & Uddin, S. J. (2014). Central-stimulating and analgesic activity of the ethanolic extract of *Alternanthera sessilis* in mice. *BMC Complementary and Alternative Medicine, 14*(1), 1–9. https://doi.org/10.1186/1472-6882-14-398
18. Khanongnuch, C., Unban, K., Kanpiengjai, A., & Saenjum, C. (2017). Recent research advances and ethno-botanical history of miang, a traditional fermented tea (Camellia sinensis var. assamica) of northern Thailand. *Journal of Ethnic Foods, 4*(3), 135–144. https://doi.org/10.1016/j.jef.2017.08.006
19. Boldrin, P. K., Resende, F. A., Höhne, A. P. O., de Camargo, M. S., Espanha, L. G., Nogueira, C. H., Melo, M. D. S. F., Vilegas, W., & Varanda, E. A. (2013). Estrogenic and mutagenic activities of

Crotalaria pallida measured by recombinant yeast assay and Ames test. *BMC Complementary and Alternative Medicine, 13*(1), 1–10. https://doi.org/10.1186/1472-6882-13-216
20. Kviecinski, M. R., David, I. M. B., Fernandes, F. D. S., Correa, M. D. R., Clarinda, M. M., Freitas, A. F., Silva, J. D., Gava, M., Müller, S. D., Florentino, D., & Petronilho, F. (2017). Healing effect of *Dillenia indica* fruit extracts standardized to betulinic acid on ultraviolet radiation-induced psoriasis-like wounds in rats. *Pharmaceutical Biology, 55*(1), 641–648. https://doi.org/10.1080/13880209.2016.1266672
21 Semwal, P., Painuli, S., Painuli, K. M., Antika, G., Tumer, T. B., Thapliyal, A., Setzer, W. N., Martorell, M., Alshehri, M. M., Taheri, Y., & Daştan, S. D. (2021). Diplazium esculentum (Retz.) Sw.: ethnomedicinal, phytochemical, and pharmacological overview of the Himalayan ferns. *Oxidative Medicine and Cellular Longevity, 2021*, 1–15. https://doi.org/10.1155/2021/1917890
22. Hannah, L., Aguilar, G., & Blanchon, D. (2019). Spatial distribution of the Mexican daisy, *Erigeron karvinskianus*, in New Zealand under climate change. *Climate, 7*(2), 24. https://doi.org/10.3390/cli7020024
23. Chrystomo, L.Y., Karim, A.K., Nugroho, L.H., Wahyuono, S. and Nohno, T. (2012). Antiproliferative effect of *Eupatorium riparium* Reg. leaves benzene extract against C2C12 and MKN45 cell line in vitro. International Conference: Research and Application on Traditional Complementary and Alternative Medicine in Health Care (TCAM) June, 22nd -23rd 2012 Surakarta Indonesia, pp 127–132. http://hdl.handle.net/11617/2426
24. Baruah, S., Barman, P., Basumatary, S., & Bhuyan, B. (2021). Diversity and ethnobotany of genus Garcinia L (Clusiaceae) in Assam. Eastern Himalaya. *Ethnobotany Research and Applications, 21*(1), 1–14. https://doi.org/10.32859/era.21.33
25. Bora, N. S., Kakoti, B. B., Bairy, P. S., & Gogoi, B. (2014). *Garcinia lanceifolia* Roxb; an endemic medicinal plant of assam relieves pain and delays nociceptive response: An assay for its analgesic and anti-inflammatory activity. *International Journal of Pharmaceutical Sciences and Drug Research, 6*(3), 216–219.
26. Chung, M. S., Bae, W. J., Choi, S. W., Lee, K. W., Jeong, H. C., Bashraheel, F., Jeon, S. H., Jung, J. W., Yoon, B. I., Kwon, E. B., & Oh, H. A. (2017). An Asian traditional herbal complex containing Houttuynia cordata Thunb, Perilla frutescens Var. acuta and green tea stimulates hair growth in mice. *BMC Complementary and Alternative Medicine, 17*(1), 1–11. https://doi.org/10.1186/s12906-017-2003-x
27. Wang, J.H., Bose, S., Shin, N.R., Chin, Y.W., Choi, Y.H. and Kim, H. (2018). Pharmaceutical impact of *Houttuynia Cordata* and metformin combination on high-fat-diet-induced metabolic disorders: Link to intestinal microbiota and metabolic endotoxemia. *Frontiers in Endocrinology 620*. https://doi.org/10.3389/fendo.2018.00620
28. Manvar, M. N., & Desai, T. R. (2013). Phytochemical and pharmacological profile of *Ipomoea aquatica*. *Indian Journal of Medical Sciences, 67*, 1–12. https://doi.org/10.4103/0019-5359.121115
29. Varma, R. S., Ashok, G., Vidyashankar, S., Patki, P., & Nandakumar, K. S. (2011). Ethanol extract of Justicia gendarussa inhibits lipopolysaccharide stimulated nitric oxide and matrix metalloproteinase-9 expression in murine macrophage. *Pharmaceutical Biology, 49*(6), 648–652. https://doi.org/10.3109/13880209.2010.527993
30. Sheam, M., Haque, Z., & Nain, Z. (2020). Towards the antimicrobial, therapeutic and invasive properties of *Mikania micrantha* Knuth: a brief overview. *Journal of Advanced Biotechnology and Experimental Therapeutics, 3*(2), 92–101. https://doi.org/10.5455/jabet.2020.d112
31. Badwaik, H., Singh, M. K., Thakur, D., Giri, T. K., & Tripathi, D. K. (2011). The botany, chemistry, pharmacological and therapeutic application of *Oxalis corniculata* Linn-a review. *International Journal of Phytomedicine, 3*(1), 01.
32. Junejo, J. A., Zaman, K., Rudrapal, M., & Hussain, N. (2020). Antidiabetic and antioxidant activity of hydro-alcoholic extract of *Oxalis debilis* Kunth. leaves in experimental rats. *Bioscience Biotechnology Research Communications, 13*, 860–867.
33. Tynsong, H., Dkhar, M., & Tiwari, B. K. (2013). Domestication, conservation, and livelihoods: A case study of piper peepuloides roxb.—An important nontimber forest product in South Meghalaya, Northeast India. *International Journal of Biodiversity, 2013*, 1–7. https://doi.org/10.1155/2013/987914
34. Islam, S. M. A., Ahmed, K. T., Manik, M. K., Wahid, M. A., & Kamal, C. S. I. (2013). A comparative study of the antioxidant, antimicrobial, cytotoxic and thrombolytic potential of the fruits and leaves of *Spondias dulcis*. *Asian Pacific Journal of Tropical Biomedicine, 3*(9), 682–691. https://doi.org/10.1016/S2221-1691(13)60139-2
35. Rather, M. A., Deori, P. J., Gupta, K., Daimary, N., Deka, D., Qureshi, A., Dutta, T. K., Joardar, S. N., & Mandal, M. (2022). Ecofriendly phytofabrication of silver nanoparticles using aqueous extract of *Cuphea carthagenensis* and their antioxidant potential and antibacterial activity against clinically important human pathogens. *Chemosphere, 300*, 134497. https://doi.org/10.1016/j.chemosphere.2022.134497

36. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356. https://doi.org/10.1021/ac60111a017
37. Miller, E. V., Winston, J. R., & Schomer, H. A. (1940). Physiological studies of plastid pigments in rinds of maturing oranges. *Journal of Agricultural Research, 60*, 259–267.
38. AOAC. (2019). International Official Methods of Analysis. 21st Edition. Available online: https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/. Accessed March 2022
39. Biswas, A. K., Sahoo, J., & Chatli, M. K. (2011). A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. *LWT - Food Sci. Technol., 44*(8), 1809–1813. https://doi.org/10.1016/j.lwt.2011.03.017
40. de Carvalho, L. M. J., Gomes, P. B., de Oliveira Godoy, R. L., Pacheco, S., do Monte, P. H. F., de Carvalho, J. L. V., Nutti, M. R., Neves, A. C. L., Vieira, A. C. R. A. & Ramos, S. R. R. (2012). Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. *Food Research International, 47*(2), 337–340. https://doi.org/10.1016/j.foodres.2011.07.040
41. Classics Lowry, O., Rosebrough, N., Farr, A., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J. boil. Chem., 193*(1), 265–275.
42. Kara, M., Assouguem, A., Fadili, M. E., Benmessaoud, S., Alshawwa, S. Z., Kamaly, O. A., Saghrouchni, H., Zerhouni, A. R., & Bahhou, J. (2022). Contribution to the evaluation of physicochemical properties, total phenolic content, antioxidant potential, and antimicrobial activity of vinegar commercialized in Morocco. *Molecules, 27*(3), 770. https://doi.org/10.3390/molecules27030770
43. González-Palma, I., Escalona-Buendía, H. B., Ponce-Alquicira, E., Téllez-Téllez, M., Gupta, V. K., Díaz-Godínez, G., & Soriano-Santos, J. (2016). Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages. *Frontiers in Microbiology, 7*, 1099. https://doi.org/10.3389/fmicb.2016.01099
44 Rather, M. A., Gupta, K., & Mandal, M. (2021). Inhibition of biofilm and quorum sensing-regulated virulence factors in Pseudomonas aeruginosa by Cuphea carthagenensis (Jacq.) JF Macbr. leaf extract: an in vitro study. *Journal of Ethnopharmacology., 269*, 113699. https://doi.org/10.1016/j.jep.2020.113699
45. Famuyide, I. M., Aro, A. O., Fasina, F. O., Eloff, J. N., & McGaw, L. J. (2019). Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African Eugenia and *Syzygium* (Myrtaceae) species and their selectivity indices. *BMC Complementary and Alternative Medicine, 19*(1), 1–13. https://doi.org/10.1186/s12906-019-2547-z
46. Singh, V. K., Mishra, A., & Jha, B. (2017). Anti-quorum sensing and anti-biofilm activity of *Delftia tsuruhatensis* extract by attenuating the quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa*. *Frontiers in Cellular and Infection Microbiology, 7*, 337. https://doi.org/10.3389/fcimb.2017.00337
47. Das, A., Das, M. C., Sandhu, P., Das, N., Tribedi, P., De, U. C., Akhter, Y., & Bhattacharjee, S. (2017). Antibiofilm activity of *Parkia javanica* against *Pseudomonas aeruginosa*: A study with fruit extract. *RSC Advances, 7*(9), 5497–5513. https://doi.org/10.1039/C6RA24603F
48. Moradi, F., Hadi, N., & Bazargani, A. (2020). Evaluation of quorum-sensing inhibitory effects of extracts of three traditional medicine plants with known antibacterial properties. *New microbes new infect, 38*, 100769. https://doi.org/10.1016/j.nmni.2020.100769
49. Preetha, T. S., Anju, S., Anilkumar, S., & Mini, I. (2018). Nutritional analysis of selected species of *Alternanthera Forsskal* (Amaranthaceae). *Indian Journal of Experimental Biology, 56*(1), 48–53.
50. Ayam, V. S. (2011). *Allium hookeri*, Thw. Enum. A lesser known terrestrial perennial herb used as food and its ethnobotanical relevance in Manipur. *African Journal of Food, Agriculture, Nutrition and Development, 11*(6). https://doi.org/10.18697/ajfand.47.9330
51. Choudhury, J., Majumdar, S., Roy, S. and Chakraborty, U. (2017). Antioxidant activity and phytochemical screening of two edible wetland pteridophytes *Diplazium esculentum* (Retz) Sw and *Marsilea minuta* L.–a comparative study. *World Journal of Pharmaceutical and Medical 3*(9), 195–203.
52. Devi, O. A., Das, M., Saikia, A., & Das, P. (2016). *AJHS. Asian Journal of Home Science, 11*(1), 127–135.
53. Fiedor, J., Fiedor, L., Haeßner, R., & Scheer, H. (2005). Cyclic endoperoxides of β-carotene, potential pro-oxidants, as products of chemical quenching of singlet oxygen. *Biochimica et Biophysica Acta – Bioenergetics, 1709*(1), 1–4. https://doi.org/10.1016/j.bbabio.2005.05.008
54. Ukil, S., Laskar, S., & Bandyopadhyay, D. (2017). Isolation, purification and partial characterization of *Crotalaria pallida* Aiton seed proteins. *International Journal of Peptide Research and Therapeutics, 23*(4), 461–467. https://doi.org/10.1007/s10989-017-9578-4
55. Singh, G., Passsari, A. K., Leo, V. V., Mishra, V. K., Subbarayan, S., Singh, B. P., Kumar, B., Kumar, S., Gupta, V. K., Lalhlenmawia, H., & Nachimuthu, S. K. (2016). Evaluation of phenolic content

variability along with antioxidant, antimicrobial, and cytotoxic potential of selected traditional medicinal plants from India. *Frontiers in Plant Science, 7*, 407. https://doi.org/10.3389/fpls.2016.00407
56. Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine, 12*(1), 1–12. https://doi.org/10.1186/1472-6882-12-221
57. Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants., 8*(4), 96. https://doi.org/10.3390/plants8040096
58. Chavan, J. J., Gaikwad, N. B., Kshirsagar, P. R., & Dixit, G. B. (2013). Total phenolics, flavonoids and antioxidant properties of three *Ceropegia* species from Western Ghats of India. *South+A1443 African Journal of Botany, 88*, 273–277. https://doi.org/10.1016/j.sajb.2013.08.007
59. Elisha, I. L., Botha, F. S., McGaw, L. J., & Eloff, J. N. (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complementary and Alternative Medicine, 17*(1), 1–10. https://doi.org/10.1186/s12906-017-1645-z
60. Rather, M. A., Gupta, K., Bardhan, P., Borah, M., Sarkar, A., Eldiehy, K. S., Bhuyan, S., & Mandal, M. (2021). Microbial biofilm: A matter of grave concern for human health and food industry. *Journal of Basic Microbiology, 61*(5), 380–395. https://doi.org/10.1002/jobm.202000678
61. El-Sayed, N. R., Samir, R., Abdel-Hafez, J. M., & Ramadan, M. A. (2020). Olive leaf extract modulates quorum sensing genes and biofilm formation in multi-drug resistant *Pseudomonas aeruginosa*. *Antibiotics, 9*(9), 526. https://doi.org/10.3390/antibiotics9090526
62. Alam, K., Al Farraj, D. A., Mah-e-Fatima, S., Yameen, M. A., Elshikh, M. S., Alkufeidy, R. M., Mustafa, A. E. Z. M., Bhasme, P., Alshammari, M. K., Alkubaisi, N. A., & Abbasi, A. M. (2020). Anti-biofilm activity of plant derived extracts against infectious pathogen-*Pseudomonas aeruginosa* PAO1. *Journal of Infection and Public Health, 13*(11), 1734–1741. https://doi.org/10.1016/j.jiph.2020.07.007
63. Kalia, V. C., Patel, S. K., Kang, Y. C., & Lee, J. K. (2019). Quorum sensing inhibitors as antipathogens: Biotechnological applications. *Biotechnology Advances, 37*(1), 68–90. https://doi.org/10.1016/j.biotechadv.2018.11.006
64. Kalia, V. C., Gong, C., Patel, S. K., & Lee, J. K. (2021). Regulation of plant mineral nutrition by signal molecules. *Microorganisms., 9*(4), 774. https://doi.org/10.3390/microorganisms9040774
65. Parasuraman, P., Devadatha, B., Sarma, V. V., Ranganathan, S., Ampasala, D. R., Reddy, D., Kumavath, R., Kim, I. W., Patel, S. K., Kalia, V. C., & Lee, J. K. (2020). Inhibition of microbial quorum sensing mediated virulence factors by *Pestalotiopsis sydowiana*. *Journal of Microbiology and Biotechnology, 30*(4), 571–582. https://doi.org/10.4014/jmb.1907.07030
66. Rather, M. A., Saha, D., Bhuyan, S., Jha, A. N. & Mandal, M. (2022). Quorum quenching: A drug discovery approach against *Pseudomonas aeruginosa*. *Microbiological Research*, *127173*.https://doi.org/10.1016/j.micres.2022.127173

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