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Qualitative Estimation, Total Phenol Content, Antioxidant and Antibacterial Activity from the Fruits Extracts of *Evodia Fraxinifolia*

Bhoj Raj Chhetri[1],Nayan Kamal Bhattacharyya[*1], Deepjyoti Dutta[1], Karma Gyurmey Dolma[2], Sangeeta Jha[1], Archana Moni Das[3], Joydeep Biswas[1]

[1]Sikkim Manipal Institute of Technology (Sikkim Manipal University), Majitar, Rangpo Sikkim, India

[2]Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, 5th Mile, Tadong, Gangtok, Sikkim India

[3]CSIR-North East Institute of Science and Technology, Jorhat, India

*Corresponding author: **Nayan Kamal Bhattacharyya**

Abstract: In Sikkim, *Evodia fraxinifolia* stands out as one of the important and commonlyused plant in traditional medicine. This study aims to assess the chemical composition of different crude (petroleum ether, chloroform, ethyl acetate, methanol and aqueous) extracts from fruit *of Evodia fraxinifolia.* Herein, Fourier transform infrared spectrometry (FTIR) was used to identify analyze the presence of important functional groups in fruit extracts of *Evodia fraxinifolia*. Additionally, the extracts were screened for phytochemical metabolites, total phenol content, antioxidant activity and antibacterial activity. Phytochemicals such as alkaloids, phenols, proteins, saponins, amino acids, carbohydrates, and sterols were present in the extracts. The highest phenol content was found in ethyl acetate extract with 26.816 ± 0.42 mg of GAE/g, followed by chloroform extract with 24.984 ± 0.48 mg of GAE/g and aqueous extract with 23.67 ± 0.42 mg of GAE/g of plant sample. The antioxidant properties were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay where ethyl acetate extract exhibit the lowest DPPH radical scavenging activity with IC_{50} value of 33.62 µg/mL and highest by aqueous extract with 165.78µg/mL. The chloroform extract showed the bacterial inhibitionzone of 11 mm against *S. aureus*and *E. coli* respectively. The ethyl acetate extract was more effective, with 18 mm inhibition zone against *S. aureus* and a 15 mm zone against *E. coli*. The lowest Minimum Inhibitory Concentration (MIC) value of 16.28 µg/ml was given by ethyl acetate extract against *S. aureus* and the MIC value of 37.56 µg/ml by methanol extract against *E. coli*. To our knowledge, a study on different extracts from the fruit extract of *E. fraxinifolia* with phytochemicals screening, functional groups detection, antioxidant and antimicrobial activity was done for the first time. The results suggest that *E. fraxinifolia* extract possesses bioactive substances with antioxidant and antimicrobial properties and could be a probable herbal medicine soon.

Keywords: *Evodia fraxinifolia*, Sikkim, total phenol content, antioxidant, antimicrobial activity

1. Introduction

Natural products have played a major role in the history of medicine, with diverse cultures harnessing the therapeutic properties for healing purposes(Alkowni et al., 2023).The World Health Organization acknowledges that plants serve as a fundamental cornerstone of primary health for more than half of the global population(Famuyide et al., 2019).This can be attributed to their widespread availability across various regions and their lower risk of adverse effects compared to synthetic drugs(Sangma et al., 2023).Many plant species have been recorded to exhibit pharmacological properties including antioxidant, antibacterial, anticancer and antidiabetic effects due to their rich content of diverse secondary metabolites such as alkaloids, steroids glycosides, tannins, saponins, flavonoids and terpenes(Deepjyoti Dutta et al., 2021). Consequently, these plants should be harnessed for their ability to combat pathogens that cause diseases(D Dutta et al., 2022). The use of plant-derived substances for therapeutic purposes dates back centuries, forming the basis of traditional medicine systems worldwide (Sbieh et al., 2022). In the context of the ever-evolving landscape of pharmaceutical research, natural products continue to capture the attention of scientists and researchers for their potential in drug discovery and development.*Evodia fraxinifolia* is one of the important traditional medicinal plants available in Sikkim. It is a deciduous tree from the Rutaceae family, commonly known as ‘‘Khanakpa’’ in Sikkim. It grows to 2-16 m high and is often found in loamy soil. The fruits are mostly 4-carpellate, connate at base forming a four lobed shape about 11 mm wide. The seeds are small, black, and shaped like a triangle with three sides. The flowers typically begin to `bloom between August and September, and the fruiting period extends from October to December. It is a native plant of China, Korea, Nepal, Bhutan, Myanmar, Vietnam, Thailand, and India. In India, it is likely to be found in Sikkim, Arunachal Pradesh, West Bengal, Nagaland, Assam, Meghalaya, and Tamil Nadu(Puri, 1956).

The fruits, leaves, and roots of*E. fraxinifolia* have been traditionally used in Sikkim's folk medicine to treat a wide range of health issues. These include gastrointestinal disorders like diarrhea, dysentery, indigestion, and stomach ulcers, as well as conditions such as nausea, fever, skin diseases, and abdominal pain. The plant has also been employed to treat vomiting and birth control purposes (Chanda et al., 2007; Chhetri, 2004; Green, 2009). Phytochemicals like iso-bauerenol, β-sitosterol, limonoids, fraxinifoline A-F, B-seco limonoids, pentacyclic triterpenes have been identified from the stem bark and leaves of *E. fraxinifolia*(Chhetri, 2004; K. Talapatra et al., 1968). Previous studies have highlighted the plant’s antibacterial, anti-inflammatory, and wound healing properties(Chanda et al., 2007; Chhetri, 2004; Sharma, 2013).However, scientific evidence supporting these traditional uses remains limited. Furthermore, plant

species can exhibit varying biological properties based on regional environmental conditions(Sharma, 2013), and despite numerous studies on phytochemical content and biological properties, the vast diversity of plants globally and in Sikkim makes existing research insufficient(Durhan et al., 2022). Plants also produce different secondary metabolites to adapt to their specific environments, resulting in variations in phytochemical content and biological effects even within the same species growing in different regions(Kurt et al., 2023). To the best of our knowledge, this is the first paper in which we have explored the phytochemical content, functional groups through Fourier transform infrared spectrometer (FTIR), antioxidant and antibacterial activity of *E. fraxinifolia* fruitextracts. The rise of drug-resistant bacterial strains has created an urgency to discover new antimicrobial agents.*E. fraxinifolia* is traditionally used for its medicinal properties, but until now, there has been little to no scientific data validating these uses. This research serves as an essential first step in understanding the plant's potential, particularly in light of antibiotic resistance. By screening the antibacterial properties of *E. fraxinifolia*, we contribute to the ongoing discourse on natural products as a source of effective and sustainable solutions for combating infectious.

2. Materials and methods

2.1. General

Extraction of fruit was done in Soxhlet apparatus Borosil®. The absorbance was measured on a Shimazu UV-19020I at a wavelength of 725 nm. FT-IR was recorded in Shimazu Attenuated Total Reflection Fourier Transform Infra-Red Spectrophotometer on a thin film using chloroform. The microbial strains *Staphylococcus aureus* ATCC 43300 and gram-negative bacteria *Escherichia coli* ATCC 25922S were obtained from the Microbiology Department, Sikkim Manipal Institute of Medical Science. Muller Hinton Agar was purchased from HIMEDIA® M173-500G. Petroleum ether, Chloroform, Ethyl acetate and Methanol are of analytical grade with 99% purity while distilled water was used for the aqueous extraction.

2.2. Plant collection and identification

The fruits of *E. fraxinifolia*were collected in the last week of September 2022 from the area of Uttarey, West Sikkim. The botanical identification and voucher specimen was deposited at the Botanical Survey of India- SRH, Gangtok, Sikkim with accession no. BRC/15. The collected fruits were thoroughly washed and kept in a dry shade. After the complete drying, it was ground and kept in an airtight container for further use.

2.3. Phytochemicals Assays

2.3.1. Preparation of plant extract

100 gm of *E. fraxinifolia*dried fruits was successively extracted with petroleum ether, chloroform, ethyl acetate and methanol using Soxhlet Apparatus. After extraction, the residue was separated by filtration using Whatman filter paper No. 42. The solvent was then evaporated using a rotary vacuum pump at 40°C, and the resulting concentrated extract was utilized for phytochemical analysis.

2.3.2. Qualitative phytochemical analysis

Standard qualitative analyses were carried out to conduct phytochemical screening tests for various plant metabolites, including proteins, carbohydrates, phenols, tannins, flavonoids, saponins, steroids, terpenoids, amino acids and anthraquinone(Bakir Çilesizoğlu et al., 2022).

2.3.3. Determinations of total phenols content

The total polyphenol content was established following the method outlined with slight adjustments(Makkar, 2003). In brief, 0.5 mL of sample solution was combined with 0.25 mL of Folin-Ciocalteu reagent, which was previously diluted tenfold. After 2 minutes of incubation, 1.25 mL of 20% sodium carbonate solution was added to the mixture and was allowed to stand for 1 hour before measuring the absorbance at 725 nm. A calibration curve using gallic acid was established as the standard of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml and 10 µg/ml. The concentration of total polyphenols was calculated by converting the optical density (OD) reading for each sample, accounting for the dilution carried out. The results are presented as gallic acid equivalents (GAE) mg of extract.

2.3.4. Fourier transform infrared spectrometer (FTIR)

The analysis of various functional groups in the various extracts of the fruit of *E. fraxinifolia* was examined from ATR-FTIR (Shimazu Attenuated Total Reflection Fourier Transform Infra-Red Spectrophotometer).

2.4. Antimicrobial Assays

2.4.1. Preparation of crude extract

The fruits extraction of *E. fraxinifolia*was done successively using the Soxhlet apparatus by increasing the polarity from petroleum ether, chloroform, ethyl acetate and methanol. After extraction, it was filtered using standard filter paper and finally, it was concentrated with a rotatory vapor at 40-45°C. The aqueous extraction was done through maceration and was concentrated in a water bath. Then, each extract was dissolved in 10% dimethyl sulfoxide and stored at 4°C for the antimicrobial evaluation.

2.4.2. Microbial strains

The antimicrobial strains used in this study include gram-positive (*Staphylococcus aureus* ATCC 43300) and gram-negative bacteria (*Escherichia coli* ATCC 25922S. These strains were cultured on a Nutrient Agar, incubated at 37°C for 24-72 h. One or two colonies were suspended in peptone water and its turbidity was maintained at 0.5 standard McFarland (prepared by mixing 1% H_2SO_4 in 9.95 ml and 1% of 0.05 ml $BaCl_2$ in distilled water) with an inoculum of 1×10^8 CFU/mL for the experiment.

2.4.3. Antimicrobial Susceptivity Assays

*Well Diffusion Method:*The antimicrobial activity screening of *E. fraxinifolia*extracts against the microbial strain was tested using the well diffusion method(Deepjyoti Dutta et al., 2022). In this method, the strains were grown on the nutrient plate and were obtained in vile-containing peptone. Then, the resulting suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard. The test strains were then cultured in the Mueller-Hinton agar using cotton swap. After drying for 15 minutes a whole of 6 mm was made in the agar plate with the help of a cork borer. In the subsequent step, 100 µL, 80 µL, 60 µL, 40 µL, and 20 µL of each prepared plant extract were added to separate wells, with Gentamycin (10 mcg) antibiotic serving as the positive control. The plates were incubated for 18-24 h in aerobic conditions at 37 ± 2 °C. After incubation, the zone of inhibition was measured in millimeters using a scale. All experiments were performed in triplicate, and the results were reported as the mean ± standard deviation.

Macro-broth dilution for MIC evaluation: The Minimum Inhibitory Concentration of the extract was done by macro-broth dilution assay(Balouiri et al., 2016). In this method, 1 mL of Muller Hinton broth was added to all sterile test tubes followed by 100 µL of adjusted 0.5 standard microbial strains. Then, 100 µL of plant extract from 50 mg stock was added to the first test tube to get a concentration of 4.545 mg/ml and serial dilution was done to prepare a range of concentration in all remaining test tubes. For the positive control, nutrient broth and bacterial strains were used, while for the negative control, plant extract and nutrient broth were utilized. The tubes were incubated for 18-24 h at 37 ± 2 °C and after incubation, the tubes were assessed for their turbidity caused by bacteria. If the tubes are transparent, it was concluded that the plant extract restricts bacterial growth and measured the Minimum Inhibitory Concentration(Neipihoi et al., 2021).

3. Antioxidant Activity

The radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was measured using a spectrophotometer, following a slightly modified version of the method by

Jaradat et al.(Sultana et al., 2009)as outlined below.. Stock solutions of the plant extracts and Ascorbic acid (used as a positive control) were prepared at a concentration of 1 mg/mL in methanol. Serial dilutions in methanol were made to obtain working concentrations of 20, 40, 60 and 80 µg/mL. After incubation for 30 mins in dark, absorbance of these solutions was measured at 517 nm. The antioxidant activity of the extract was represented as the IC_{50} value, which is the concentration required to inhibit 50% of DPPH radical formation. The experiment was conducted in triplicate, and the activity was determined by calculating the percentage of DPPH radicals scavenged. The DPPH radical scavenging activity for each plant extract was then compared to that of ascorbic acid. The antioxidant activity of the ascorbic acid standard and plant extracts was determined using the following formula:

$$\text{DPPH radical scavenging activity}(\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \qquad (1)$$

Where $A_{control}$= Absorbance of blank (methanol + DPPH) and A_{sample}= Absorbance of sample.

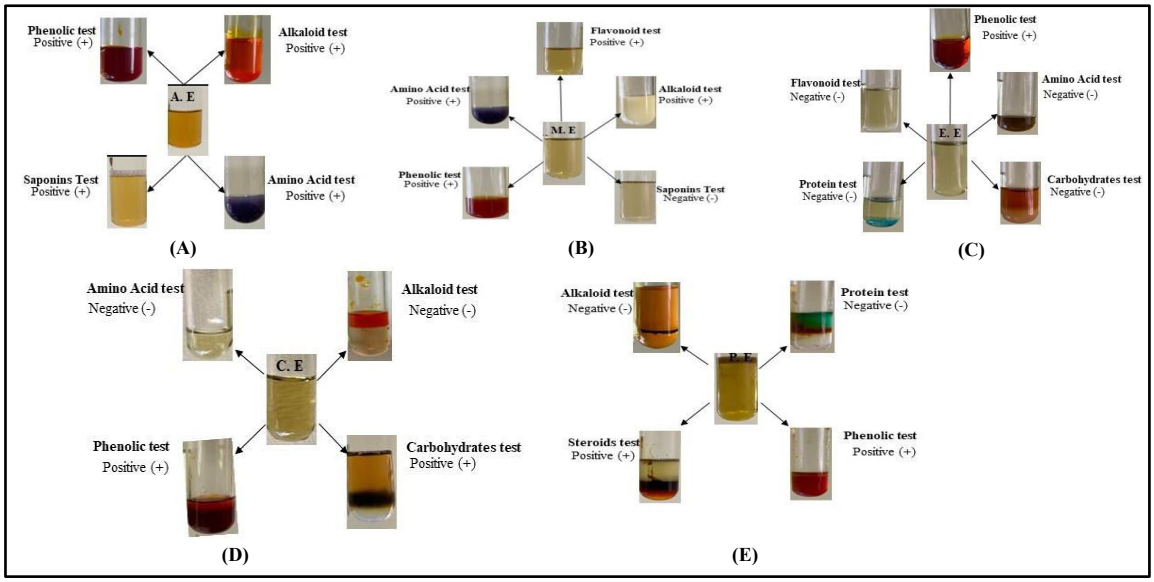
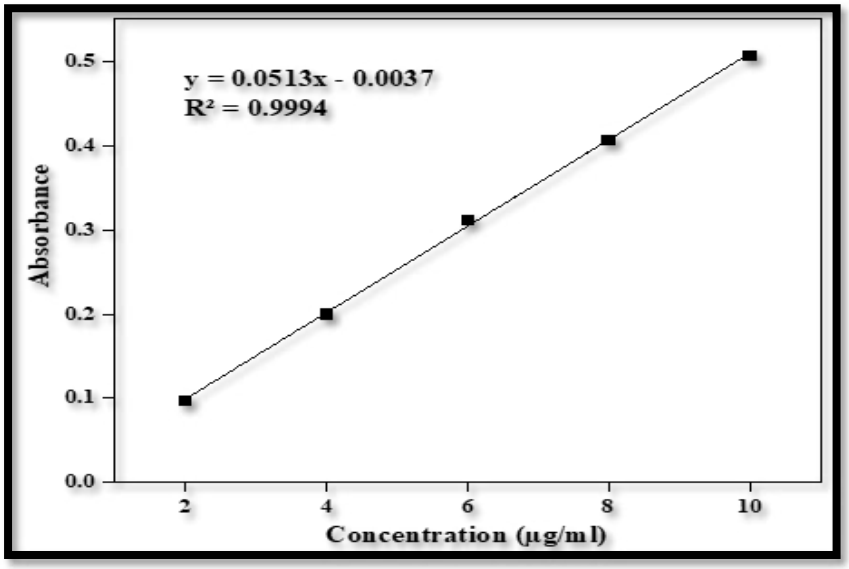


Figure 1: Qualitative analysis of *E. fraxinifolia*. A.E indicates extraction with aqueous **(A),** M.E with methanol **(B)**, E.E with ethyl acetate **(C)**, C.E with chloroform **(D)** and P.E indicates Petroleum ether **(E)** respectively.

4. Results and discussion

Throughout human antiquity, plants have served as the primary source for numerous medicinal preparations and remain a significant reservoir of natural products undergoing clinical trials, particularly in anti-bacterial, antifungal, anticancer and other antimicrobial agents. This is evident from the statistic that, between 1981 and 2014, 75% of medications approved by the US Food and Drug Administration (FDA) were acquired from natural sources, with 49%being natural products or their derivatives, and 1% being defined mixtures of plants(Atanasov et al., 2015).There has been a significant focus on exploring natural sources for antimicrobial agents, with



considerable efforts for identifying compound that could serve as effective alternatives to synthetic ones. Phytochemicals obtained from plant products are being studied as a model to develop medications that are both less harmful and more potent in managing microbial growth(Mohamed, 2017). Many studies have investigated the antimicrobial activity with the extract of various plants as well as the discovery of new antimicrobial compounds(Ahmad & Beg, 2001).

Figure 2: Calibration Curve of Gallic acid for total phenol content determination.

*E. fraxinifolia*is animportant traditional medicinal plant in Sikkim. In this study, various fruit extracts of *E. fraxinifolia*were examined for qualitative and quantitative analyses, identification of functional groups using FTIR spectroscopy, and evaluation of antibacterial activity against both Gram-positive and Gram-negative bacteria. The susceptibility of each extract was evaluated through the serial macro broth dilution method (MIC), while the agar well diffusion method was used to assess their effectiveness.

In the present investigation, the qualitative phytochemical screening of the fruit extracts of *E. fraxinifolia* revealed the presence of various bioactive compounds, including steroids, alkaloids, tannins, amino acids, saponins, carbohydrates, and terpenoids, among others(Figure 1). These metabolites were identified through traditional assays and confirmed by FTIR analysis.In addition,phenols were present in all the extracts, while saponins

were observed only in aqueous extract. The presence of terpenoids, steroids and tanninswereexclusively presentonly in petroleum ether extract. In addition, onlymethanol and aqueous extract contained alkaloids and amino acids respectively. Carbohydrates were present only in chloroform and aqueous extract of *E. fraxinifolia*, while proteins and anthraquinones were absent in all the tested extracts (Table 1).

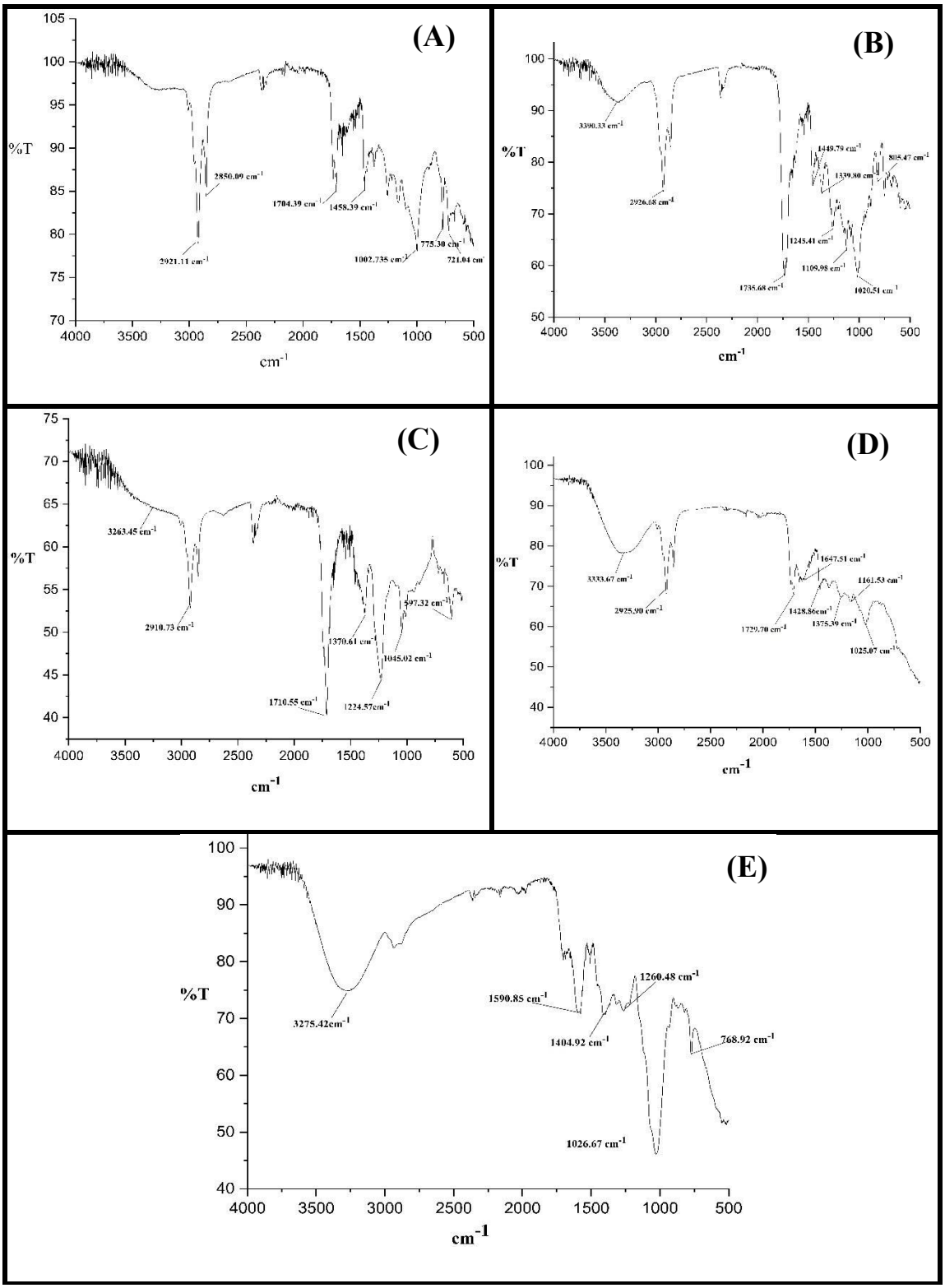


Figure 3: FTIR spectrum:(**A**) petroleum ether extract (**B**) chloroform extract (**C**) ethyl acetate extract (**D**) methanol extract (**E**) aqueous extract of *Evodia fraxinifolia*.

FTIR spectroscopy, an essential analytical technique, was employed to identify the functional groups present in the fruit extracts of *E. fraxinifolia*. The technique is crucial for detecting molecular vibrations that correspond to specific functional groups, associated with various phytochemicals, aiding in the identification of bioactive compounds in plant species(Coates, 2000). From the IR spectra (Figures 3), various characteristic absorption bands were observed, which corresponded to different classes of compounds, confirming their presence.Alkanes were identified with C-H stretching vibrations at 2921-2926 cm^{-1}, a feature common in fatty acids, terpenoids, and other lipid-based metabolites(*(2) Spectrometric Identification of Organic Compounds, 7ed 2005 – Silverstein, Webster & Kiemle | Request PDF*, n.d.).Phenols and alcohols were confirmed by the broad O-H stretching band around 3390-3275 cm^{-1}, indicating the presence of flavonoids and other polyphenoliccompounds(Stuart, 2005).Carbonyl groups (C=O stretching) were observed around 1710-1729 cm^{-1}, suggesting the presence of aldehydes, ketones, esters, and carboxylic acids, which are common in steroids, tannins, and triterpenoids.Amines and amines derivatives showed bands at 1020-1045 cm^{-1}, further supporting the presence of alkaloids.Aromatic compounds were identified by peaks around 1449-1458 cm^{-1}, which is indicative of phenolic compounds or other aromatic rings typically found in saponins and tannins(Konappa et al., 2020).These IR spectral results confirm the presence of several major classes of phytochemicals: phenolics (flavonoids and tannins), terpenoids, alkaloids, and steroids which are consistent with previous phytochemicals studies using spectroscopic methods(Awadelkareem et al., 2022; Punetha & Vuppu, 2023). Each of these classes plays a significant role in the biological activities of *E. fraxinifolia*, contributing to its reported antioxidant, antimicrobial, and anti-inflammatory properties(Richardson & Harborne, 1990).

For determination of total phenol content, the standard calibration curves were plotted using the various concentrations of gallic acid (mg of GAE/g). From the calibration curve, the equation$y = 0.0513x - 0.0037$ $(R^2 = 0.9994)$ obtained was used for determining the total phenol content in the fruit extract of *E. fraxinifolia*(Figure 2). The highest phenol content wasshowed by ethyl acetate extract of 26.816 ± 0.42 mg of GAE/g, followed by chloroform extract of 24.984 ± 0.48 mg of GAE/g and aqueous extract of 23.67 ± 0.42 mg of GAE/g of plant sample. The pet. ether and methanol showed the lowest content 5.315± 0.38 and 7.440 ± 0.41 mg of GAE/g of plant sample respectively. A variation in total phenolic content was observed across different *E. fraxinifolia* extracts, which can be attributed to the differential solubility of phenolic compounds in solvents with varying

polarities. The ethyl acetate extract, a moderately polar solvent, exhibited a significantly higher phenolic content compared to the non-polar petroleum ether extract. This suggests that phenolic compounds, being diverse in nature, are more efficiently extracted by polar solvents.In contrast, methanol and petroleum ether extracts exhibited lower phenolic content. Methanol, despite being a polar solvent, may have extracted fewer phenolics due to specific solvent-phenol interactions or the poor solubility of certain phenolic compounds in methanol. Petroleum ether, a non-polar solvent, was the least effective, which aligns with the general understanding that phenolic compounds are more soluble in polar or moderately polar solvents. Aqueous extracts, while polar, may have resulted in lower phenolic content due to solubility limitations or interference from other co-extracted compounds such as sugars and proteins.

These results are consistent with previous research. Sultana et al. and Wang et al. (Sultana et al., 2009) reported that solvent polarity significantly influences the extraction of phenolic compounds, with higher phenolic content being extracted by moderately polar solvents like ethyl acetate compared to non-polar solvents such as petroleum ether. Similarly, Wang et al. (2013) demonstrated that ethyl acetate is particularly effective in extracting polyphenols from plant materials. This supports the conclusion that ethyl acetate's intermediate polarity makes it an ideal solvent for the extraction of phenolic compounds from *E. fraxinifolia*.

The fruit extracts of *E. fraxinifolia* were investigated for their antioxidant effectiveness by DPPH assay. The DPPH radical scavenging assay is a commonly used method for assessing the overall antioxidant potential of plant extracts, particularly those rich in phenolic compounds such as flavonoids and phenolic acids(De Marino et al., 2007; McCune & Johns, 2007).In the present study,the methanol extract exhibited the highest percentage radical inhibition activity of 60% and that of ascorbic acid was 97%. This was followed by ethyl acetate (50%), aqueous (46%), and chloroformextracts (43%) respectivelywhereas, petroleum ether extract showed the lowest activity of 24% (Figure 4). The DPPH radical scavenging activity of the plant extracts increased as their concentration was raised. This suggests an enhanced capacity to donate hydrogen ions, leading to a lighter solution, which is directly related to the number of electrons accepted(Silva et al., 2005; Villaño et al., 2007).The lowest IC_{50} value was observed in ethyl acetate extract followed by petroleum ether, methanol, chloroform and aqueous extract with IC_{50} values 33.62 (µg/mL), 59.9(µg/mL), 65.04(µg/mL), 69.86(µg/mL) and 165.78(µg/mL) respectively. As the lowerIC_{50}value has a higher antioxidant activity, the ethyl acetate extract has a higher ability to scavenge free radicalby donating more electrons to the unstable DPPH free radical, transforming it into diamagnetic molecule as compared to other extracts(Bhandari et al.,

2016).The high antioxidant activity observed in the ethyl acetate extract correlates positively with its total phenolic content (TPC). A higher TPC is associated with increased antioxidant capacity, reflecting a linear relationship between these two factors. Previous research has demonstrated a strong link between antioxidant capacity and the total flavonoid and phenolic content in crude extracts of plant(Mustafa et al., 2010). Finding from this study was supported by the findings reported by Sharif et al., where the crude ethyl acetate extract showed the lowest IC_{50} with a value of 21.567 ± 1.34 mg/mL which was comparable to the IC_{50} value of ascorbic acid, which was 19.453 ± 1.32 mg/mL(Narayanan et al., 2023).

The antibacterial activity of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extract was investigated using a well diffusion method against *E. coli* and *S. aureus*(Figure 5 and Figure 6). The result exhibited that ethyl acetate extract showed the highest zone of inhibition of 18 ± 0.02 mm and lowest by chloroform extract with 11 ± 0.18 mm against S. *aureus*respectively. The petroleum ether was only sensitive against *S. aureus* with 12 ± 0.25 mm zones of inhibition.In addition, chloroform, ethyl acetate and methanol extract showed a zone of inhibition with 11 ± 0.02 mm, 15 ± 0.01 and 13 ± 0.15 mm against *E. coli* respectively. The aqueous extract was not sensitive to both strains (Table 2).

Table 1: Summary of the phytochemicals present in different fruit extracts of *E. fraxinifolia*.

Phytochemicals	**Pet. ether extract**	**Chloroform extract**	**Ethyl acetate extract**	**Methanol extract**	**Aqueous extract**
Alkaloid	--	--	--	**++**	**++**
Flavonoid	--	--	--	**++**	--
Tannins	**++**	--	--	--	--
Saponins	--	--	--	--	**++**
Terpenoids	**++**	--	--	--	--
Phenols	**++**	**++**	**++**	**++**	**++**
Anthraquinone	--	--	--	--	--
Steroids	**++**	--	--	--	--
Proteins	--	--	--	--	--
Carbohydrates	--	++	--	--	++
Amino acid	--	--	--	++	++

Positive (++) indicates the presence of phytochemicals. Negative mark (--) indicates the absence of phytochemicals

Table 2: Antimicrobial activity with Zone of Inhibition of extracts (mean ± SD) for different crude extracts of *E. fraxinifolia* against Gram-positive and Gram-negative bacteria.

Extract	**ZOI against microbes (mean ± SD) (mm)**		**MIC ($mgmL^{-1}$)**	
	E. coli.	*S. aureus*	*E. coli.*	*S. aureus*
Pet. ether	NF	12 ± 0.25	ND	3.28
Chloroform	11 ± 0.02	11 ± 0.18	12.5	8.25
Ethyl acetate	15 ± 0.01	18 ± 0.02	6.25	1.56
Methanol	13 ± 0.15	14 ± 0.31	4.12	5.12
Aqueous	NF	NF	ND	ND
Gentamicin (μgmL^{-1})	19 ± 0.15	21 ± 0.21	ND	ND

ZOI= Zone of inhibition in mm, MIC= Minimum inhibitory concentration, –: Activity not found, ND= Not Determined

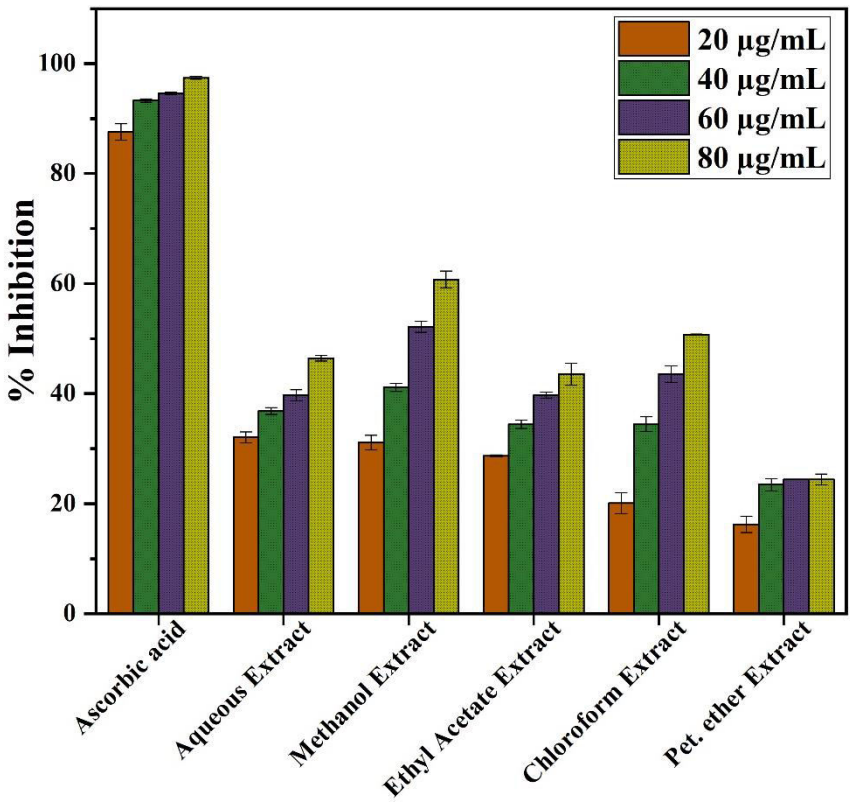


Figure 4: DPPH radical scavenging activity by the fruit extract of *E. fraxinifolia.*

For the positive control, Gentamycin (10 mcg) was used as standard antibioticfor both strains. The antibacterial Minimum inhibitory concentrations (MIC) of methanol, ethyl acetate, chloroform and petroleum ether extract were performed against two strains (*E. coli* and *S. aureus*) by macro broth dilution. Antibacterial MIC tests showed that the highest inhibition activities (lowest MIC value) were for ethyl acetate extract against *S. aureus* with MICof 1.56mg/ml and methanol extract with lowest MIC value of 4.12mg/ml against *E. coli*respectively. In addition, chloroform extract showed the highest inhibition activity with a MIC of 12.5mg/ml and 8.25 mg/ml in both the strains.However, petroleum ether extract gave a MIC value of 3.28mg/ml against *S. aureus*. Unfortunately, the aqueous extract was inactive against the *E. coli*and *S. aureus* strains used in this study (Table 2). The variation in antibacterial activity observed across the different extracts of *E. fraxinifolia* can be attributed to the selective extraction of bioactive phytochemicals influenced by the polarity of the solvents used. A previous study has highlighted the critical role of solvent selection in extracting phytochemicals from plants, as solvent polarity directly impacts the extraction of active compounds (Mohamed, 2017).An earlier investigation reported that the fruit extract of *E. fraxinifolia* in 50% ethanol showed no antimicrobial activity against *Bacillus subtilis, S. aureus, E. coli,* and *Salmonella typhi*(Sharma, 2013).This variation in activity may be due to differences in the characteristics of the extracted phytochemicals, the extraction rate, the method used, geographic variation of the plant, and the specific solvent employed.In this study, extraction with ethyl acetate,

chloroform, and methanol yielded promising antibacterial activity

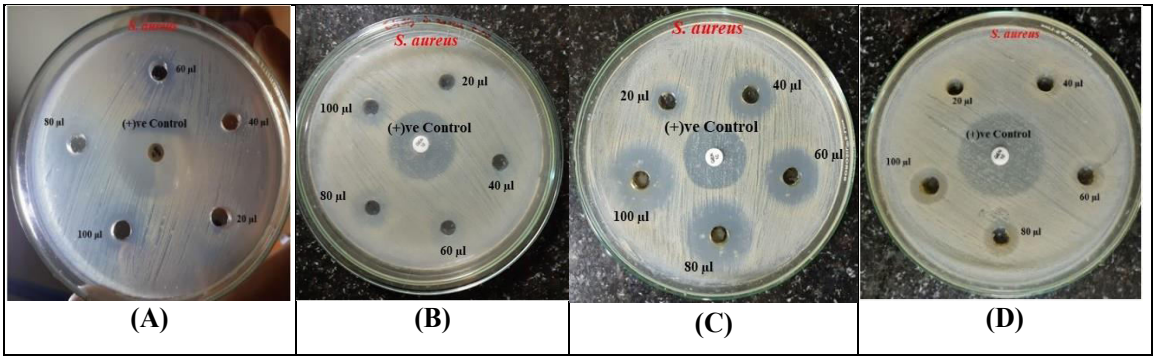


Figure 5: Antibacterial activity by Well Diffusion Method: (**A**) Petroleum ether (**B**) Chloroform (**C**) Ethyl acetate (**D**) Methanol extract against *S. aureus*.

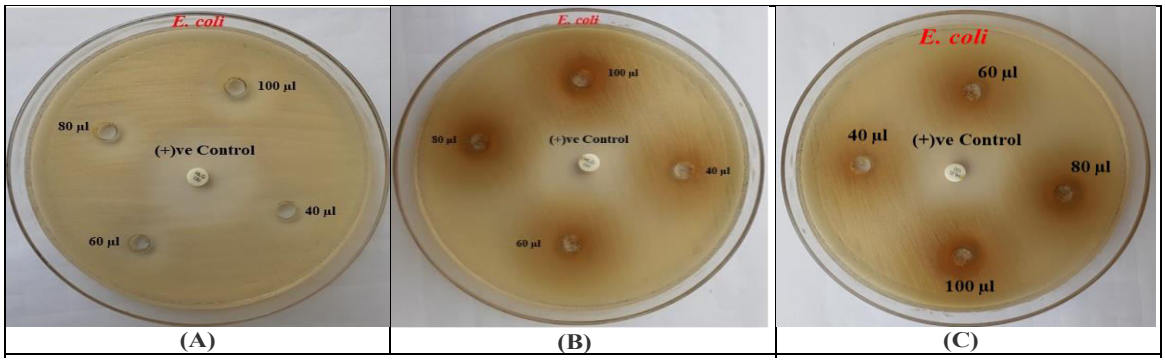


Figure 6: Antibacterial activity by Well Diffusion Method: (**A**) Chloroform (**B**) Ethyl acetate (**C**) Methanol extract against *E. coli*.

compared to otherextracts, possibly due to

differences in the bioactive compounds present in these extracts. Ethyl acetate demonstrated the highest antibacterial activity against *S. aureus* with a MIC value of 1.56 mg/ml, while methanol showed significant activity against *E. coli* with a MIC value of 4.12 mg/ml. Chloroform extracts displayed moderate antibacterial effects with MIC values of 12.5 mg/ml and 8.25 mg/ml against both bacterial strains, whereas the petroleum ether extract showed notable activity against *S. aureus* (MIC of 3.28 mg/ml). In contrast, the aqueous extract did not exhibit antibacterial activity against either strain, suggesting that non-polar residues in the extracts, such as those obtained using chloroform or petroleum ether, may contribute to increased bacteriostatic capabilities. These findings align with other studies, which suggest that most plant-derived antibiotic compounds are aromatic or saturated organic molecules that readily dissolve in organic solvents (Evans & Cowan, 2016). The study revealed that the *E. fraxinifolia* plant harbors a significant reservoir of pharmacologically active constituents, including therapeutic phytochemicals that exhibit a range of pharmacological effects such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic, analgesic, antiaging, and anticancer properties.

In this study, the isolation of the active compounds from *E. fraxinifolia* was not conducted. This was primarily due to the unavailability of sufficient plant material required for column chromatography. As a result, chemical structure elucidation, *in-vivo* investigation and the identification of the most bioactive compounds from *E. fraxinifolia* extracts were not addressed in this investigation. However, these experiments are planned for future research.

4. Conclusions

The results obtained from this study have provided evidence that different extracts of fruits of *E. fraxinifolia*containimportant functional groups like alkanes, amines, aldehyde, ketone, and alcohols. Further extracts exhibited saponins, tannins, sterols, phenols, amino acids as a secondary metabolite. The highest phenol content was shown by ethyl acetate extract of 26.816 ± 0.42 mg of GAE/g of plant sample. The *in vitro* antibacterial evaluation of different extracts significantly varies among the extracts. The ethyl acetate extract showed excellent antibacterial activity with MIC 16.28 µg/mL against *S. aureus* and MIC 37.56 µg/mL by methanol extract against *E. coli*. This may be due to the concentration and nature of the metabolite content in the extract. Overall, this is the first report of qualitative, quantitative, and antibacterial activity of the fruit of *E. fraxinifolia,* an important role in ethno-medicinal plant of Sikkim. Hence, further studies must be done to isolate novel bioactive compounds, and *in vivo* investigation needs to be done to validate the pharma logical benefits.

5. Conflicts of interest

The authors declare that they do not have any identifiable conflicting financial interests or personal relationships that might have influenced the findings presented in this paper.

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8. References

1. (2) Spectrometric identification of organic compounds, 7ed 2005 – silverstein, webster & kiemle | Request PDF. (n.d.). Retrieved September 10, 2024, from www.researchgate.net
2. Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology, 74(2), 113–123.
3. Alkowni, R., Jaradat, N., & Fares, S. (2023). Total phenol, flavonoids, and tannin contents, antimicrobial, antioxidant, vital digestion enzymes inhibitory and cytotoxic activities of Verbascum fruticulosum. European Journal of Integrative Medicine, 60, 102256.
4. Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances, 33(8), 1582–1614.
5. Awadelkareem, A. M., Al-Shammari, E., Elkhalifa, A. E. O., Adnan, M., Siddiqui, A. J., Snoussi, M., Khan, M. I., Azad, Z. R. A. A., Patel, M., & Ashraf, S. A. (2022). Phytochemical and In Silico ADME/Tox Analysis of Eruca sativa Extract with Antioxidant, Antibacterial and Anticancer Potential against Caco-2 and HCT-116 Colorectal Carcinoma Cell Lines. Molecules, 27(4).
6. Bakir Çilesizoğlu, N., Yalçin, E., Çavuşoğlu, K., & Sipahi Kuloğlu, S. (2022). Qualitative and quantitative phytochemical screening of Nerium oleander L. extracts associated with toxicity profile. Scientific Reports, 12(1), 1–16.
7. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79.
8. Bhandari, P., Kumar, N., Khan, S. M., & Bhutani, K. K. (2016). Online antioxidant activity and ultra-performance LC-electrospray ionisation-quadrupole time-of-fight mass spectrometry for chemical fingerprinting of Indian polyherbal formulations. Natural Product Research, 30(5), 530–535.
9. Chanda, R., Mohanty, J., Bhuyan, N., Kar, P., & Nath, L. (2007). Medicinal pants used against gastrointestinal tract disorders by the traditional healers of Sikkim Himalayas. Indian Journal of Traditional Knowledge, 6(4), 606–610.
10. Chhetri, D. R. (2004). Medicinal plants used as antipyretic agents by the traditional healers of Darjeeling Himalayas. Indian Journal of Traditional Knowledge, 3(3), 271–275.
11. Coates, J. (2000). Interpretation of Infrared Spectra, A Practical

Approach. Encyclopedia of Analytical Chemistry.

12. De Marino, S., Gala, F., Borbone, N., Zollo, F., Vitalini, S., Visioli, F., & Iorizzi, M. (2007). Phenolic glycosides from Foeniculum vulgare fruit and evaluation of antioxidative activity. Phytochemistry, 68(13), 1805–1812.
13. Durhan, B., Yalçın, E., Çavuşoğlu, K., & Acar, A. (2022). Molecular docking assisted biological functions and phytochemical screening of Amaranthus lividus L. extract. Scientific Reports, 12(1).
14. Dutta, D, Chhetri, B. R., Biswas, J., & Bhattacharyya, N. K. (2022). COVID-19 : A REVIEW. 15(2), 853–860.
15. Dutta, Deepjyoti, Bordoloi, M. J., & Bhattacharyya, N. K. (2021). Chemical constituents from Sarcostemma acidum (Roxb.) Voigt. Egyptian Journal of Chemistry, 64(9), 4815–4819.
16. Dutta, Deepjyoti, Narzary, B., Saikia, S., Tamuli, K. J., Bordoloi, M., Lhouvum, N., Nath, S., Sahoo, R. K., Gogoi, B., Saikia, S., & Bhattacharyya, N. K. (2022). Major chemical constituents from Illicium griffithii Hook. f. & Thoms of North East India and their cytotoxicity and antimicrobial activities. Turkish Journal of Chemistry, 46(5), 1468–1476.
17. Evans, S. M., & Cowan, M. M. (2016). Plant products as antimicrobial agents. Cosmetic and Drug Microbiology, 12(4), 205–231.
18. 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.
19. Green, K. (2009). Biodiversity Conservation in the Kangchenjunga Landscape. Mountain Research and Development, 29(3), 294.
20. K. Talapatra, S., Sengupta, S., & Talapatra, B. (1968). A new pentacyclic triterpene alcohol from evodia fraxinifolia hook f. Tetrahedron Letters, 9(57), 5963–5968.
21. Konappa, N., Udayashankar, A. C., Krishnamurthy, S., Pradeep, C. K., Chowdappa, S., & Jogaiah, S. (2020). GC–MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. Scientific Reports, 10(1), 1–23.
22. Kurt, D., Yalçin, E., & Çavuşoğlu, K. (2023). GC-MS and HPLC supported phytochemical analysis of watercress and the protective role against paraben toxicity. Environmental Science and Pollution Research International, 30(3), 6033–6046.
23. Makkar, H. P. S. (2003). Treatment of Plant Material, Extraction of Tannins, and an Overview of Tannin Assays Presented in the Manual. In Quantification of Tannins in Tree and Shrub Foliage.
24. McCune, L. M., & Johns, T. (2007). Antioxidant activity relates to

plant part, life form and growing condition in some diabetes remedies. Journal of Ethnopharmacology, 112(3), 461–469.

25. Mohamed, A. (2017). Evaluation of Antimicrobial Activity of Different Solvent Extracts of Saussurea Lappa. World Journal of Pharmacy and Pharmaceutical Sciences, 4(2), 12–18.
26. Mustafa, R. A., Hamid, A. A., Mohamed, S., & Bakar, F. A. (2010). Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants. Journal of Food Science, 75(1).
27. Narayanan, M., Chanthini, A., Devarajan, N., Saravanan, M., Sabour, A., Alshiekheid, M., Chi, N. T. L., & Brindhadevi, K. (2023). Antibacterial and antioxidant efficacy of ethyl acetate extract of Cymodocea serrulata and assess the major bioactive components in the extract using GC-MS analysis. Process Biochemistry, 124(July 2022), 24–32.
28. Neipihoi, Narzary, B., Saikia, S., Saikia, S., Tamuli, K. J., Sahoo, R. K., Dutta, D., & Bordoloi, M. (2021). Anticancer and antimicrobial compounds from Croton caudatus Gieseler and Eurya acuminata DC: Two edible plants used in the traditional medicine of the Kuki tribes. Natural Product Research, 35(24), 6025–6029.
29. Punetha, S., & Vuppu, S. (2023). GC–MS, FTIR and physico-chemical analysis of phytochemicals from Vellore floral waste and its in-silico studies. Chemical Papers, 77(11), 6873–6905.
30. Richardson, P. M., & Harborne, J. B. (1990). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Second Edition. Brittonia.
31. Sangma, S. R., Phukan, M. M., Chongloi, V., Verma, D. K., Bora, P., Kumari, S., & Pankaj, P. P. (2023). Phytochemical profiling, antioxidant and antimicrobial investigations on Viburnum simonsii Hook. f. & Thoms, an unexplored ethnomedicinal plant of Meghalaya, India. Future Journal of Pharmaceutical Sciences, 9(1).
32. Sbieh, R., Al-Lahham, S., & Jaradat, N. (2022). Antioxidant, antimicrobial and cytotoxic properties of four different extracts derived from the aerial parts of Chiliadenus iphinoides. European Journal of Integrative Medicine, 54(May), 102149.
33. Sharma, B. C. (2013). In vitro antibacterial activity of certain folk medicinal plants from Darjeeling Himalayas used to treat microbial infections. ~ 1 ~ Journal of Pharmacognosy and Phytochemistry, 2(4), 1–4. www.phytojournal.com
34. Silva, C. G., Herdeiro, R. S., Mathias, C. J., Panek, A. D., Silveira, C. S., Rodrigues, V. P., Rennó, M. N., Falcão, D. Q., Cerqueira, D. M., Minto, A. B. M., Nogueira, F. L. P., Quaresma, C. H., Silva, J. F. M., Menezes, F. S., & Eleutherio, E. C. A. (2005). Evaluation of antioxidant activity of Brazilian plants. Pharmacological Research, 52(3), 229–233.

35. Stuart, B. H. (2005). Infrared Spectroscopy: Fundamentals and Applications. Infrared Spectroscopy: Fundamentals and Applications, 1–224.
36. Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules 2009, Vol. 14, Pages 2167-2180, 14(6), 2167–2180.
37. Villaño, D., Fernández-Pachón, M. S., Moyá, M. L., Troncoso, A. M., & García-Parrilla, M. C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta, 71(1), 230–235.