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Phytochemical profiling, antioxidant and antimicrobial investigations on *Viburnum simonsii* Hook. f. & Thoms, an unexplored ethnomedicinal plant of Meghalaya, India

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Abstract

Background *Viburnum simonsii* Hook. f. & Thoms is one of the 17 *Viburnum* species reported from India. *Viburnum* species such as *Viburnum opulus* and *Viburnum grandiflorum* have been used since time immemorial to treat various ailments and their therapeutic claims have been scientifically validated. However, the species under investigation despite having a long traditional usage history for the treatment of various illnesses in Meghalaya, India has grossly remained unexplored to date. No scientific report validating its therapeutic claim has been reported thus far. Therefore, the present study was mainly focused on investigating the antioxidant and antimicrobial properties of *V. simonsii* and its phytochemical profile.

Result Preliminary phytocompound assessment revealed the presence of alkaloids, phenolics, steroids, glycoside and terpenoids. The fruit extract displayed good antioxidant activity with phenolic and flavonoid content of 250.20 ± 8.12 mgGAE/g and 40.65 ± 1.31 mgQE/g respectively, and IC_{50} value of 131.35 ± 1.71 µg/ml. In antimicrobial assay, inhibitory activity was observed against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) with 17.80 ± 0.80 mm and 15.78 ± 2.62 mm zone of inhibition respectively. However, no activity was observed against gram-negative bacteria (*Escherichia coli and Salmonella enterica*) as well as fungus (*Candida albicans*). The absorption bands in the FTIR spectra of the sample corresponded to the presence of primary and secondary alcohols, alkanes, amines, aliphatic ethers, etc. Further, the GC–MS analysis revealed the presence of phytocompounds such as neophytadiene, β-sitosterol, α-amyrin, lupeol, etc., which have bioactivity especially anticancer, antimicrobial, antioxidant and anti-inflammatory activities.

Conclusions The findings of the present study demonstrated that *V. simonsii* possessed appreciable antioxidant and antimicrobial activity and may be a potential target for pharmaceutical research.

Keywords Ethnomedicine, *Viburnum simonsii*, Meghalaya, Antioxidant, FTIR, GC–MS, Antimicrobial

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Background

Mankind's reliance on plants predates recorded history. In addition to basic human needs such as food, clothing, shelter etc. plants are also an important source of medicines. Medicinal plants have been used to prevent and treat various diseases since time immemorial and their therapeutic usages have been passed on to subsequent generations among human communities [26]. Currently, 80% of the world's population depends on medicinal plants for therapeutic purposes [79]. It can be attributed to their extensive geographical availability and lesser adverse effects compared to synthetic drugs. Traditional medicinal plants have an exquisite impact on the evolution of the human health care system [79], since nearly 70% of all the presently available prescribed pharmaceutical drugs are made of plant-origin compounds [32].

Currently, concerns regarding the harmful impacts on health are intensifying as a result of industrial synthetic product use. Certain artificial antioxidants, such as butylated hydroxyanisole and dibutyl hydroxytoluene, have been demonstrated to stimulate carcinogenic activity [9, 65]. While naturally occurring, phenolic antioxidants have been proven to exhibit a range of health-beneficial biological actions [9, 12]. Recently, there has been a lot of attention focused on natural plant-based compounds including terpenoids, alkaloids, and flavonoids because of their numerous pharmacological qualities, which include antibacterial, antioxidant, and anticancer effects [2]. Enough dietary antioxidant consumption can strengthen the body's defences against free radicals since natural antioxidants derived from plants can scavenge harmful free radicals produced by the body. Furthermore, eating a diet high in antioxidant-rich foods is thought to be crucial in preventing or delaying the beginning of degenerative illness [29]. Additionally, multidrug-resistant (MDR) bacteria are also posing a serious threat to global health. This has prompted scientific research activities to prioritise the development of novel antibacterial drugs capable of combating antibiotic-resistant microorganisms [15, 17]. In this regard, naturally occurring bioactive compounds specifically derived from plants have surfaced as promising prospective medicinal agents [2].

With increase in health-related issues, there has been a scientific resurgence in medicinal plant research, mostly as a source of herbal medicine. Among the medicinal plants, the plants of the Genus *Viburnum* (family Adoxaceae) are considered very important for their ornamental as well as therapeutic potential [13]. *Viburnum*, a genus from Adoxaceae family (formerly Caprifoliaceae) comprises more than 230 species [62]. This genus is naturally predominant in temperate regions of the northern hemisphere and subtropical regions of Asia and Latin America [1]. The Eastern Himalayas harbour the majority of the species from this genus, as this range has all the elements for their comfortable accommodation. Majority of these species are endemic [1, 82]. They are primarily used in folk medicines for the treatment of diseases, such as splenic asthenia, rheumatic arthritis, diabetes mellitus, cough, diarrhoea, tumefaction, kidney cramps and swelling [27, 75]. For instance, *V. opulus* and *V. prunifolium* (popularly known as cramp bark and black haw) have a long history of therapeutic usage for pregnancy issues within the Native American tribes [73]. The other *Viburnum* species which showed potential therapeutic properties were *V. lantana*, *V. macrocephalum, V. grandiflorum, V. odoratissimum*, *V. dilatatum,* etc.,[73]. The therapeutic properties they exhibit include; antioxidant, antimicrobial, antidiabetic, antimalarial, hepato-protective, anti-inflammatory, anticancer, etc., [63, 73].

Phytochemically, diterpenes, triterpenes, monoterpenes, sesquiterpenes, flavonoids, phenols, iridoids, lignans, coumarins and alkaloids are the major chemical compounds found in the *Viburnum* plants [64]. Vibsane-type diterpenes are the characteristic chemical compounds of this genus. Owing to their limited distribution (found in *Viburnum* plants only), they are considered to be rare natural products. In terms of pharmacological interest, vibsane-type diterpenes possess neurotrophic activity, anti-inflammatory, antileukemic and anticancer properties [64, 83].

Literature review confirms that species such as *V. awabuki* [34], *V. dilatatum* [77], *V. fordiae* [13], *V. odoratissimum* [81], and *V. opulus* [27] have a maximum quantity of bioactive compounds in comparison to other species of the genus. It is also reported that fruits of *Viburnum* plants are rich in polyphenols, ascorbic acid, malic acid, oxalic acid and vitamin C [10]. Further, some in vitro and in vivo studies have revealed the antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer and neuroprotective properties of some species from this genus [27, 59, 81]. For such diverse chemical components and biological activities across the genus, the study of *Viburnum* simonsii Hook. f. & Thoms (synonym *Viburnum odoratissimum var. odoratissimum*) is of scientific curiosity pertaining to novel drug discovery and as a source for nutraceutical product development. *Viburnum simonsii* Hook. f & Thoms is a small tree with a height of up to 40 ft, thin greyish bark, leaves are oppositely arranged, elliptic, distantly cuspidate, dentate and glabrous with lateral nerves of 5–8. Flowers are white or tinged red and sweet-scented. Fruits are 0.4–0.8 cm in diameter with bright red colour. Figure 1 shows the tree, flower, fruits and leaf of *Viburnum simonsii* Hook. f & Thoms. Flowering time is between March-June, whereas fruiting time is July–October [23, 28]. It is endemic to the

Fig. 1 Tree, flower, fruit and leaf of *V. simonsii*

Eastern Himalayan range. In India, the distribution is limited only to the state Meghalaya [42, 72]. The Khasi people (local tribe of Meghalaya) locally call it *Sohlang-eit-ksew.* Traditionally the fruits of this species are used as tonic and anti-spasms [41]. Besides, the species is neglected and unexplored, which is evident with the declining population graph in the state.

Interestingly, the species under investigation is closely related to *V. odoratissimum* (possess diverse biological activities and a high number of phytocompounds) [58]. It is quite arguable to expect that the species under investigation (*Viburnum simonsii* Hook. f. & Thoms) will have diverse biological activities owing to the likely presence of varied phytocompounds. The local tribes of Meghalaya have been using and claiming the therapeutic value of this species [41], and this prompted to undertake the present investigation pertaining to scientific validation. In addition, no scientific reports concerning the biological and pharmacological activities of this species have been reported so far. Therefore, the current investigation aims to highlight *Viburnum simonsii* Hook. f. & Thoms as a potential medicinal plant with unexplored therapeutic potential. The investigation attempts to highlight the biological activities of *V. simonsii* and validate its biochemical attributes specifically emphasizing on its phytochemical, antioxidant and antimicrobial aspects.

Methods

Plant materials

The fresh leaves and fruits of the plant *V. simonsii* Hook. f. &Thoms. were collected from Cherrapunjee [25°14′42.1′′N, 91°43′28.4′′E], East Khasi Hills, Meghalaya. The plant species was identified by referring to the existing literatures [23, 24, 28] and authenticated by comparing the prepared herbarium specimen to the existing herbarium specimen of the species under investigation (Reference accession no: 90420) present in the Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, Meghalaya, India.

Chemicals and reagents

DPPH, TPTZ, Folin-Ciocalteu reagent, Anthrone and Gallic acid were purchased from SRL, India. Ascorbic acid, Bovine serum albumin (BSA), Quercetin and Ferric chloride were obtained from LOBA Chemie, India. Whereas, DMSO, Methanol, Sodium acetate, Aluminum Chloride and Mueller Hinton Agar (MHA) were procured from Merck Lifesciences and Himedia, India, respectively.

Extract preparation

The collected leaves and fruits were washed well, shade dried and powdered with an electric grinder. The extract

preparation was carried out following the procedure described in the previous work [54] with slight modifications. Soxhlet extraction was performed at 65 °C using 80% CH_3OH (Methanol) as the extraction solvent. The solvent following collection was cooled and subjected to rotary vacuum evaporation (Equitron Roteva 66 series – 8766.V0). The final concentrated extracts were methanolic leaf (MEVL) and fruit (MEVF) extracts. The extract was further subjected to lyophilization. These extracts were finally stored at 4 °C for further analysis.

Qualitative phytochemical screening

The qualitative phytochemical screening was carried out to determine the presence of different phytochemicals in the *V. simonsii* extracts. Phytocompounds such as alkaloids (Mayer's test), phenols and tannin (Ferric chloride test), flavonoid (Shinoda test), terpenoids (Saldowski test), glycoside (Libermann's test), cardiac glycoside (Keller-Kilani test), saponins, steroids and anthraquinone were screened following the standard methods [22].

Carbohydrate content

The carbohydrate content was determined following the Anthrone's method [80]. Briefly, the powdered plant sample was acid hydrolyzed with HCl (2.5N) for 3 h and filtered. 1 ml of filtrate was mixed with 4 ml of Anthrone reagent and kept in a water bath (100 °C) for 10 min. Absorbances were recorded at 620 nm. Glucose of various concentrations (20–100 µg/ml) was used to plot a standard calibration curve graph. The carbohydrate content of the sample was calculated from the calibration curve graph and expressed as mg glucose equivalent/g of sample. The assay was carried out in triplicates and only the mean values are reported.

Protein content

The protein content of the samples was determined following Lowry's method [39]. The protein from the powdered sample was extracted using phosphate buffer as a solvent and filtered. The alkaline reagent was prepared by mixing reagent A (2% Na_2CO_3 in 0.1N of NaOH) and reagent B (0.5% $CuSO_4$ and 1% potassium sodium tartrate in distilled water) at a ratio of 50:1. To 1 ml of filtrate, 4 ml of alkaline reagent and 0.5 ml Folin-Ciocalteu reagent was added and incubated for 30 min at 37 °C. The absorbances were recorded at 660 nm. Bovine serum albumin (BSA) was used to plot a standard calibration curve graph. Protein content of the sample was expressed as mg BSA equivalent/g of the sample. The assay was carried out in triplicates and only the mean values are reported.

Total phenolic content

The total phenolic content (TPC) of the methanolic extracts of *V. simonsii* leaf and fruit was carried out by the method described by Neupane and Lamichhane [44] with slight modification. To 100 µl of methanolic extract, 0.5 ml of Folin-Ciocalteu and 1.5 ml of Na_2CO_3 (7.5%) were added and incubated at 37 °C for 2 h. The absorbances of the mixture were taken at 750 nm. A standard calibration curve graph was obtained using gallic acid as a standard. The TPC was calculated from the standard calibration curve graph and expressed in mg gallic acid equivalent (GAE)/g of dried extract. The assays were carried out in triplicates and only the mean values are reported.

Total flavonoid content

The total flavonoid content (TFC) of the methanolic extracts of the samples was estimated by the Aluminum Chloride method [44]. Briefly, to 1 ml of methanolic extract, 1 ml of 10% $AlCl_3$ and 1 ml of sodium acetate (1 M) were added and incubated at room temperature for 45 min in a dark chamber. The absorbances were taken at 415 nm. The TFC was calculated from the standard calibration curve graph which was obtained by using quercetin as a standard. The final result was expressed in mg quercetin equivalent (QE)/g of dried extract. The assays were carried out in triplicates and only the mean values are reported.

DPPH radical scavenging activity

A DPPH radical scavenging activity of the extracts of *V. simonsii* leaf and fruit was determined following the method described by Clarke et al. [14] with minor modifications. 1 ml of methanolic extract was mixed with 3 ml of DPPH reagent (0.2 mM) and incubated for 30 min at 37 °C in a dark chamber. A control was prepared by mixing 1 ml methanol (95%) and 3 ml DPPH reagent. The absorbances were read at 517 nm. Ascorbic acid was used as the standard antioxidant agent to compare the DPPH radical scavenging activity of the sample. The percentage of radical scavenging activity was calculated using the formula given in Eq. (1).

$$\%RSA = \frac{Ac - As}{Ac} \times 100 \quad (1)$$

where, %RSA = Radical scavenging activity percentage, Ac = Absorbance of control, As = Absorbance of sample.

IC_{50} of the sample and standard was calculated from the regression curve equation obtained by plotting %RSA against various extract concentrations.

FRAP assay

The FRAP assay of the extracts was done following the methods described by Benzie and Strain [4]. To 0.1 ml of methanolic extract, 3 ml of FRAP reagent (mixture of 0.3 M sodium acetate, 10 mM of TPTZ solution and 20 mM of $FeCl_3$ at a ratio of 10:1:1) was added and incubated for 15 min at 37 °C. The absorbances were recorded at 593 nm. A standard calibration curve graph was obtained using ascorbic acid of various concentrations (0.2–1.0 mM) as standard. The FRAP value of the extracts was calculated using Eq. (2) and expressed in µM ascorbic acid equivalent (AAE)/g of dried extract.

$$\text{FRAP value}\left(\mu\text{MAAE/g}\right) = \text{c} \times \text{V} \times \frac{\text{t}}{\text{m}} \quad (2)$$

where, c = Ascorbic acid equivalent in µM/ml, V = sample volume, t = dilution factor, m = weight of dried extract in gram (g).

Antimicrobial activity assay

Antimicrobial activity assay of the crude methanolic extracts was done through agar-well diffusion method [56]. The assay was carried out against gram-positive (*Staphylococcus aureus* MTCC 11949, *Bacillus cereus* MTCC 8361), gram-negative (*Escherichia coli* MTCC 593, *Salmonella enterica* MTCC 1166) bacterial strains and fungus (*Candida albicans* MTCC 13013). The samples were prepared in Dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml. A 24 h culture was diluted with normal saline till the concentration of 1×10^8 cells/ml. Mueller Hinton Agar (MHA, HiMedia) for bacteria and Mueller Hinton Agar supplemented with 2% glucose and 5 µg/ml Methylene blue for fungus were used as the culture media for the assay. 100 µl of the diluted culture was spread onto solidified agar, and wells (8 mm) were bored. 100 µl of extract, DMSO (as negative control) and a ciprofloxacin 5 µg/100 µl (bacteria) and fluconazole 10 µg/100 µl (fungus) [as positive control] were placed onto the wells. After 24 h incubation at 37 °C, the diameter (mm) of the zone of growth inhibition was measured.

FTIR analysis

The IR spectra of leaf and fruit sample of *V. simonsii* were recorded on a PerkinElmer Spectrum 100 spectrometer at ambient temperature. A small quantity of powdered plant sample was mixed with potassium bromide (KBr) and pressed into pellets. The spectra were recorded in the mid infra-red region (4000–400 cm^{-1}). The spectral data were interpreted with the help of standard IR spectrum chart described by Pavia et al. [48].

Gas chromatography-mass spectroscopy (GC–MS) analysis

A GC–MS analysis of the *V. simonsii* extracts (MEVL, MEVF) was carried out in a Thermo-Fischer Scientific model GC with a split/split less injection port and a split ratio of 1/100, coupled with an ISQ7000 mass spectrometer system and TG-5MS fused silica capillary column (30 m × 0.25 mm i.d, 0.25 µm film thickness). The temperature program was isothermal for 3 min at 60 °C, then increased to 230 °C at a rate of 5 °C/min. The injector temperature and transfer line temperature were set at 290 °C and 230 °C respectively. Ultrapure Helium was used as a carrier gas at a flow rate of 1 ml/min. The components were identified and confirmed by comparing the retention time (RT) index and mass spectrum with the authentic references provided in the NIST library. The peak area percentages of the spectrum were calculated automatically by using Chromeleon™ Software provided along with the instrument setup.

Results

Qualitative phytochemical screening

The qualitative phytochemical screening of the extracts revealed the presence of alkaloids, phenols, flavonoids, glycoside, tannin, steroids, terpenoids and saponins. The results for qualitative phytochemical screening of *V. simonsii* extracts are furnished in Table 1.

Carbohydrate and protein content

The carbohydrate and protein content of the extracts were estimated by Anthrone's and Lowry's methods respectively. A carbohydrate content of 312.6 ± 0.74 mg/g (31.2%) was obtained in the fruit and 232.9 ± 0.64 mg/g (23.2%) in the leaf sample. Similarly, fruit and leaf samples showed 65.9 ± 1.79 mg/g and 34.6 ± 0.77 mg/g of protein content respectively. The measured carbohydrate and protein content of the *V. simonsii* extracts is furnished in Table 2.

Table 1 Results of the qualitative phytochemical screening of *V. simonsii* extract

Tests	Leaf extract	Fruit extract
Alkaloid	+	+
Anthraquinone	–	–
Glycoside	+	+
Flavonoid	+	+
Phenolic	+	+
Steroids	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+

(+) indicates presence and (-) indicates absence

Table 2 Carbohydrate and Protein content of *V. simonsii*

Samples	Total carbohydrate (mg/g)	Total Protein (mg/g)
Leaf	232.9 ± 0.64	34.6 ± 0.77
Fruit	312.6 ± 0.74	65.9 ± 1.79

Values are present as mean ± SD

Total phenols and flavonoid content

Phenols and flavonoids have a strong correlation with the antioxidant activities [9, 70]. The TPC and TFC of the leaf and fruit extracts of *V. simonsii* were determined by Folin-Ciocalteu and Aluminum Chloride methods respectively. The total phenol and flavonoid content of the extracts were calculated using the standard calibration curve graph (Fig. 2 and Fig. 3). The fruit extract displayed 250.20 ± 8.12 mgGAE/g of phenolic contents and 40.65 ± 1.31 mgQE/g of flavonoid contents. Whereas, leaf extract showed 127.03 ± 1.62 mgGAE/g and 45.83 ± 3.93 mgQE/g of phenolic and flavonoid content (Table 3).

DPPH radical scavenging assay

The DPPH radical is a stable free radical. This is because of an odd electron delocalizing across the molecule which consequently inhibits dimer formation. The DPPH radical scavenging assay uses these free radicals to measure the ability of antioxidants to neutralize those free radicals. The reduction of the free radicals to a non-radical state is indicated by the colour change from dark purple to yellow [68]. The reducing capacities of the extracts increase accordingly with the increase in concentration. IC_{50} (half maximal inhibitory concentration) of free radical scavenging activity was calculated using various concentrations of the extracts. Fruit extract (MEVF) showed an IC_{50} value of 131.35 ± 1.71 µg/ml and 872.71 ± 2.86 µg/ml was observed in leaf extract (MEVL) (Table 3). Further, the DPPH free radical scavenging activities of fruit and leaf extracts and standard ascorbic acid at various concentrations are displayed in Fig. 4.

FRAP assay

The ferric reducing antioxidant power (FRAP) is based on the reduction of ferric-tripyridyl triazine (Fe^{3+}-TPTZ) complex to ferrous-tripyridyl triazine (Fe^{2+}-TPTZ) by antioxidants at low pH (3.6) [4]. The fruit extract showed highest ferric reducing ability with a FRAP value of 94.31 ± 0.67 µMAAE/g, whereas 32.57 ± 0.85 µMAAE/g of FRAP value was observed in the leaf extract (Table 3).

Antimicrobial assay

The antimicrobial activities of the *V. simonsii* extracts were performed by agar-well diffusion method. The tested extracts showed varied inhibitory actions against tested bacteria and fungus. The results of the antimicrobial activity assay of the extracts are furnished in Table 4 and Fig. 5. Leaf extract (100 mg/ml) showed highest

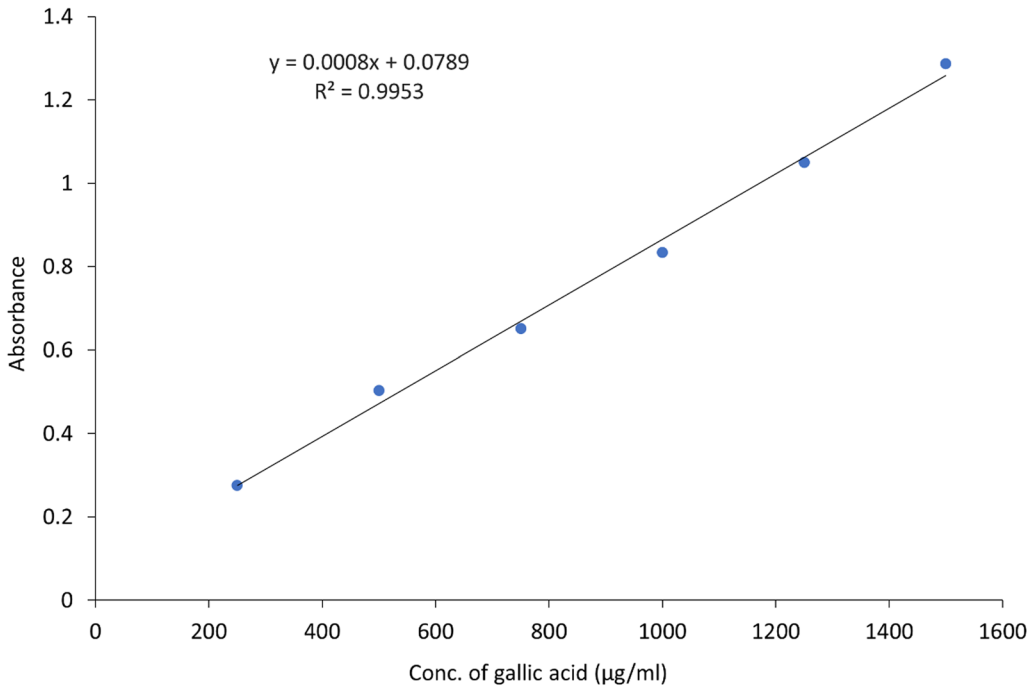

Fig. 2 Calibration curve graph of gallic acid for TPC

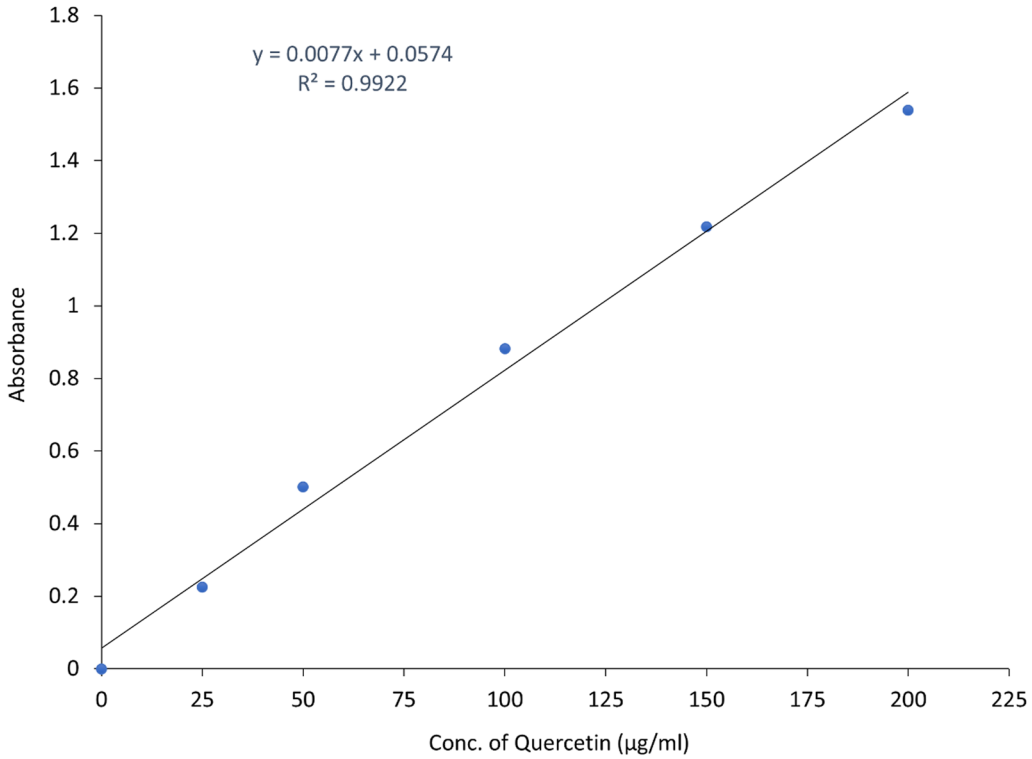

Fig. 3 Calibration curve graph of quercetin for TFC

Table 3 Phenolic, flavonoid, DPPH and FRAP values of *V. simonsii* extracts

Samples	Phenolic (mgGAE/g)	Flavonoid (mgQE/g)	DPPH IC_{50} value (µg/ml)	FRAP value (µMAAE/g)
Leaf (MEVL)	127.03 ± 1.62	45.83 ± 3.93	872.71 ± 2.86	32.57 ± 0.85
Fruit (MEVF)	250.20 ± 8.12	40.65 ± 1.31	131.35 ± 1.71	94.31 ± 0.67

Values are present as mean ± SD

inhibition zone against *S. aureus* (17 mm) and lower zone against *B. cereus* (15 mm). Similarly, fruit extract (100 mg/ml) showed inhibition zone against *S. aureus* (13 mm) and *B. cereus* (15 mm). However, no inhibition zone was observed against gram-negative bacteria (*E. coli* and *S. enterica*) and fungus (*C. albicans*).

FTIR analysis

FTIR was done to identify the functional groups in the chemical constituents of the plant extracts. FTIR spectrum and spectral values of the extracts of *V. simonsii* is furnished in Fig. 6 and Table 5. Twelve (12) significant bands were detected in the range of 4000–400 cm^{-1}. The peaks were observed at 3426 cm^{-1}, 2927 cm^{-1}, 1649 cm^{-1}, 1387 cm^{-1}, 1251 cm^{-1} and 1066 cm^{-1}. A broad absorbance at 3500–3400 cm^{-1} denotes stretching vibration of OH in H bonded alcohol. Additionally, stretching vibration was observed at 1085–1050 cm^{-1}, thereby confirming the presence of phenolic compounds [45]. A C-H stretching vibration at 3000–2840 cm^{-1} implies the presence of lipid. Absorption at 1649 cm^{-1} can be referred to C=O vibration of carboxylic acid [20]. A stretching vibration of C-N was observed at 1321 cm^{-1} which corresponds to amines. The stretching vibration of C-O at 1251 cm^{-1} and 1148 cm^{-1}implies the presence of ether. From Fig. 6, it can be seen that leaf (MEVL) and fruit (MEVF) extracts displayed peaks at the same band, however their absorption intensity was different.

GC–MS analysis

The GC–MS analysis of the methanolic leaf (MEVL) and fruit (MEVF) extracts of *V. simonsii* reveals the presence of a total of 21 and 13 bioactive compounds respectively. The chromatograms are displayed in Figs. 7 and 8, whereas the phytocompounds of MEVL and MEVF with their retention time (RT) and concentration (Peak area

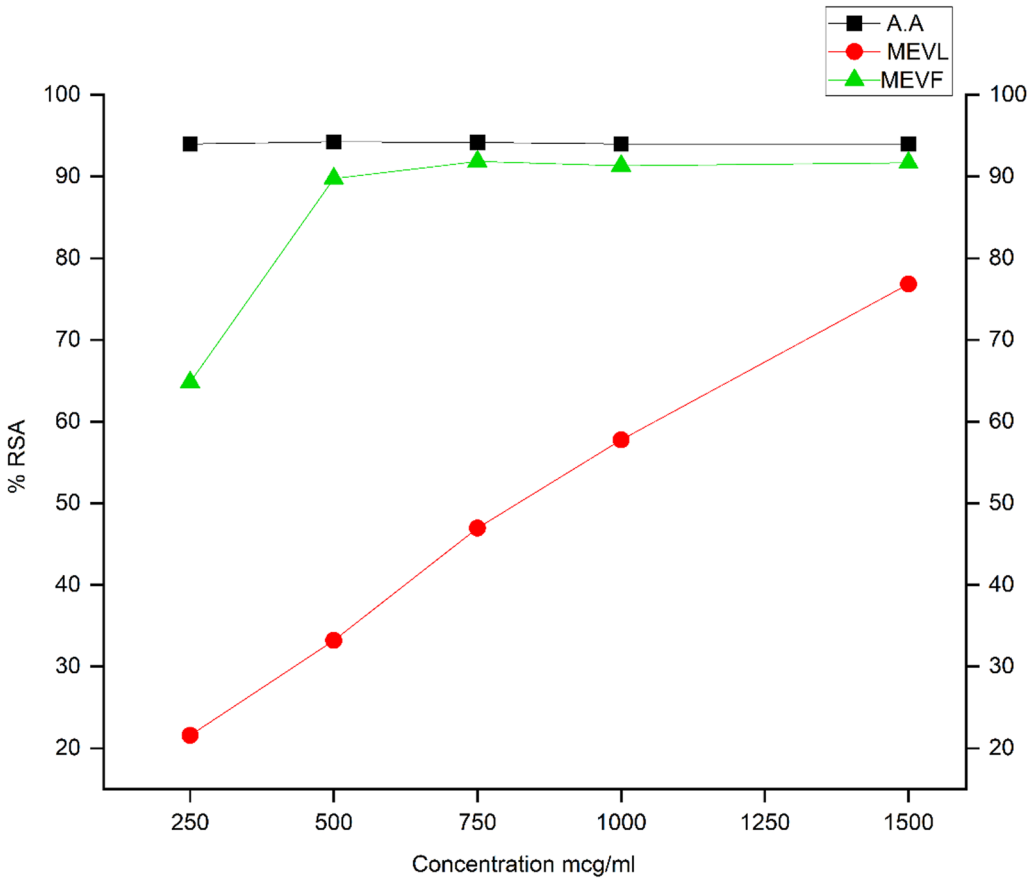

Fig. 4 DPPH assay of MEVL and MEVF. 'AA' denotes ascorbic acid

Table 4 The antimicrobial activity of *V. simonsii* extracts against gram-positive, gram-negative bacteria and fungus

Test sample	Concentration of extracts	Diameter of zone of inhibition (mm)				
		E. coli	*S. aureus*	*B. cereus*	*S. enterica*	*C. albicans*
MEVL (leaf)	100 mg/ml	ND	17.80 ± 0.80	15.78 ± 2.62	ND	ND
MEVF (Fruit)	100 mg/ml	ND	13.12 ± 2.52	14.84 ± 1.83	ND	ND
Positive control (Ciproflaxacin/ Fluconazole)	–	40.57 ± 2.32	27.79 ± 0.75	30.08 ± 1.36	35.49 ± 1.85	30.38 ± 1.60
Negative control (DMSO)	–	NA	NA	NA	NA	NA

Values are present as mean ± SD, ND = Not Detected, NA = No Activity

%) are presented in Tables 6 and 7 respectively. Compounds obtained in MEVL are: 1,2,3,4-Cyclohexanetetrol (16.86%), α- Amyrin (15.95%), ß-Amyrin (4.41%), ß-Sitosterol (5.70%), Neophytadiene (4.96%), Lup-20(29)-en-3-ol, acetate, (3ß)- (9.22%), Ergosta-5,22-dien-3-ol, acetate, (3ß,22E)-, Benzoic acid, 4-ethoxy-, ethyl ester, Tridecanoic acid, 12-methyl-, methyl ester, cis-Z-a-Bisabolene epoxide, 1-Heptatriacotanol (2.07%), a-Tocopheryl acetate (1.48%). Similarly, compounds obtained in MEVF are: Glycerin (37.38%), Lupeol; Lup-20(29)-en-3-ol, acetate, (3ß)- (35.12%), Propanoic acid, 2-methyl-, 2-ethylhexyl ester (8.20%), Lupeol (2.84%), -, Benzoic acid, 4-ethoxy-, ethyl ester (3.90%), Benzene acetaldehyde, a,2,5-trimethyl, Eicosane, 2-methyl-, Pentadecanoic acid, 14-methyl-, methyl ester.

Discussion

Bioprospecting of medicinal plants strictly demands pharmacognostic investigation of crude plant materials. Such investigations include screening of phytochemicals

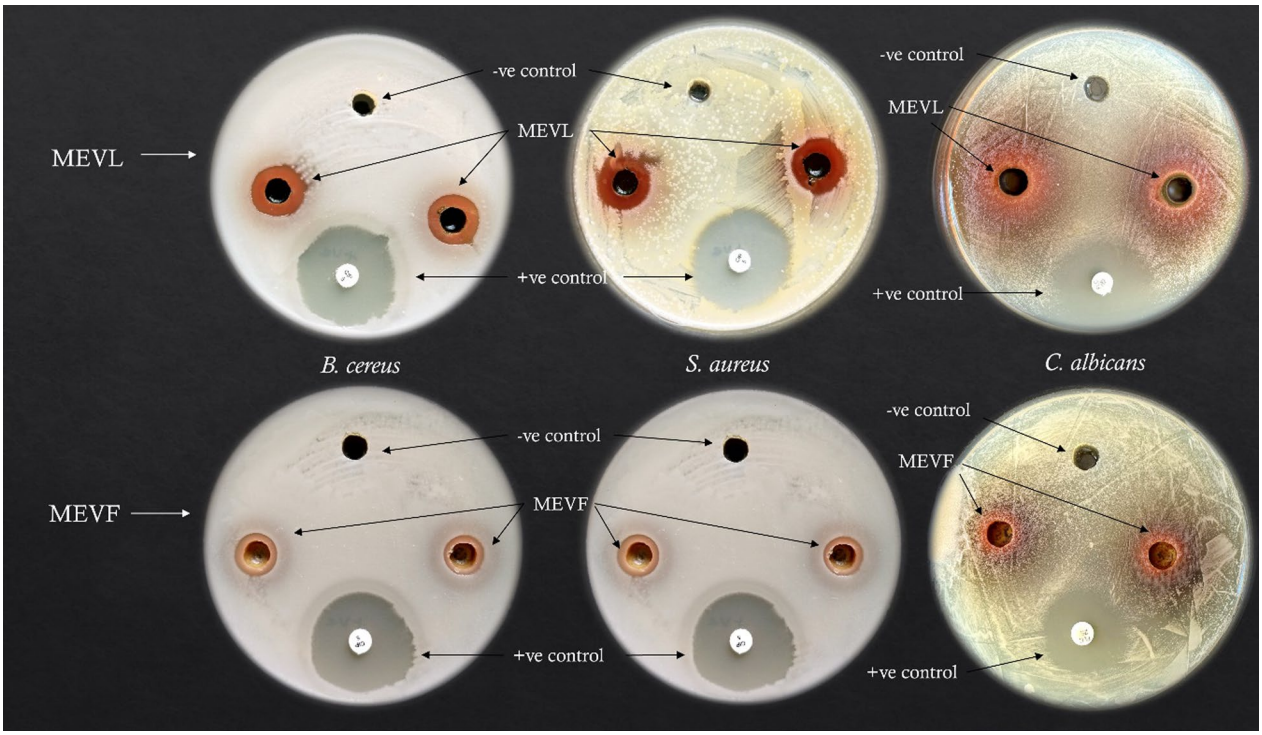

Fig. 5 Antimicrobial activities of *V. simonsii* extracts

and biological activities [6]. The present study investigates the phytochemicals, antioxidant and antimicrobial activities of *V. simonsii* to validate its pharmaceutical potential. The presence or absence of phytochemicals play a crucial role in the biological activities of specific plant species [69]. In the present study, qualitative phytochemical screening revealed the presence of major phytochemicals such as alkaloid, phenols, flavonoid, steroids, etc. (Table 1). The result was concordant with the findings of the previous studies carried out on related species such as *V. opulus* [33], *V. coriaceum* [73], *V. foetens* [7] and *V. grandiflorum* [71]. However, anthraquinones, which were found in the aqueous and methanolic fraction of the aerial parts of *V. foetens* [7] and roots of *V. grandiflorum* [71], were absent in the species under investigation. This might be due to the fact that phytochemical distribution is diverse among the different parts of the same plant [53]. On the contrary, cardiac glycoside, which was reported absent in previously studied *Viburnum* species (*V. opulus* and *V. coriaceum*), was present in *V. simonsii*. Nevertheless, it is confirmed by previous studies that phenols, flavonoids, alkaloids, tannins and saponins are chiefly responsible for antioxidant and antimicrobial activities [3, 65].

It is worth mentioning that the majority of the therapeutically valued plants are employed in the development of nutraceutical products. Therefore, investigation of the nutritional profile of plants including carbohydrate, protein, fats and minerals are indispensable. As these nutritional compounds execute an essential role in making healthy organ systems in human beings [52]. Also, they are crucial elements in the selection of plant species for nutraceutical significance [45]. In comparison to the other related species such as *Viburnum mullaha* [40], the carbohydrate content of the species under study was found higher. However, it was found lower in comparison to wild vegetables such as *Dryopteris filixmas, Corchorus capsularis, Ipomoea aquatica* etc. [60] and wild edible plants such as *Gnetum gnemon, Prenanthes hookeri, Smilax perfoliata, Blumea lanceolaria* etc., consumed by the local tribes of Meghalaya [61]. The protein content of the studied species was in close proximity to a previous report on *V. opulus* [50] and *V. mullaha* [40]. Furthermore, Vishwakarma and Karma [74], reported protein content of edible herbs in the range of 3.1–13.6% and regarded them as a good protein source. Therefore, owing to the considerable content of carbohydrates and proteins, plant materials of *V. simonsii,* (both fruit and leaf) can be utilized in dietary supplement products.

Qualitative phytochemical analysis of an extract is not sufficient enough to validate its pharmaceutical potential. The quantitative investigations of some of the important compounds such as phenols, flavonoids, alkaloids, terpenoids also account most in this regard. Besides, a strong

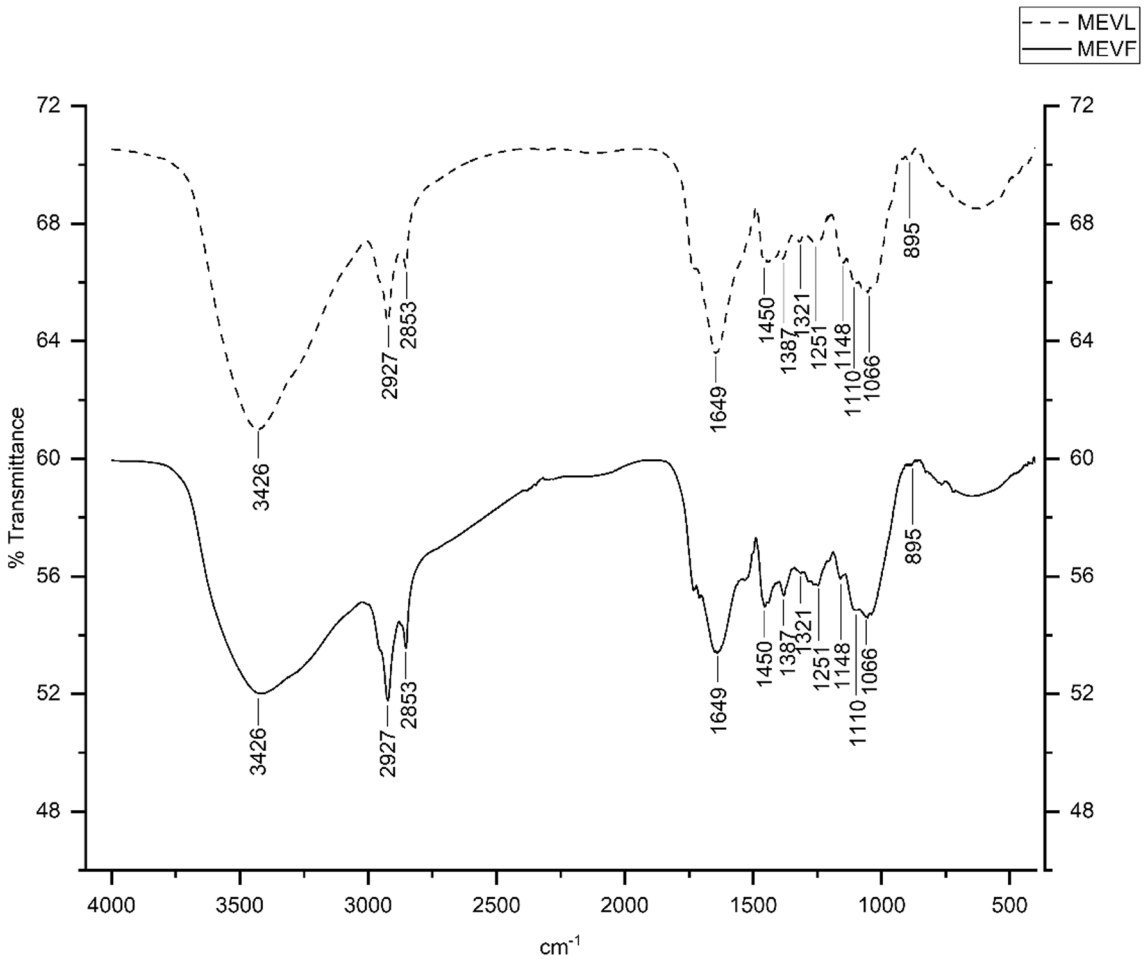

Fig. 6 FTIR spectrum of MEVL and MEVF

correlation also exists between the quantity of phenolic compounds and antioxidant activities [9, 70]. Flavonoids, the most diverse and widely distributed phenol of natural products, possess free radical scavenging and antimicrobial activities [15]. Similarly, it is crucial to analyse the free radical scavenging potential of the extracts along with phenolic content assay to substantiate antioxidant property. Since free radicals are of different chemical entities, different tests are required to prove the free radical scavenging capability through various mechanism [29]. In this regard, DPPH and FRAP assay methods are often employed. In terms of phenolic content, the values for *V. simonsii* fruit extract (250.20 mg/g) were higher than the fruit extract of *V. opulus* (131.99 mg/g) [59], *V. mullaha* (12.57 mg/g) [66] and *V. coriaceum* (29.75 mg/g) [73]. In contrast, the flavonoid content of *V. simonsii* fruit extract (40.65 mg/g) was higher than the fruit of *V. mullaha* (35.03 mg/g) [66]. Further, Česonienė et al. [12] and Polka et al. [50] demonstrated that *V. opulus* fruit has significant antioxidant activity with 10.07–37.3 mg/g of phenolic content and FRAP value of 0.19 µM/g. Undeniably, *V. opulus* was the most studied among the *Viburnum* species so far [66]. Besides, Levent et al. [35] also

Table 5 FTIR spectral values and their corresponding functional groups in *V. simonsii*

	Absorption (cm^{-1})	Appearance	Group	Compound class
1	3500- 3400	Stretching	O–H	Alcohol
2	3000–2840	Stretching	C-H	Alkane
3	3000–2840	Stretching	C-H	Alkane
4	1650–1600	Stretching	C=O	Carboxylic acid
5	1450–1375	Bending	C-H	Alkane
6	1450–1375	Bending	C-H	Alkane
7	1390–1310	Stretching	C-N	Amine
8	1275–1200	Stretching	C-O	Alkyl aryl ether
9	1150–1085	Stretching	C-O	Aliphatic ether
10	1124–1087	Stretching	C-O	Secondary alcohol
11	1085–1050	Stretching	C-O	Primary alcohol
12	895–885	Bending	C=O	Alkene

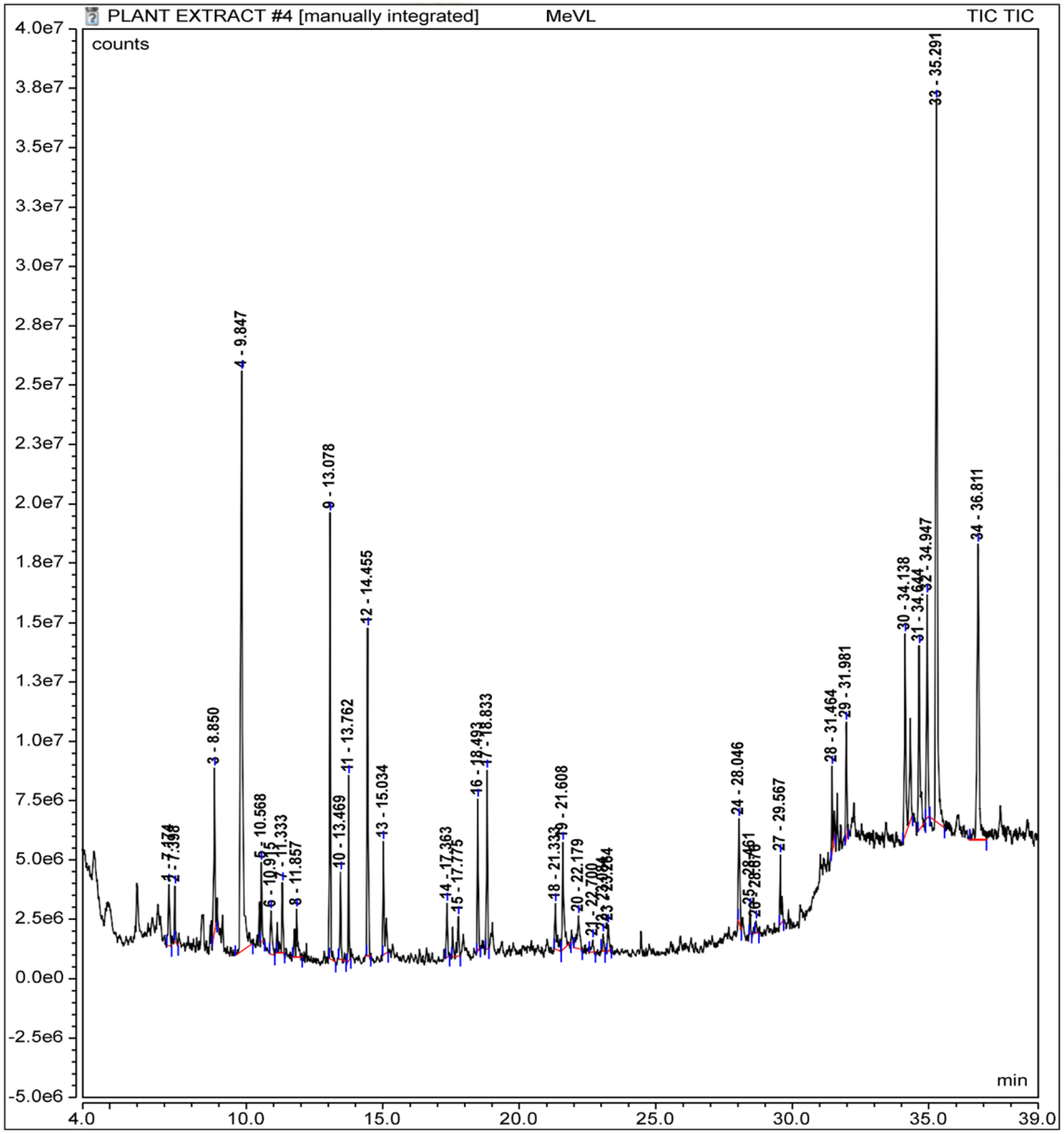

Fig. 7 GC–MS chromatogram of MEVL

demonstrated the strong DPPH inhibition potency (IC_{50} 85 µg/ml) of *V. lantana.* Vijaytha et al. [73] have also demonstrated a weak antioxidant activity of *V. coriaceum* (IC_{50} 1500 µg/ml). However, considering the standard antioxidant property, the antioxidant values of the *V. simonsii* fruit extracts (MEVF) represent moderately strong free radical scavenging activity (Table 3). Because, IC_{50} values in the range of 10–100 µg/ml represent very strong antioxidant properties, whereas > 100 represent moderate strong and > 500 represents weak properties [49].

In wake of recent advancements in modern medicine, there has been a rapid upsurge in multiple antibiotic resistant bacteria. Especially, gram-negative pathogenic bacteria are known to be resistant to many antibiotics [54]. Therefore, to combat antibiotic resistance of the pathogenic bacteria, alternative strategies are much essential. Further, it has been verified that medicinal plants exhibit good antimicrobial activity [55]. The antimicrobial activities of any plant extracts were found to have correlation with the presences of some phytocompounds such as alkaloid, tannins, steroids, phenols,

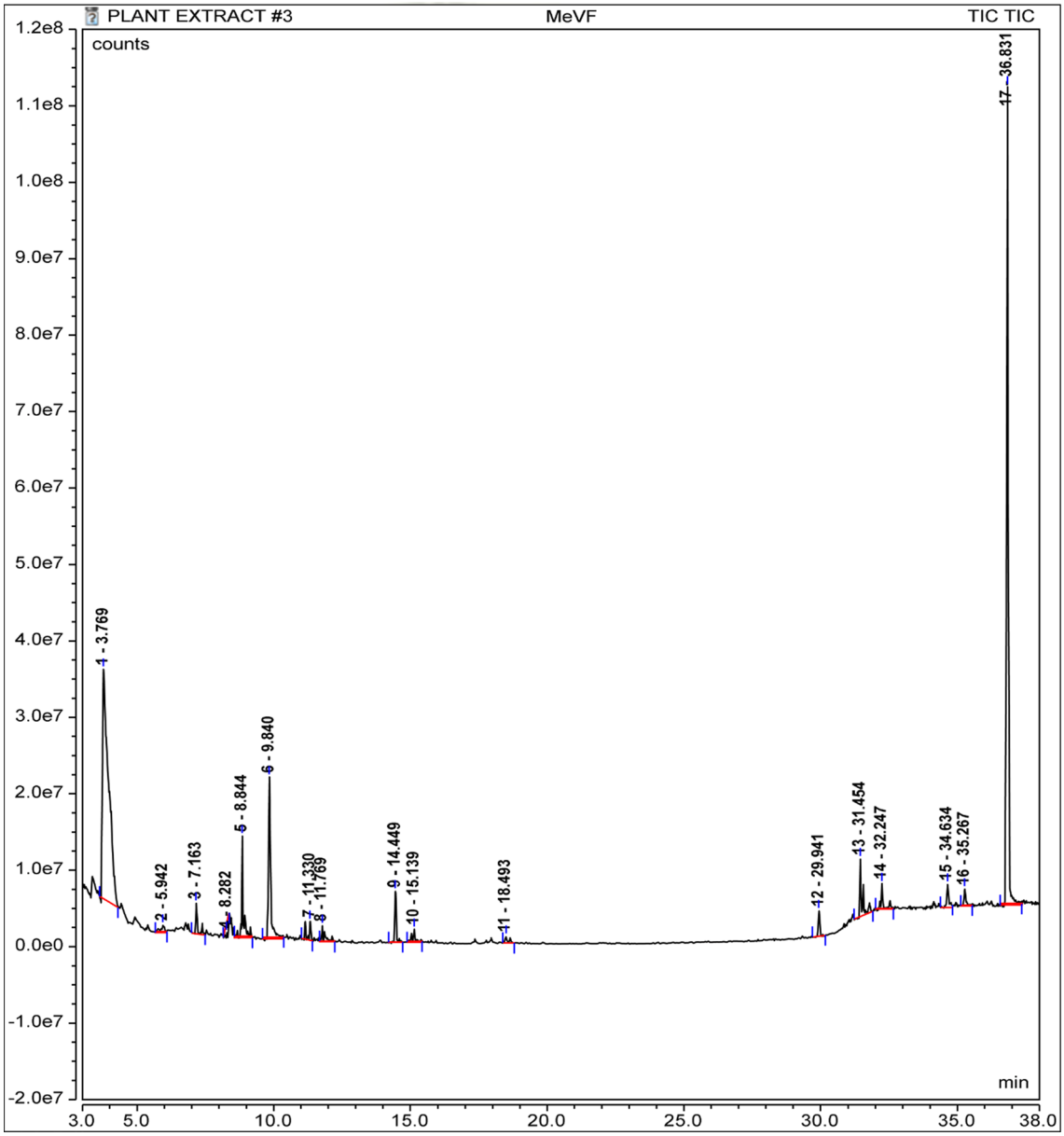

Fig. 8 GC–MS chromatogram of MEVF

etc., [30]. Different mechanisms by which phytocompounds inhibit microbial growths was mentioned earlier [2]. For instance, destruction of cell membrane, inhibition of cell-wall and protein biosynthesis, DNA replication and repairing. Plants rich in polyphenolic compounds exhibited antibacterial activities against a broad spectrum of bacteria [8, 67]. These polyphenolic compounds were found to inhibit bacterial growth by restricting the activities of virulence factor, biofilm formation and cell-wall formation, and by reducing pH values [30]. The present findings showed that fruit and leaf extract showed inhibitory action against gram-positive bacteria only (Table 4 and Fig. 5). These findings were in agreement with the findings reported by Wintola et al. [76], in which anti-dysenteric plant extracts showed 12–15 mm inhibitory zone against the same bacteria that were used in the present study. Additionally, Sagdic et al. [59] demonstrated that methanolic extracts of *V. opulus* showed 19–22 mm zone of inhibition against *B. cereus* and *S. aureus.* Roy et al. [57] reported that methanolic fractions of *V. foetidum* showed 18–20 mm inhibitory zone against the *B. cereus* and *S. aureus* bacteria. No activity against

Table 6 GC MS spectral analysis of methanolic leaf extract of *Viburnum simonsii* (MEVL)

Sl. No	RT (min)	Name of the Compounds	Peak Area %
1	7.174	8-Methyl-3-oxo-2-oxabicyclo (4.4.0) deca-4,9-diene-6,8-carbolactone	1.32
2	7.398	t-Butyl-(2-[3-(2,2-dimethyl-6-methylene-cyclohexyl)-propyl]-[1, 3] dithian-2-yl)-dimethyl-silane	0.87
3	8.850	Benzoic acid, 4-ethoxy-, ethyl ester	2.51
4	9.847	1,2,3,4-Cyclohexanetetrol	16.86
5	10.568	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1a,2a,3ß,6a)]-	0.94
6	11.333	Tridecanoic acid, 12-methyl-, methyl ester	1.59
7	13.078	Neophytadiene	4.96
8	13.762	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.23
9	14.455	Hexadecanoic acid, methyl ester	4.04
10	15.034	n-Hexadecanoic acid	2.22
11	17.363	11-Octadecenoic acid, methyl ester	0.92
12	18.493	1-Heptatriacotanol	2.07
13	18.833	Doconexent	2.41
14	21.608	cis-Z-a-Bisabolene epoxide	2.50
15	28.046	Ergosta-5,22-dien-3-ol, acetate, (3ß,22E)-	1.70
16	31.464	W-18	1.45
17	31.981	a-Tocopheryl acetate	1.48
18	34.138	ß-Sitosterol	5.70
19	34.644	ß-Amyrin	4.41
20	35.291	a-Amyrin	15.95
21	36.811	Lup-20(29)-en-3-ol, acetate, (3ß)-	9.22

Table 7 GC MS spectral analysis of methanolic fruit extract of *Viburnum simonsii* (MEVF)

Sl. No	R. T (min)	Name of the Compounds	Peak Area %
1	3.769	Glycerin	37.38
2	7.163	Benzeneacetaldehyde, a,2,5-trimethyl-	1.33
3	8.282	3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-oxo-, ethyl ester	1.13
4	8.844	Benzoic acid, 4-ethoxy-, ethyl ester	3.90
5	9.840	Propanoic acid, 2-methyl-, 2-ethylhexyl ester	8.20
6	11.330	Cyclopentanetridecanoic acid, methyl ester	1.00
7	11.769	Eicosane, 2-methyl-	1.27
8	14.449	Pentadecanoic acid, 14-methyl-, methyl ester	1.49
9	15.139	Decane, 2,3,5,8-tetramethyl-	0.91
10	29.941	Lupeol, trifluoroacetate	0.76
11	31.454	Lupeol	2.84
12	32.247	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	1.37
13	36.831	Lup-20(29)-en-3-ol, acetate, (3ß)-	35.12

gram-negative bacteria and fungus might be due to the reason that some phytocompounds are target specific or might be due to the fact that gram-negative bacteria show highest resistance to broad spectrum of antibiotics [54]. For instance, coumarin (polyphenolic compound) shows activity against gram-positive and no activity against gram-negative bacteria [17]. In addition, Iqbal et al. [25], recently isolated three alkaloid compounds (Viburnoate A, B, C) from *V. grandiflorum* and demonstrated that these alkaloid compounds showed maximum inhibition against gram-positive bacteria than gram-negative bacteria.

Many scientific investigations concerning antimicrobial and antioxidant activities of other *Viburnum* species [25, 50, 57, 73] have been undertaken previously. However, there is lack of scientific documentation on the efficacy

of currently studied species, especially on the aspects of antimicrobial and antioxidant activities. Therefore, the findings pertaining to free radical scavenging and antimicrobial potential of the *V. simonsii* extracts in the present study necessitates the need for identifying active antioxidant as well as antimicrobial components present in the extracts and speculating their subsequent commercial application.

FTIR was done in order to determine the functional groups present in the crude extract. The FTIR spectra implies the presence of alcohols (primary and secondary), ethers, amines, carboxylic acids and hydrocarbons (alkane, alkene and alkyne). In the FTIR spectra the fruit extract showed more absorption intensity than leaf extracts, which may be attributed to the presences of phytocompounds in larger quantity than the leaf extracts. The phytoconstituents of the *V. simonsii* extracts were identified by GC–MS analysis. The analysis revealed the presence of 21 compounds in fruit extract and 13 compounds in leaf extract. 1,2,3,4-Cyclohexanetetrol are polyols having highest peak area percentage (16.86%) in MEVL which act as glucosidase inhibitor [47]. Lup-20(29)-en-3-ol, acetate, (3ß)- found in both MEVL (9.22%) and MEVF (35.12%) have anticancer, anti-inflammatory, antituberculosis, antimalarial, antimicrobial, antinociceptive and antioxidant activities [38, 51]. ß-Sitosterol (5.70%), have antitumor activities against breast, lung, prostrate and colon cancer [31]. α, ß-Amyrins (15.95%, 4.41%) were reported to have antinociceptic, anti-inflammatory, antioxidant, anti-diabetic and anticancer, antihyperglycemic, gastroprotective and anticonvulsant properties [19, 46]. Neophytadiene (4.96%) have anti-inflammatory, anti-microbial, antioxidant, antipyretic, good analgesic properties [5, 11]. Further, Benzoic acid, 4-ethoxy-, ethyl ester, which is found in both MEVL and MEVF has cardioprotective, antimicrobial, antioxidant and anti-inflammatory properties [37]. Pentadecanoic acid, 14-methyl-, methyl ester, Tridecanoic acid, 12-methyl-, methyl ester, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 11-Octadecenoic acid, methyl ester and 1-Heptatriacotanol were reported to have antimicrobial, anti-inflammatory, hypocholesterolemic, cancer preventive, antioxidant, hepatoprotective, and antiarthritic properties [18, 21]. cis-Z-a-Bisabolene epoxide increase sex hormone activity and has antitumor property [18]. In one of the previous studies on the related species such as *Viburnum odoratissimum var odoratissimum* dehydrovibsanin G (diterpenoid), (+)-9'-*O*-senecioyllariciresinol (lignan) were isolated and reported to possess anticancer activity against breast cancer cell lines (human A431, T47D) [36]. Recently, five new terpenoids (two vibsane-type diterpenoids, three iridoid allosides) were isolated from *Viburnum odoratissimum var sessiliflorum*, these compounds showed efficient anti-inflammatory and anticancer activities against colon cancer [78].

However, it is worth mentioning herein that FTIR and GC–MS studies are not conclusive for phytochemical investigations. Since IR and GC–MS studies cannot elucidate the structure of the bioactive compounds, higher techniques such as NMR spectroscopy is required. NMR provides insight into the different structures and functionalities of the various components and, hence, assists the interpretation of the results of the other analytical methods [43]. The study also necessitates the need for NMR spectroscopy (a part of planned future research activity) which is highly sought after in drug development for both molecule identification and structural elucidation [16].

Conclusion

The use of plant-based medicine as an alternative to allopathic medicine is now becoming an increasingly attractive option for the management of human diseases. The medicinal plants secrete a diverse array of high value bio-actives (polyphenols, flavonoids, alkaloids, glycosides, terpenoids etc.) and consequently possess significant bioactivity. Additionally, medicinal plants are believed to impart lesser side effects in comparison to conventional allopathic medicine. *V. simonsii* in the present study showed high phenolic content and appreciable antioxidant and antibacterial activities. Qualitative phytochemical screening revealed the presence of high value bioactives. FTIR study also confirms the presence of functional groups which are usually present in phytocompounds. Further, GC–MS analysis demonstrated the presence of compounds which have anticancer, anti-inflammatory, antidiabetic, antimalarial, antituberculosis, and anti-nociceptic properties. Since the plant parts are already in use by the indigenous populace of Meghalaya, there lies tremendous scope for new area of investigations. Therefore, the present study warrants greater research capacities with specific emphasis on pure compound isolation, bioactivities (*in-vitro*, in vivo investigation of anticancer, antiviral, anti-diabetic, anti-inflammatory activity etc.) and in silico drug designing studies in order to validate its pharmaceutical potential.

Abbreviations

V. simonsii:	Viburnum simonsii
V. opulus	Viburnum opulus
MEVL	Methanolic extract of V. simonsii leaf
MEVF	Methanolic extract of V. simonsii fruit
FTIR	Fourier transform infrared
GC–MS	Gas chromatography mass spectrometry
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-Diphenyl-1-picrylhydrazyl

FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
QE	Quercetin equivalent
RSA	Radical scavenging activity
AAE	Ascorbic acid equivalent
DMSO	Dimethyl sulfoxide
NMR	Nuclear magnetic resonance
MHA	Mueller Hinton agar

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Plant authentication
The plant species was identified and authenticated by referring to the existing herbarium specimen (Reference accession no: 90420) available in Botanical Survey of India, Eastern Region, Shillong, India

Author contributions
SRS: Formal analysis, investigation, methodology, writing original draft and editing. MMP: Visualization, conceptualization, formal analysis, investigation, methodology, writing-original draft, reviewing and editing. VC: Formal analysis, investigation, methodology, writing original draft. DKV: Formal analysis, investigation, methodology and writing original draft. PB: Formal analysis, investigation, methodology and writing original draft. SK: Formal analysis and writing original draft. PPP: Formal analysis and writing original draft.

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Availability of data and materials
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Declarations

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Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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