NBUJPS
NBU Journal of Plant Sciences

ISSN No. 0974-6927
Vol. 16 (2024), pp. 74-89

Research Article

Evaluation of anti-bacterial potential and characterization of phytoconstituents of leaf extracts of *Anisomeles indica* (L.) Kuntze, a pharmacologically important herb from Terai-Duars region of West Bengal

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Abstract

Medicinal plants have been a great source of herbal drugs, and with the advent of biotechnology, enormous novel herbal compounds have now been identified that may have a vast application in the pharmaceutical industry. Anisomeles indica (L.) Kuntze is an important medicinal plant found growing in the wild in the Terai Duars region of North Bengal, belonging to the family Lamiaceae. Although some of the studies have suggested the potential application of this plant extract for the treatment of bacterial diseases, none of the studies have studied the chemical composition of leaf extracts in great detail. *In lieu* of this, the present work has been framed to evaluate the phytochemical composition of leaf extracts of A. indica in two different solvents, i.e. ethyl acetate and ethanol, and the qualitative screening for the presence of various phytochemicals namely terpenoids, carbohydrates, steroids, flavonoids, alkaloids, phenols, tannins, cardiac glycosides, saponins, and lignins. Additionally, the antioxidant potential of ethanolic leaf extract was studied by analyzing DPPH activity and ABTS assay. The freehand sections of fresh *A. indica* leaves were carried out, and localization of the bioactive phytoconstituents inside the leaf was highlighted. One of the major approaches followed in this work was the evaluation of the antibacterial activity of leaf extracts, and it was found that ethanolic leaf extracts were highly effective against all tested bacterial strains (Bacillus subtilis ATCC 11774, Salmonella typhimurium ATCC 25241, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 11229). Further, GC-MS profiling of the ethanolic extract confirmed the presence of a total of 15 compounds, of which 13 were found to be biologically active.

Article info

Received **21 September 2025**
Revised **23 December 2025**
Accepted **29 December 2025**

Keywords: Anti-bacterial, Anti-microbial, Anti-oxidant, Secondary metabolites, Phytochemistry

DOI: ***https://doi.org/10.55734/NBUJPS.2024.v16i01.010***

Introduction

In the present scenario, a large number of pharmaceutical drugs have been employed for the treatment of many human pathogenic diseases. Although in the short term, these drugs are highly effective, their consistent use has a significant effect on human health. Due to which herbal medicines have gained utmost importance due to their targeted action and fewer side effects in curing various diseases and ailments. Medicinal plants are an excellent source of herbal medicines as they constitute a wide variety of secondary compounds that have bioactive properties such as anti-oxidant, anti-inflammatory, anti-microbial, anti-tumor etc. Thus, plant-derived phytochemicals are of great significance with reduced side effects against a wide range of microorganisms (Azalework et al., 2017). These phytochemicals have diverse functional properties that assist them in combating with different pathogenic microorganisms. A number of such phytochemicals have been isolated from variety of medicinal plants, such as phenolics, alkaloids and terpenoidal compounds. All these secondary

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metabolites possess strong anti-oxidant activity that provides resistance to attack from a number of pathogenic microorganisms. Apart from their role in biotic stress alleviation, these secondary metabolites also augment plant growth and development under different abiotic stresses, such as drought, salinity, and high temperature.

Most of the research studies in the current scenario have focused on the detection of novel secondary compounds from plants that have significant importance in herbal drugs. Extraction of phytochemical compounds from plant tissues is carried out by the utilization of different solvents such as methanol, ethyl acetate, DMSO, etc. This variation in solvent for the extraction of phytochemicals depends upon the nature of the compound to be isolated. It has been observed that different researchers have utilized different solvents for the isolation of phytochemicals from the same type of tissue, but in different plant species. Therefore, it is crucial to select the solvent beforehand for the isolation of particular phytochemicals. As is evident, these secondary metabolites occupy different plant tissues and vary in anatomical location in various plant tissues. Previous histochemical studies have also confirmed the accumulation of bioactive secondary metabolites within the tissues of plant parts examined under microscopes using different stains, thus contributing greatly to the isolation process of phytochemicals (Badria et al., 2019).

Gas chromatography-mass spectrometry (GC-MS) is a combined technique that plays a vital role in phytochemical analysis for its precision in identifying the bioactive secondary metabolites present in the leaf extracts (Starlin et al., 2019). A plethora of studies have stated the application of GC-MS in the identification and separation of bioactive secondary compounds from different plant extracts. Correspondingly, the GC-MS analysis demonstrates the presence of different functional groups/moieties in extracts from a multitude of plant extracts that have no prior information available in the literature. And as such, these compounds can be purified, characterized and may be further tested in different plant and animal models for their functional traits.

With this background, the present work has been designed for the isolation and characterization of phytochemicals from an important medicinal plant, *A. indica* (L.) Kuntze, commonly known as Indian catmint. The plant is an aromatic perennial herb that belongs to the family Lamiaceae, native to Asia. In India, it is predominantly found in the north and northeastern regions during the months of March to October and has been used as folk medicine for years (Antil et al., 2019a). As a traditional medicine, the leaves of *A. indica* were used to treat toothache, snakebite, paralysis, and epilepsy. The whole plant is also identified for its anti-inflammatory, anti-oxidative, anti-bacterial, analgesic, and herbicidal properties (Dharmasiri et al., 2003; Kundu et al., 2013). The biological activity of the extracts indicated the presence of phytochemicals such as diterpenoids, flavonoids, steroids and terpenoids (Basappa et al., 2015; Antil et al., 2019b). The increase in the resistance potential of antimicrobials demands the exploration of more plants of medicinal importance, which may provide significant results due to the presence of various bioactive compounds (Ismail et al.,, 2020). Antil et al., (2019b) have shown the inhibitory activity of its phyto-constituent against pathogenic bacteria Helicobacter pylori. The plant has also been found to be effective as an anticancer agent (Bich-Loan et al., 2021). The histochemical study of the leaf section of *A. indica* has provided strong evidence for the presence of polyphenols, terpenoids, tannins and alkaloids (Sathyaand Phawa, 2018). GC-MS analysis of the methanolic extract of A. indica indicated the presence of different phytoconstituents (Nasrin et al.,, 2022).

To our knowledge, this is the first complete report documenting the major micromorphological aspects of *A. indica* along with the chemical composition in the various leaf structures from the plants growing in this region of India. This allows us to identify functional and structural aspects of tissues, such as metabolite localization, investigate enzyme activity of secondary metabolites, explore cellular specialization, and study environmental influences. Moreover, advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) have facilitated the identification of diverse secondary compounds with biological activities such as anti-bacterial, anti-oxidant, anti-cancer, anti-inflammatory, etc. The work will lay the foundation for the isolation of novel metabolites from the leaf extracts that will play an important role in the development of pharmaceutically active herbal drugs against microbial diseases with no or little side effects.

Materials and Methods

Fresh and healthy leaves of *A. indica* (L.) were harvested from the campus of the University of North Bengal (NBU) (26.7095° N, 88.3542° E) during July and August of Year 2022 and stored at

4° C for further use. Further, running tap water was used to remove impurities from the surface of the collected leaf samples. The leaf samples were washed and rinsed with double-distilled water (ddH_2O) followed by 70% ethanol for 1 min. The leaf samples were further dried in a hot air oven until no moisture could be observed. The leaves were then pulverized using a mortar and pestle to prepare fine powder (Baskaran et al., 2012). The powder was dissolved in two diverse solvents (mainly ethanol and ethyl acetate) in a 1:10 (w/v) ratio and kept at 4° C until further use. The prepared extracts were centrifuged at 9000 rpm for 10 min. The supernatant was collected and dried with the help of a rotary evaporator at 40° C to prepare a crude extract. The extracted residue was re-dissolved in ethanol and ethyl acetate to prepare the stock solution and stored for further analysis.

Phytochemical evaluation of the leaf extracts was qualitatively assessed according to the standard protocols for tannins (Ugochukwu et al., 2013), terpenoids (Rajesh et al., 2014), alkaloids (Ugochukwu et al., 2013), flavonoids (Shaikh and Patil 2020), phenols (Rajesh et al., 2014), carbohydrates (UC and Nair 2013), cardiac glycosides (Zahoor et al., 2020), steroids (Antil et al., 2019), saponins (Devmurari 2010), and lignins (Bhatt and Dhyani 2012).

The total phenolic content in the leaf tissue extracts was determined by the Folin-Ciocalteu spectrophotometric method by Singleton and Rossi (1965). A standard curve was prepared with a gallic acid solution (20 to160 µg ml^{-1}). To 1 ml of extract was added 1 ml of 95% ethanol, 5 ml of distilled water, and 0.5 ml folin ciocalteu solution. After 5 minutes, 1 ml of 5% Na_2CO_3 was added to the solution mixture. Then, the mixture was incubated for 1 h, and the absorbance was measured in the spectrophotometer at a wavelength of 725 nm.

The aluminium chloride ($AlCl_3$) colorimetric method was adopted for evaluation of the total flavonoid content of the plant extracts (Durai et al., 2016). 1 ml of leaf extract and 4 ml dH_2O were mixed in a test tube and after 5 minutes, 0.3 ml of 5% $NaNO_2$ and 0.3 ml of 10% $AlCl_3$ were added to this mixture and mixed thoroughly. Again, after 5 min, 2 ml of 1M NaOH was added to the mixture, and the entire content was diluted with dH_2O to make a final volume of 10 ml. A standard curve was developed with quercetin solution (20 to 100 µg ml-1) for the calculation of total flavonoids. The readings of the absorbance were recorded in a spectrophotometer at a wavelength of 510 nm.

Total tannin content was examined by folin-ciocalteu spectrophotometric method by Durai et al., (2016). In a test tube, 0.1 ml extract along with 7.5 ml of dH_2O, 0.5 ml Folin- Ciocalteu reagent and 1 ml of 35% Na_2CO_3 were taken and mixed well. The entire mixture was diluted with 10 ml dH_2O and shaken well. Further, the absorbance values were recorded at 725 nm in a spectrophotometer after incubating the mixture for 30 min. Gallic acid (with a concentration of 20 to 600 µg ml^{-1}) was used as a standard for the determination of total tannin content.

The DPPH assay was executed following the standard protocol of Hsu et al., (2007) with some modifications. However, 1.5 ml of various concentrations of leaf extracts (10 to 100 µg ml-1) and 1.5 ml of 0.1mM DPPH solution were mixed in a test tube. The mixture was then incubated for 30 min in the dark, and the reduction of DPPH free radical was calculated by measuring the absorbance of a solution at 517 nm in a UV-VIS spectrophotometer. The control was prepared without adding the extract. Ascorbic acid, a natural antioxidant, was used as a standard. The experiment was carried out in three replicates, and the percentage inhibition of DPPH free radicals was calculated as follows

$$\text{Inhibition (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} * 100$$

where A_Control is the absorbance of the control, and A_Sample is the absorbance of the sample.

ABTS assay is associated with the ability of the sample to scavenge (2, 2′ azino-bis ethylbenzthiazoline-6-sulfonic acid $ABTS^{+}$) radical. Preparation of the ABTS solution was done by mixing 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$). The solution was allowed to stand for 4-16 h until the reaction was complete, and the solution was diluted with ethanol to record an absorbance of 0.700 ± 0.05 at 734 nm in a UV-VIS spectrophotometer (Re et al., 1999). The leaf extract (0.3 ml) of different concentrations (10 to 100 µg $m-^{1}$) was taken in a test tube to which 2.7 ml ABTS solution was added and mixed well. The solution without extract was considered a control. The experiment was carried out in triplicate. Moreover, the antioxidant activity of the sample was evaluated through identify the decrease in the absorbance at different concentrations by using the equation

$$\text{Inhibition (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} * 100$$

where A_Control is the absorbance of the control, and A_Sample is the absorbance of the sample.

Fresh young leaves of *A. indica* were collected and examined by cross-sections using surface preparations. The cut leaves were stained with suitable reagents to detect the presence of phytochemicals in different parts of the leaves. To check the presence of alkaloids, the sections were stained with Dragendroff's reagent and washed with dH_2O (Gomez et al., 2019). For lignins, the sections were stained with safranin and washed with 50% alcohol after 15 min (Demarco, 2017; Gangaram et al., 2020). Similarly, to study terpenoids, the sections were stained with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl (Boix et al.,, 2011). To detect the presence of phenols, the sections were stained with FeCl3 (Gangaram et al., 2020), and the excess stains were washed with dH_2O. Tannins were localized by staining the leaf sections with a 10% potassium bichromate (K2Cr2O7) solution (Muravnik and Shavarda 2012). After mounting the coverslips, the slides were observed under a microscope and analyzed.

The anti-bacterial potential of leaf extracts was examined using the agar well diffusion method (Baskaran et al., 2012). Four bacterial strains, two gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 11774) and two gram-negative (*Salmonella typhimurium* ATCC 25241; Escherichia coli ATCC 11229) strains were used as test organisms against the activity of extracts. The culture was grown in fresh nutrient broth (NB) media and incubated for 72 h at 37 °C and used as a source of inoculum for the experiment. The culture was swabbed uniformly on nutrient agar plates, and wells were filled with 30 µl each of leaf extract (200 mg ml^{-1} and 100 mg ml^{-1}). Streptomycin was selected as a standard positive control, ethanol, and ethyl acetate as a negative control. After a 48-hour incubation period at 37°C, the inhibition zone diameter (IZD) was measured to assess the antibacterial activity.

Based on the observations from both qualitative and quantitative phytochemical assays, the ethanolic extract was then selected to perform a GC-MS evaluation for the identification of bioactive compounds. The analysis was accomplished using an Agilent 8890 GC system coupled with a 5977 MSD. The compounds were separated on an HP-5ms ultra-inert capillary column (30m×250µm×0.25µm). The initial oven temperature was maintained at 75°C withhold time of 0.5 min. MS operating parameters, such as the ion source temperature and quad temperature, were maintained at 230°C and 150°C, respectively, and the separation was carried out at 70 eV in electron ionization (EI) mode. The total run time of GC was 53.5 min. The oven temperature was augmented from 75°C to 350°C. Further, Helium was explored as the carrier gas with a split flow of 6 ml min^{-1}, which corresponded to a split ratio of 5:1. The identification of compounds analyzed by GC-MS was further determined by associating the MS spectrum patterns with the standard mass spectra accessible at the National Institute of Standards and Technology (NIST) Mass Spectra Database.

Results

Phytochemical screening of leaf extracts of A. indica was performed using two different solvents and the presence of various phytochemicals was noticed in two separate solvents (Figure 1).

Among the phytochemicals screened, terpenoids, carbohydrates and steroids were found to be present in leaf extracts of both the solvents while, tannins, saponins, alkaloids, flavonoids, lignins, phenols and cardiac glycosides were present only in the ethanolic extract (Table 1).

Table 1. Screening for the presence of phytochemicals in leaf extracts of *A. indica* prepared in two different solvents

Phytochemicals	Test	Ethanol	Ethyl acetate
Saponins	Foam test	+	-
Alkaloids	Wagner's test	+	-
Flavonoids	Lead acetate test	+	-
Phenols	Folin test	+	-
Tannins	-	+	-
Cardiac glycosides	Keller-Killani test	+	-
Lignins	Labat test	+	-
Terpenoids	Salkowski test	+	+
Carbohydrates	Molisch's test	+	+
Steroids	-	+	+

'+' indicates presence '-' indicates absence of phytochemical in extract.

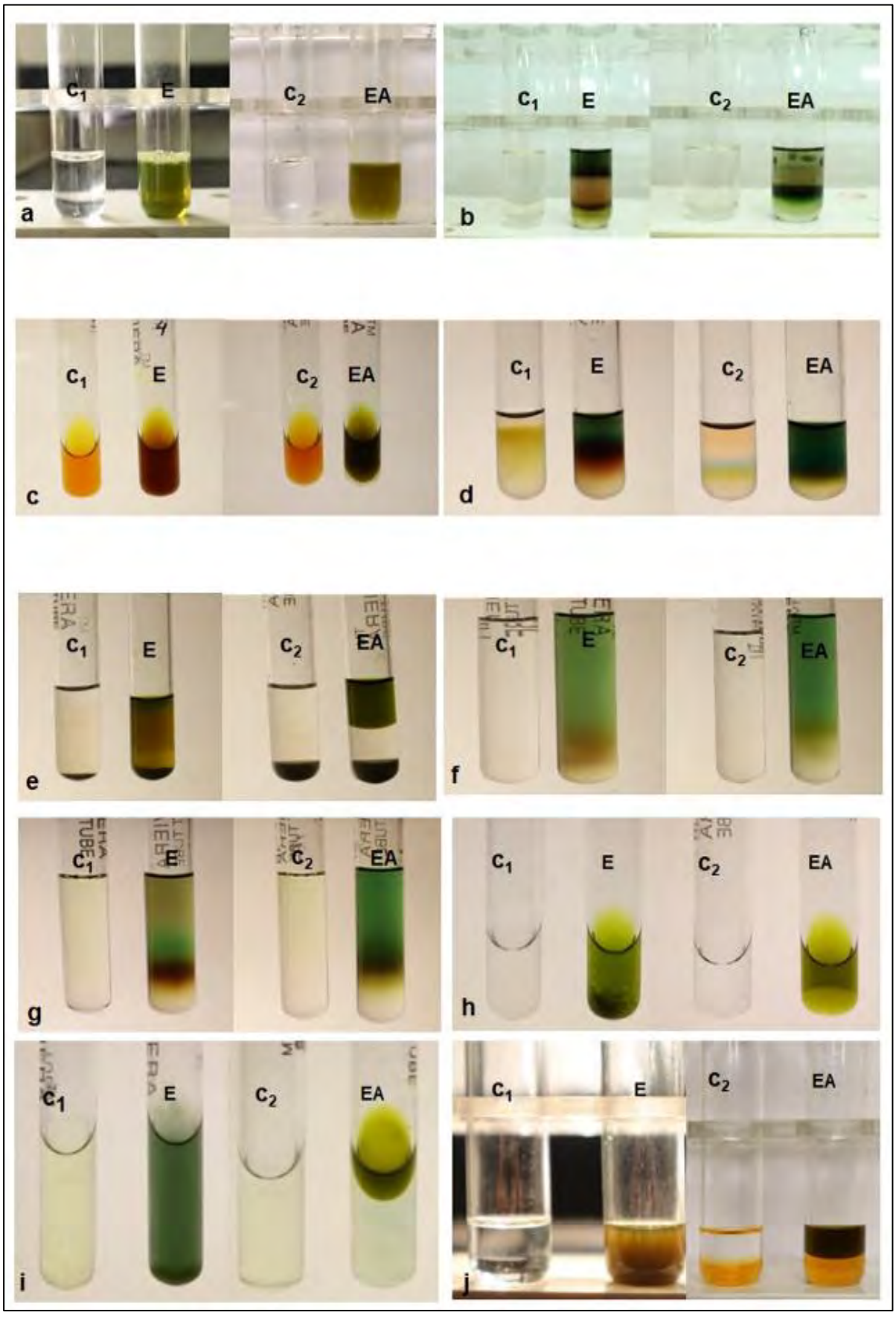

Figure 1. Phytochemical screening of leaf extract of *A. indica*. C_1= ethanolic control, E= ethanolic extract, C_2=ethyl acetate control, EA= ethyl actetate extract.

The quantitative assay of leaf extract prepared in ethanol exhibited higher flavonoid content (1.15 ± 0.07 mg ml^{-1}) as compared to ethyl acetate (0.083 ± 0.03 mg ml^{-1}) (Table 2). In the case of tannins, a concentration of 0.66 ± 0.013 mg ml^{-1} was achieved with ethanol, but ethyl acetate only resulted in 0.12 ± 0.03 mg ml^{-1} (Table 2). Similarly, higher phenol content was observed in leaf extract prepared in ethanol (0.103 ± 0.003 mg ml^{-1}) as compared to leaf extract prepared in ethyl acetate (0.024 ± 0.001 mg ml^{-1}) (Table 2). The results showed that the total phenols, total flavonoids, and total tannins contents differed among the two leaf extracts, and high occurrence was observed in the leaf extract prepared in ethanol.

Table 2. Quantitative estimation of phytochemicals content in leaf extracts of *A. indica* prepared in two different solvents.

Phytochemicals	Ethanolic extract (mg ml^{-1})	Ethyl acetate extract (mg ml^{-1})
Total phenolic content	0.103 ± 0.003	0.024 ± 0.001
Flavonoid content	1.15 ± 0.07	0.083 ± 0.03
Tannins	0.66 ± 0.013	0.12 ± 0.03

The values represent is a mean of three replicates and are presented as mean ± SD

The antioxidative potential of *A. indica* was determined spectrophotometrically through the DPPH reduction assay. The inhibition concentration (IC_{50}) parameter is the amount of sample necessary to decrease the absorbance of DPPH by 50%. It was used to calculate the anti-oxidative activity of the extract using the inhibition curve. Results showed that the antioxidant activity of ethanolic extract of A. indica in terms of IC_{50} was found at the concentration of 34.791 µg ml^{-1} (Fig. 2a). The scavenging effect can be attributed to the presence of active phytoconstituents in the ethanolic leaf extract of *A. indica*.

The antioxidative activity of the ethanolic leaf extract was determined in terms of IC_{50} from the inhibition curve through the ABTS reduction assay. The change in absorbance was plotted against the concentration of the sample to calculate the IC_{50} value. The antioxidant activity of the ethanolic extract in terms of IC_{50} was found at the concentration of 84.019 µg ml^{-1}, indicating the presence of bioactive compounds contributing to its anti-oxidative property (Figure 2b).

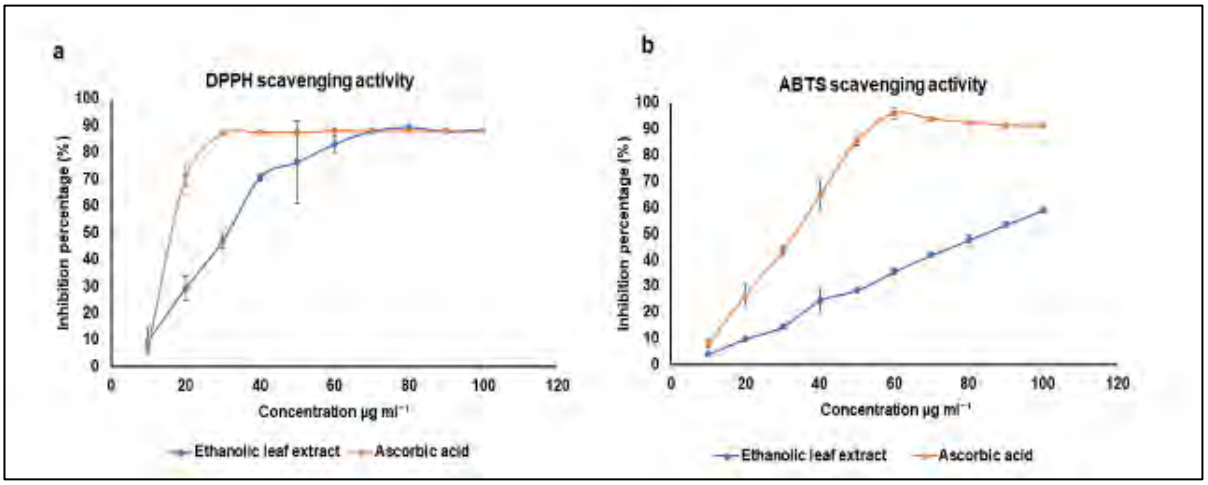

Figure 2. Antioxidant activity of leaf extract of *A. indica* relative to that of ascorbic acid. (a) DPPH scavenging radical assay; (b) ABTS scavenging activity

The freehand sections of fresh *A. indica* leaves using different reagents highlighted the localization of the bioactive phytoconstituents inside the leaf (Table 3). The results showed the presence of alkaloids in the cuticle layer, palisade cells, and trichomes when stained with Dragendroff's reagent (Figure 3a-c). The histochemical presence of lignins was observed in trichomes (Figure 3d,f) and xylem (Figure 3e) in leaf sections through staining with safranin. On the contrary, terpenoids in leaf sections were noticed in trichomes and xylem when stained with 2, 4-Dinitrophenylhydrazine (Figure 3g-i). Phenols were present in trichomes and cuticles through staining with FeCl3 (Figure 3j-l). When stained with 10% potassium dichromate for the detection of tannins, the leaf sections showed accumulation in leaf trichomes (Figure 3m-o). The ethanolic and ethyl acetate leaf extracts were analyzed for anti-bacterial activity against both gram-positive (*Bacillus subtilis* ATCC 11774; *Staphylococcus aureus* ATCC 25923) and gram-negative bacteria (*Escherichia coli* ATCC 11229; *Salmonella typhimurium* ATCC 25241) (Figure 4). Both the leaf extracts showed inhibitory potential against these strains of bacteria, with the ethanolic extract being more prominent (Table 4). The highest inhibition of 25.66 mm was recorded against E. coli ATCC 11229, followed by 18 mm, 14.66 mm, and 12.66 mm against *S. Typhimurium* ATCC 25241, *S. aureus* ATCC 25923 and *B. subtilis* ATCC 11774, respectively, by the ethanolic extracts (Table 4). The ethyl acetate extract showed activity against E. coli ATCC 11229, showing the highest inhibition of 24.66 mm, followed by 17 mm, 11 mm and 8.66 mm against *S. typhimurium* ATCC 25241, *S. aureus* ATCC 25923 and *B. subtilis* ATCC 11774, respectively (Table 4). The extraction capacity of organic solvents may have produced active compounds, thus enhancing the antibacterial activity.

GC-MS analysis of the ethanolic leaf extract of *A. indica* revealed the presence of total 15 compounds (phytochemical constituents), of which 13 were found to be potentially active in relation with the medicinal property of the plant (Fig. 4a). The major bioactive compounds present in the ethanolic leaf extract of *A. indica* were found to be squalene, γ-sitosterol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, neophytadiene, α-tocopheryl acetate, phytol, 9,12-octadecadienoic acid, ethyl ester, 9,12,15-octadecatrienoic acid,ethyl ester,(Z,Z, Z)-, hexadecanoic acid, 2- hydroxyl-1-(hydroxyl methyl) ethyl ester, 9,12,15-Octadecatrienoic acid,2,3-dihydroxypropylester, .psi.,.psi.-carotene,1,1',2,2'-tetrahydro-1,1' dimethoxy-, methyl glycocholate, 3 TMS derivative and hexadecanoic acid, ethyl ester (Table 5). The identification and characterization of the 15 compounds were done by comparing data with the NIST library and categorized based on their biological activity, peak area, retention time, and class (Table 5). Maximum compounds were found to possess bioactivity, including anti-bacterial, anti-oxidant, anti-cancer, and anti-inflammatory (Figure 4b).

Table 4. Screening for antibacterial activities of two different leaf extracts of *A. indica*

Solvent used	Concentration (mg ml^{-1})	Zone of inhibition (mm ± SD)			
		***Staphylococcus aureus* ATCC 25923**	***Salmonella typhimurium* ATCC 25241**	***Bacillus subtilis* ATCC 11774**	***Escherichia coli* ATCC 11229**
Ethanol	**100**	12.66 ± 0.57	12.70 ± 0.60	5.66 ± 0.57	25.66 ± 0.57
	200	14.66 ± 0.57	18 ± 1	12.66 ± 0.57	25.66 ± 0.57
Ethyl acetate	**100**	11.66 ± 0.57	12.66 ± 0.57	8.33 ± 0.57	24.66 ± 1.52
	200	11 ± 1	17 ± 1	8.66 ± 0.57	11.33 ± 0.57

The values represent zone of inhibition (mm) and are presented as mean ± SD.

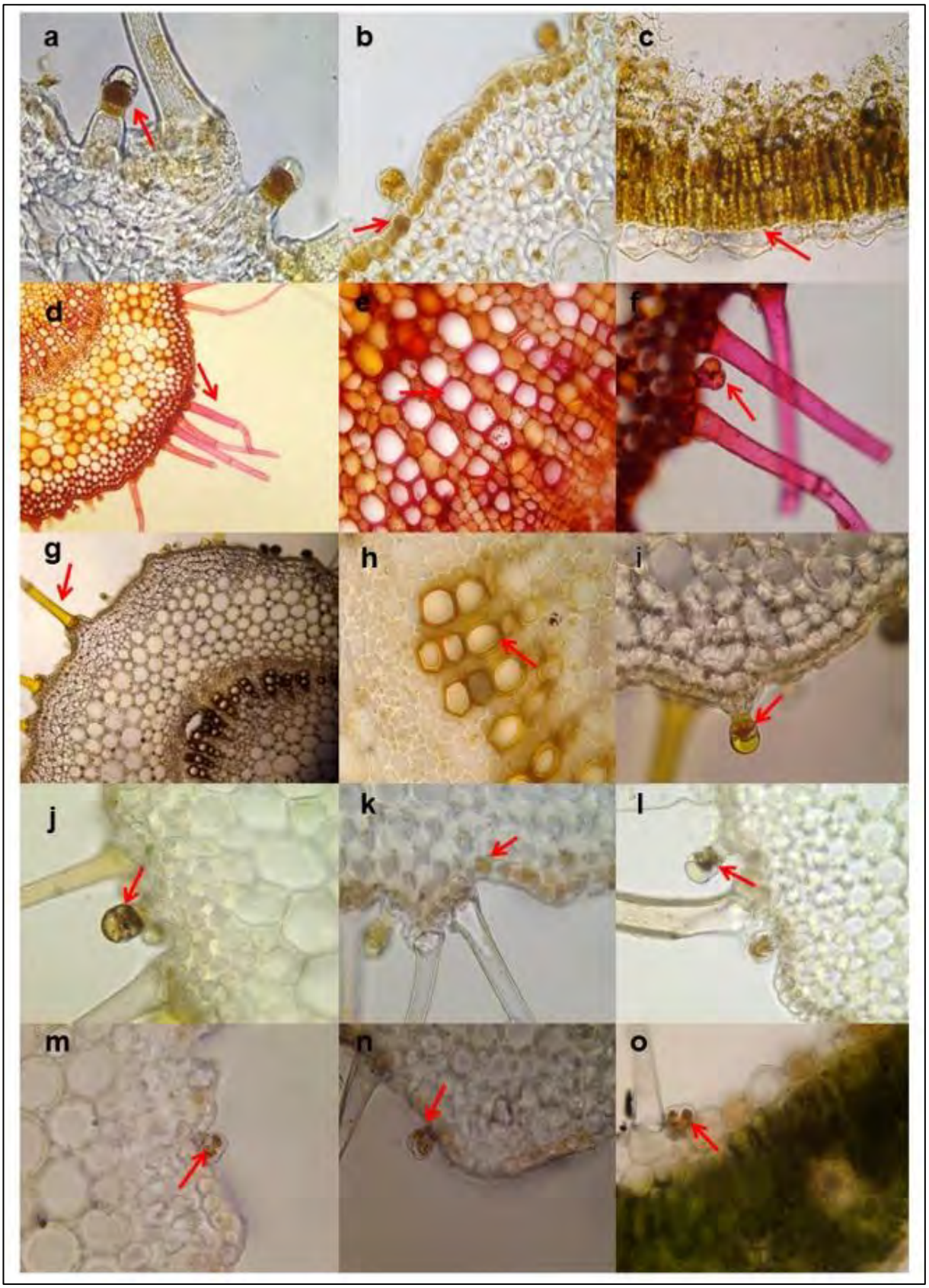

Figure 3. Transverse sections of histochemical stained leaves of *A. indica*. Dragendroff's reagent for alkaloids-stained orange in trichomes, cuticle and palisade cells (a, b, c); safranin for lignins stained pink in trichomes and xylem (d, e, f); DNPH stained orange brown for terpenoids in trichomes and xylem (g, h, i); $FeCl_3$ for phenols-stained dark brown in trichomes and cuticle (j, k, l); 10% potassium bichromate for tannins-stained brown in trichomes (m, n, o).

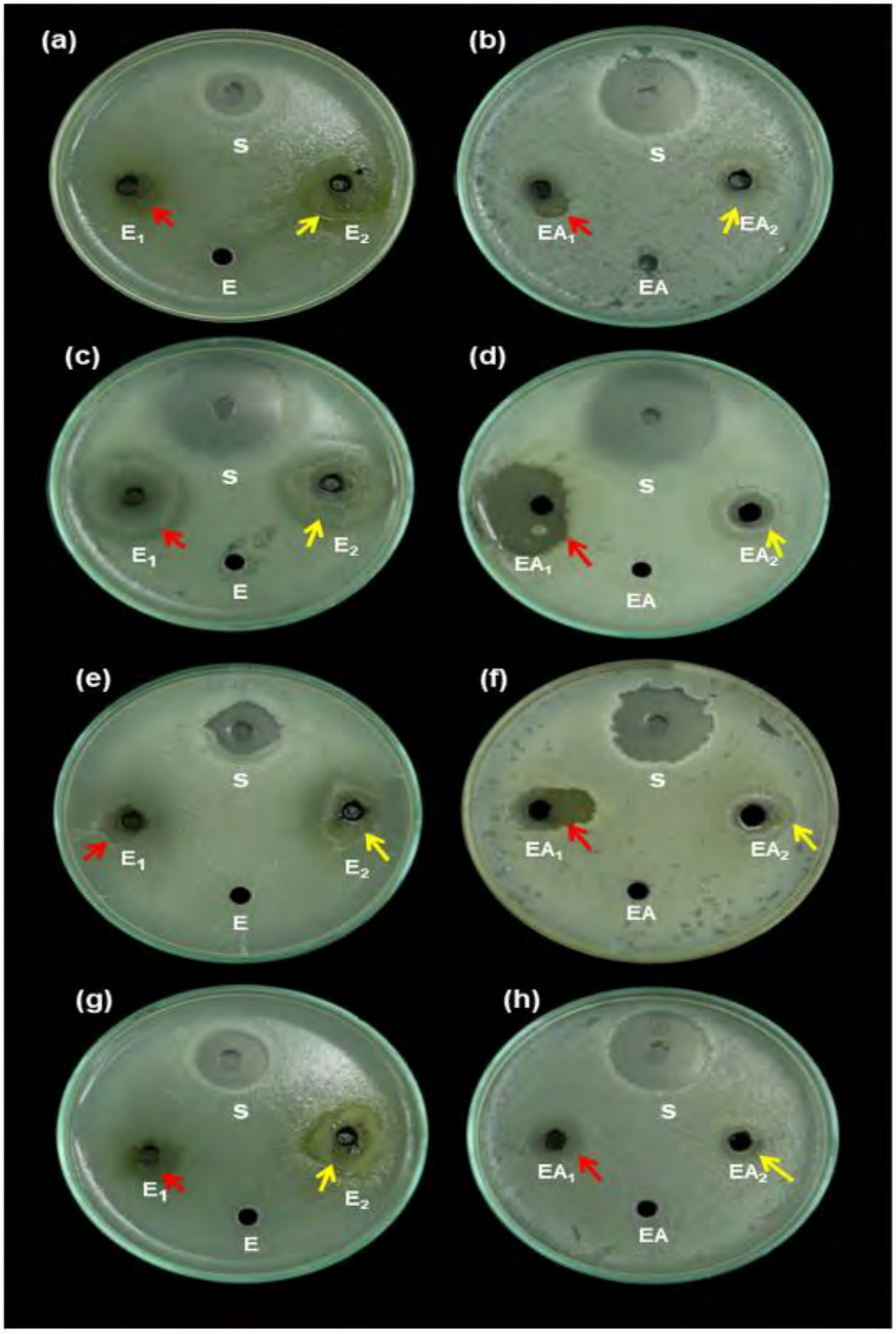

Figure 4. Antibacterial activity of ethanolic and ethyl acetate leaf extracts of *A. indica* against *Bacillus subtilis* ATCC 11774 (a), (b); *Escherichia coli* ATCC 11229 (c), (d); *Salmonella typhimurium* ATCC 25241 (e), (f) and *Staphylococcus aureus* ATCC 25923 (g), (h). S= streptomycin (positive control), E_1= ethanolic extract (100 mg ml^{-1}), E_2= ethanolic extract (200 mg ml^{-1}) E= ethanol (negative control), EA_1= ethyl acetate extract (100 mg ml^{-1}), EA_2= ethyl acetate extract (200 mg ml^{-1}), EA= ethyl acetate (negative control).

Table 5. GC-MS analysis of leaf extracts of *A. indica* prepared in ethanol for the identification of biologically active compounds

Compound name	RT (min)	Peak area %	Category	Major class	Activity	References
Neophytadiene	23.56	0.99	Sesquiterpenoids	Terpenoids	Anti-bacterial, Anti-oxidant	(Swamy et al., 2017)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	24.791	0.37	Diterpenes	Terpenoids	Anti-oxidant, Anti-inflammatory, Anti-bacterial	(Ismail et al., 2019; Nithya et al., 2018)
Hexadecanoic acid, ethyl ester	27.848	0.41	Ethyl ester	Palmitic acid ester	Anti-oxidant	(Parthipan et al., 2015)
Phytol	30.58	6.82	Acyclic diterpenoids	Terpenoids	Anti-cancer, Anti-bacterial	(Sermakanni and Thangapandian 2012)
9,12-Octadecadienoic acid, ethyl ester	31.611	0.54	Lineolic acid ethyl ester	Fatty acid	Anti-oxidant, Anti-inflammatory	(Parthipan et al., 2015)
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	31.761	1.4	Linolenic acid ethyl ester	Fatty acid	Anti-inflammatory, Cancer preventive	(Guerrero et al., 2017)
Hexadecanoic acid,2-hydroxy-1-(hydroxyl methyl)ethyl ester	37.931	1	-	Amino compound	Anti-oxidant	(Tyagi and Agarwal 2017)
9,12,15-Octadecatrienoic acid,2,3-dihydroxypropyl ester	40.838	1.33	-	-	Anti-inflammatory	(Idan et al., 2015)
Squalene	42.719	1.65	Triterpene	Terpenoids	Anti-oxidant, Anti-bacterial, Anti-inflammatory, Cancer preventive.	(Sermakanni and Thangapandian 2012)
α-Tocopheryl acetate	46.801	0.87	Vitamin E compounds	-	Anti-inflammatory, Anti-bacterial	(Adnan et al., 2019)
.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	48.358	0.4	-	Carotene	Anti-oxidant, Anti-cancer	(Khan et al., 2019)
γ-Sitosterol	49.083	4.91	Sterol lipids	-	Anti-viral, Anti-inflammatory, Anti-bacterial	(Adnan et al., 2019)
Methyl glycocholate,3 TMS derivative	50.164	0.52	-	-	Anti-oxidant	(Masek et al., 2020)
3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	32.799	0.35	-	-	Unknown	-
5HCyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy) -3-	44.357	51.54	-	-	Unknown	-

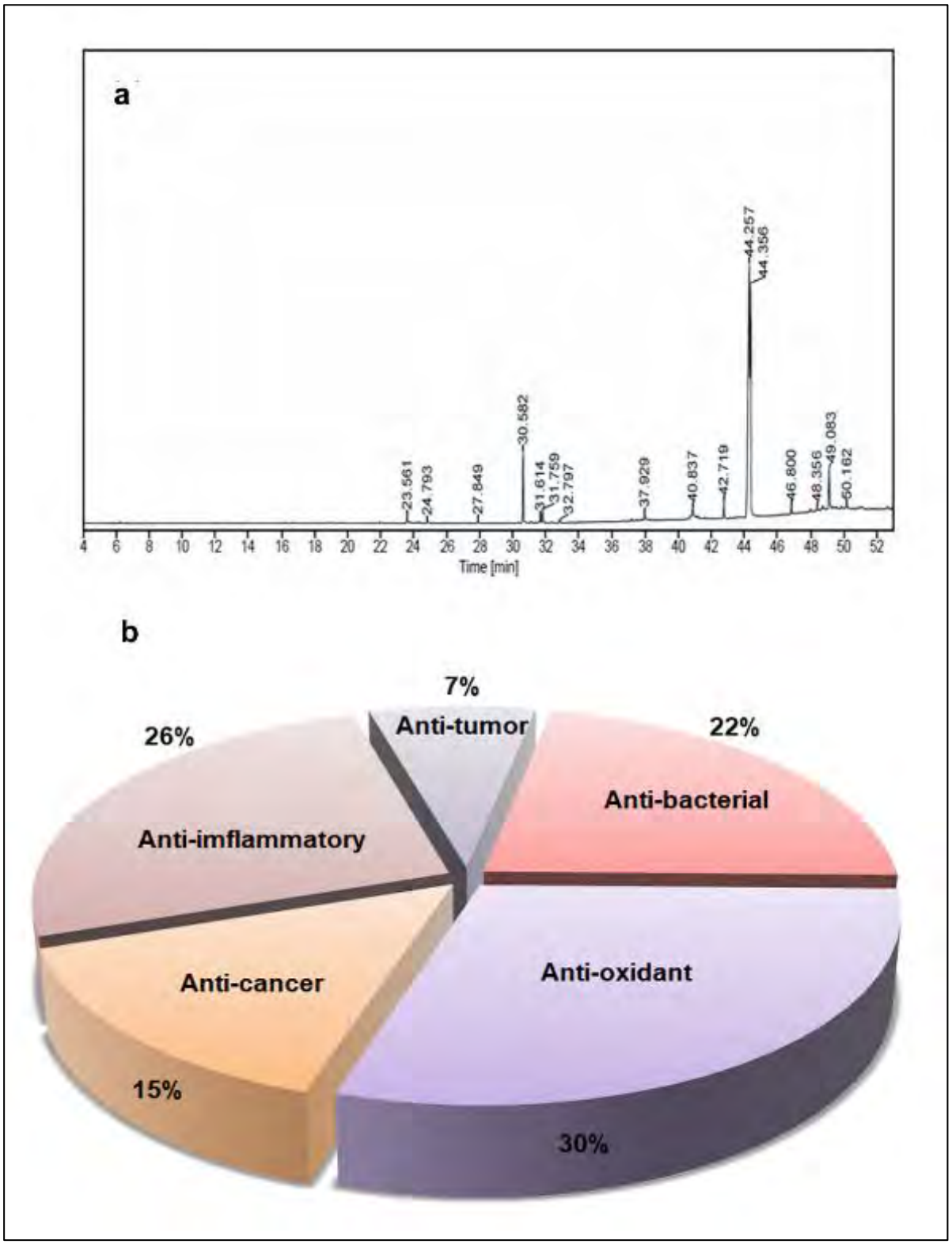

Figure 5 (a) GC-MS chromatogram of ethanolic leaf extract of *A. indica;* (b) Characterization of compounds of leaf extract of *A. indica* in terms of biological activity.

Discussion

Anisomeles indica (L.) Kuntze belonging to the family Lamiaceae, is reported to be medicinally important (with anti-inflammatory, anti-bacterial, and anti-oxidative activities (Antil et al., 2019). Medicinal plant and their secondary metabolites are the key factors for discovering and developing novel therapeutic agents (Nasrin et al.,, 2022). The therapeutic value of plants is considered to be of vital importance due to their significant uses as a primary source of bioactive constituents that could contribute to the making of novel drugs (Azwanida 2015; Islam et al., 2021). The present study investigated the key phytochemical components of the medicinal herb

A. indica and carried out their biochemical characterization.

In the present study, the qualitative phytochemical analysis revealed the presence of various phytochemicals, including flavonoids, phenols, alkaloids, terpenoids, steroids, carbohydrates, saponins, tannins, cardiac glycosides and lignins in the leaf extracts of *A. indica*. However, maximum phytochemicals were detected in the ethanolic extract, while only minor amounts were detected in the ethyl acetate extract. These phytochemicals may contribute to the pharmacological properties of leaf extracts of *A. indica*. Literature shows that plant antimicrobials have promising results with reduced side effects (Azalework et al., 2017). The phytochemicals detected with phytochemical screening have been previously reported for their biological properties. For instance, steroids function as insecticidal and antimicrobial agents and have been used in herbal medicines. Tannins have antimicrobial activity and inhibit the growth of microorganisms, including fungi, yeasts, bacteria, and viruses. Tannins exist in many plant foods as precipitated polyphenols are responsible for their remarkable antioxidant activity (Prasad et al., 2008; Gulcin et al., 2010). Flavonoids are known to be synthesized in response to microbial infections and have been found to act as effective antimicrobial agents against a wide range of microorganisms. Also, flavonoids have anti-inflammatory and antimicrobial properties (Kamaraj et al., 2020). Terpenoids are one of the compounds responsible for the fragrance of plants containing isoprene units. They are potent against various bacteria, viruses and protozoa (Cowan 1999). Saponins are known for their anti-inflammatory, anticancer, and antioxidant properties (Arulmozhi et al., 2018; Tambe et al., 2021). Similarly, phytochemicals such as alkaloids, flavonoids, and glycosides have been found to be associated with medicinal uses for centuries for their biological activities, including anti-inflammatory, antioxidant, anticancer, and antimicrobial (Dash et al., 2017). The quantitative analysis of phytochemicals resulted in a high occurrence of flavonoids, followed by phenolics and tannins. Literature reported that phenols have been found to possess antioxidant activity for their redox properties (Farag et al., 2020), which might have contributed to the antioxidant properties of the ethanolic extract in the present study. All these phytochemicals that were detected in the ethanolic extract of *A. indica* might have contributed majorly to the anti-bacterial activities of this medicinal herb in the present study.

The work showed the anti-oxidative properties of the leaf extracts, and it has been well observed that among the various plant constituents, flavonoids and phenols are considered as strong free radical scavenging compounds and thus termed as indicators of free radical scavenging activity. DPPH radical scavenging activity is known to be the most appropriate procedure to assess the antioxidant activity of plant extracts by Abbas et al., (2021). Simultaneously, ABTS is another antioxidant assay that determines the radical scavenging nature of compounds. The anti-oxidant reacts with ABTS and transfers hydrogen atoms to ABTS, thus neutralizing the free radicals (Vivek et al., 2013). The ethanolic leaf extract of *A. indica* showed significant anti-oxidative potential in terms of DPPH assay, where the maximum inhibition percentage by DPPH scavenging activity of ethanolic leaf extract of A. indica showed an IC_{50} value of 34.791 µg ml-1 compared to standard ascorbic acid. The same anti-oxidative potential was also evident from the ABTS assay, where an IC_{50} value of 84.019 µg ml^{-1} was noticed. Previous studies have shown that ABTS scavenging activity is more precise and sensitive than DPPH. However, ABTS is insensitive to pH, which makes it more viable than DPPH, which is pH-sensitive. Additionally, ABTS dissolves both in aqueous and organic solvents, while DPPH is soluble only in organic solvents (Gaber et al.,, 2021). Medicinal plants rich in phenolic compounds possess various biological activities, including antioxidant, anticancer, and anti-inflammatory activities (Asraoui et al., 2021). It is well known that plants produce ROS during various environmental stress conditions (Cao et al., 2019), where antioxidants can block the action of free radicals, which have a role in the pathogenesis of various diseases.

Histochemical localization assists in tracking different bioactive phytoconstituents for the tissue-specific extraction of foliar parts of medicinal plants. Alkaloids were detected by staining the sections with Wagner's reagent. The presence of alkaloids, as observed in trichomes, might be associated with the self-defense of plants against herbivory. Lignin, located in the cell walls of sclerenchyma tissue and xylem vessels, has been found to be important in the structural support of plants (Gangaram et al., 2020). Phenolic compounds stored in trichomes are released during damage. The antioxidant property of phenols can protect plants against bacterial infection by preventing the production of reactive oxygen species (Munien et al., 2015). The phenolic compounds in plants' epidermal cells act against

pathogen invasion and herbivory (Souza et al., 2017). Histochemical study and phytochemical analysis facilitated the localization and presence of phytoconstituents in trichomes and different tissues of plants. The terpenoid deposition in the trichomes and xylem vessels showed positive reactions of lipophilic compounds in other plant tissues, enhancing the plant defense mechanism (Boix et al., 2011). Tannins located in trichomes have been identified by staining with FeCl3 (Muravnik and Shavarda, 2012). Literature shows the abundant presence of phytochemicals in xylem (Badria et al., 2019).

The present study found that the organic solvent extracts exhibited anti-bacterial activity against the tested bacteria. This may be due to the ability of organic solvents to dissolve organic and active antimicrobial compounds efficiently (Gupta et al., 2016). The antibacterial activity may be due to the presence of phytochemicals such as glycosides, tannins, steroids, flavonoids, and saponins (Raja et al.,, 2013). Ethanol was considered the best solvent for plant yield (Alghamdi et al., 2019). The results confirmed that the extracts possessed inhibitory activity against both gram-positive and gram-negative bacteria. The phytochemicals are regarded as antimicrobials depending on the MIC range of 100-1000 mg ml^{-1} (Sivagnanasundaram et al., 2015). The highest inhibition zone of ethanolic and ethyl acetate extract was observed in *E. coli* ATCC 11229 (25.66 mm and 24.66 mm, respectively). The ethanolic extracts showed better inhibition against other tested bacterial strains. Thus, the results showed that the extracts were more effective against gram-negative bacteria, as a similar study was reported where ovatodiolide, an important constituent of *Anisomeles indica*, showed strong inhibition against gram-negative bacteria, *Helicobacter pylori* (Lien et al., 2019).

Gas chromatography-mass spectrometry (GC-MS) is a fundamental technique to identify the bioactive compounds present in the plant extract. It provides precision to phytochemical studies by identifying unknown compounds (Starlin et al., 2019). In the present study, GC-MS analysis identified 15 compounds, of which 13 were found to have biological activities, and most of these compounds are known for exhibiting various pharmacological activities. As reported by Swamy et al., (2017) the highest antioxidant and antimicrobial activities could be due to bioactive compounds including hexadecanoic acid, phytol and squalene. Similarly, neophytadiene is effective because of its anti-inflammatory, antimicrobial, and antioxidant properties. Squalene, a triterpene, has its role as a natural antioxidant. Previous investigations reported phytol as an important diterpene, known for its anti-oxidant, anti-microbial, anti-cancer and anti-inflammatory activities (Olivia et al., 2021). Phytol was observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes of the bacterium (Sermakani and Thangapandian, 2012). Literature shows squalene and γ-sitosterol possessing antibacterial and anti-inflammatory properties (Adnan et al., 2019). Likewise, among the identified compounds, hexadecanoic acid, ethyl ester and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester exhibit pesticidal and antioxidant properties (Tyagi and Agarwal, 2016). Previous studies show that methyl glycocholate, 3TMS derivatives, as found in green coffee beans, are also known to act as antioxidants (Arsana et al., 2022). 3,7,11,15-tetramethyl-2-hexadecen-1-ol has been found to have anti-inflammatory, antioxidant, and antimicrobial activities (Kumar et al., 2019). Based on the literature, these bioactive compounds may contribute to the pharmacological properties of the leaf extract of *A. indica* and can be further utilized for the synthesis of herbal medicine.

Conclusion

Phytochemical screening of the leaf extracts of *A. indica* confirmed that the extracts were rich in many phytochemicals. The extract's composition and yield varied depending on the solvent used for extraction. The ethanolic extract showed a higher number as well as the concentration of phytochemicals in comparison to ethyl acetate. The GC-MS-based analysis of the ethanolic leaf extract is of great significance for identifying various bioactive compounds that might have pharmacological properties and can be used in drug formulations. The data also indicated the extract as a potent antioxidant and antibacterial agent against both tested gram-negative and gram-positive bacteria. The localization of the phytochemicals in the leaf sections through histochemical studies provided strong evidence for the presence of phytochemicals in varied tissues, making it easier for tissue-specific extraction of specific bio constituents. Based on these results, this study might provide helpful information for extracting and applying A. indica leaf parts and extracts in the food and pharmaceutical industries.

Acknowledgments

All the authors are highly thankful to the University of North Bengal for providing the necessary

facilities and infrastructure to carry out this research work. PM acknowledges the support of SAIF-IIT Madras for assisting in the GC-MS analysis of leaf samples.

Reference

Abbas A, Naqvi SA, Rasool MH, Noureen A, Mubarik MS, Tareen RB. 2021. Phytochemical analysis, antioxidant and antimicrobial screening of *Seriphidium oliverianum* plant extracts. *Dose-response* 19:15593258211004739.

Adnan M, Nazim UCM, Mostafa Kamal AT, Azad MO, Paul A, Uddin SB, Barlow JW, Faruque MO, Park CH, Cho DH. 2019. Investigation of the biological activities and characterization of bioactive constituents of *Ophiorrhiza rugosa* var. prostrata (D. Don) & Mondal leaves through in vivo, in vitro, and in silico approaches. *Molecules* 24:1367.

Alghamdi AI, Ababutain IM. 2019. Research article phytochemical screening and antibacterial activity of *Eucalyptus camaldulensis*'s leaves and bark extracts. *Asian J Sci Res* 12:202-210.

Antil R, Singh L, Gahlawat DK, Dahiya P. 2019. Antimicrobial appraisal and antioxidant potential of *Anisomeles indica* (L.) Kuntze (Lamiaceae). *J Pharmacogn Phytochem* 8:2420-2427.

Antil R, Singh L, Gahlawat DK, Dahiya P. 2019. Investigation of chemical composition of methanolic extract of Anisomeles indica (L.) Kuntze by using FTIR and GC-MS. *J Pharmacogn Phytochem* 8:49-54.

Arsana IN, Juliasih NK, Ayu Sauca Sunia Widyantari AA, Suriani NL, Manto A. 2022. GC MS Analysis of the Active Compound in Ethanol Extracts of White Pepper (*Piper nigrum* L.) and Pharmacological Effects. *Cell Mol Biomed Rep* 2:151-161.

Arulmozhi P, Vijayakumar S, Kumar T. 2018. Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. *Microb Pathog* 123:219-226.

Asraoui F, Kounnoun A, Cadi HE, Cacciola F, Majdoub YO, Alibrando F, Mandolfino F, Dugo P, Mondello L, Louajri A. 2021. Phytochemical investigation and antioxidant activity of *Globularia alypum* L. *Molecules* 26:759.

Azalework HG, Sahabjada AJ, Md Arshad TM. 2017. Phytochemical investigation, GC-MS profile and antimicrobial activity of a medicinal plant *Ruta graveolens* L. from Ethiopia. *Int J Pharm Sci* 9:29-34.

Azwanida NN. 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants* 4:2167-0412.

Badria FA, Aboelmaaty WS. 2019. Plant Histochemistry: A versatile and indispensible tool in localization of gene expression, enzymes, cytokines, secondary metabolites and detection of plants infection and pollution. *Acta Sci Pharmaceut Sci* 3:88-100.

Basappa G, Kumar V, Sarojini BK, Poornima DV, Gajula H, Sannabommaji TK, Rajashekar J. 2015. Chemical composition, biological properties of *Anisomeles indica* Kuntze essential oil. *Ind Crops Prod* 77:89-96.

Baskaran C, Velu S, Kumaran K. 2012. The efficacy of *Carica papaya* leaf extract on some bacterial and a fungal strain by well diffusion method. *Asian Pac J Trop Dis* 2:S658-S662.

Bhatt S, Dhyani S. 2012. Preliminary phytochemical screening of *Ailanthus excels* Roxb. *Int J Curr Pharm Res* 4:87-89.

Bich-Loan NT, Kien KT, Thanh NL, Kim-Thanh NT, Huy NQ, The-Hai P, Muller M, Nachtergael A, Duez P, Thang ND. 2021. Toxicity and anti-proliferative properties of *Anisomeles indica* ethanol extract on cervical cancer hela cells and zebrafish embryos. *Life* 11:257.

Boix YF, Victório CP, Defaveri AC, Arruda RD, Sato A, Lage CL. 2011. Glandular trichomes of *Rosmarinus officinalis* L.: Anatomical and phytochemical analyses of leaf volatiles. *Plant Biosyst* 145:848-856.

Cao Y, Fang S, Fu X, Shang X, Yang W. 2019. Seasonal variation in phenolic compounds and antioxidant activity in leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja. *Forests* 10:624.

Cowan MM. 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564-582.

Dash SP, Dixit S, Sahoo S. 2017. Phytochemical and biochemical characterizations from leaf extracts from *Azadirachta Indica*: an important medicinal plant. *Biochem Anal Biochem* 6:2161-1009.

Devmurari VP. 2010. Phytochemical screening study and antibacterial evaluation of *Symplocos racemosa* Roxb. *Arch Appl Sci Res* 2:354-359.

Dharmasiri MG, Ratnasooriya WD, Thabrew MI. 2003. Water extract of leaves and stems of pre-flowering but not flowering plants of *Anisomeles indica* possesses analgesic and antihyperalgesic activities in rats. *Pharm Biol* 41:37–34.

Durai MV, Balamuniappan G, Anandalakshmi R, Geetha S, Kumar NS. 2016. Qualitative and quantitative analysis of phytochemicals in crude extract of big-leaf mahogany (*Swietenia macrophylla* King). *Int J Herbal Med* 4:88-91.

Farag RS, Abdel-Latif MS, Abd El Baky HH, Tawfeek LS. 2020. Phytochemical screening and antioxidant activity of some medicinal plants' crude juices. *Biotechnol Rep* 28:e00536.

Gaber NB, El-Dahy SI, Shalaby EA. 2021. Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. *Biomass Convers Biorefin* 26:1-0.

Gangaram S, Naidoo Y, Dewir YH. 2020. Foliar micromorphology, ultrastructure, and histochemical analysis of *Barleria albostellata* CB Clarke. *South Afr J Bot* 135:212-224.

Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. 2015. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) *L. J Food Sci Technol* 52:1212-1217.

Gomez AA, Mercado MI, Belizán MM, Ponessa G, Vattuone MA, Sampietro DA. 2019. In situ histochemical localization of alkaloids in leaves and pods of *Prosopis ruscifolia*. *Flora* 256:1-6.

Gülçin İ, Huyut Z, Elmastaş M, Aboul-Enein HY. 2010. Radical scavenging and antioxidant activity of tannic acid. *Arab J Chem* 3:43-53.

Gupta D, Dubey J, Kumar M. 2016. Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms. *Asian Pac J Trop Dis* 6:15-20.

Hsu CY, Chan YP, Chang J. 2007. Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol Res* 40:13-21.

Ismail RM, Saleh AH, Ali KS. 2020. GC-MS analysis and antibacterial activity of garlic extract with antibiotic. *J Med Plants Stud* 8:26-30.

Kamaraj M, Dhana Rangesh Kumar V, Nithya TG, Danya U. 2020. Assessment of antioxidant, antibacterial activity and phytoactive compounds of aqueous extracts of avocado fruit peel from Ethiopia. *Int J Pept Res Ther* 1549-1557.

Kathirvel A, Sujatha V, 2016. Phytochemical studies, antioxidant activities and identification of active compounds using GC–MS of *Dryopteris cochleata* leaves. *Arab J Chem* 9:S1435-42.

Kumar RS, Anburaj G, Subramanian A, Vasantha S, Selvam AP. 2019. Preliminary phytochemical investigation, Antimicrobial activity and GC-MS analysis of leaf extract of *Capparis zeylanica* Linn. *J Pharmacogn Phytochem* 8:1399-1405.

Kundu A, Saha S, Walia S, Kour C. 2013. ntioxidant and antifungal properties of the essential oil of *Anisomeles indica* from India. *J Med Plants Res* 7:1774-1779.

Lien HM, Wu HY, Hung CL, Chen CJ, Wu CL, Chen KW, Huang CL, Chang SJ, Chen CC, Lin HJ, Lai CH. 2019. Antibacterial activity of ovatodiolide isolated from *Anisomeles indica* against *Helicobacter pylori. Sci Rep* 9:4205.

Islam MS, Rashid MM, Ahmed AA, Reza AA, Rahman MA, Choudhury TR. 2021. The food ingredients of different extracts of *Lasia spinosa* (L.) Thwaites can turn it into a potential medicinal food. *NFS J* 25:56-69.

Munien P, Naidoo Y, Naidoo G. 2015. Micromorphology, histochemistry and ultrastructure of the foliar trichomes of *Withania somnifera* (L.) Dunal (Solanaceae). *Planta* 242:1107 1122.

Muravnik LE, Shavarda AL. 2012. Leaf glandular trichomes in *Empetrum nigrum*: morphology, histochemistry, ultrastructure and secondary metabolites. *Nord J Bot* 30:470 481.

Nasrin S, Islam MN, Tayab MA, Nasrin MS, Siddique MA, Emran TB, Reza AA. 2022. Chemical profiles and pharmacological insights of *Anisomeles indica* Kuntze: An experimental chemico-biological interaction. *Biomed Pharmacother* 149:112842.

Olivia NU, Goodness UC, Obinna OM. 2021. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves. *Future J Pharm Sci* 7:1-5.

Prasad RN, Viswanathan S, Devi JR, Nayak V, Swetha VC, Archana BR, Parathasarathy N, Rajkumar J. 2008. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. *J MedPlants Res* 2:268-270.

Raja W, Ovais M, Dubey A. 2013. Phytochemical screening and antibacterial activity of *Lawsonia inermis* leaf extract. *Medicine* 6.

Rajesh KD, Vasantha S, Rajesh NV, Panneerselvam A. 2014. Qualitative and quantitative phytochemical analysis in four pteridophytes. *Int J Pharm Sci Rev Res* 27:408-412.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231-1237.

Sathya C, Phawa A. 2018. Histochemical and antimicrobial activity of lamiaceae plants (*Anisomeles indica* O. Kze, Hyptis suavolens Poit, Leonotis nepetaefolia R. Br., and Leucas aspera Spr.). *EJPMR* 5:360-363.

Sermakkani M, Thangapandian V. 2012. GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian J Pharm Clin Res* 5:90-94.

Shaikh JR, Patil MK. 2020. Qualitative tests for preliminary phytochemical screening: An overview. *Int J Chem Stud* 8(2):603-608.

Singleton, VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents.*Am J Enol Vitic* 16:144-158.

Sivagnanasundaram P, Karunanayake KO .2015. Phytochemical screening and antimicrobial activity of *Artocarpus heterophyllus* and *Artocarpus altilis* leaf and stem bark extracts. *OUSL J* 9:1-17.

Souza DM, Sa RD, Araujo EL, Randau KP. 2017. Anatomical, phytochemical and histochemical study of *Solidago chilensis* Meyen. *An Acad Bras Ciênc* 90:2107-2120.

Starlin T, Prabha PS, Thayakumar BK, Gopalakrishnan VK. 2019. Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. *Bioinformation* 15:425.

Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. 2017. GC MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of *Malaysian Plectranthus* amboinicus leaves. *J Evid Based Complementary Altern Med* 2017.

Tambe BD, Pedhekar P, Harshali P. 2021. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. *Asian J Pharm Res Dev* 9:50-54.

Tyagi T, Agarwal M. 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *J Pharm Phytochem* 6:195-206.

UC R, Nair VM. 2013. Phytochemical analysis of successive reextracts of the leaves of *Moringa oleifera* Lam. *Int J Pharm Pharm Sci* 5:629-634.

Ugochukwu SC, Arukwe Uche I, Ifeanyi O. 2013. Available online at www. pelagiaresearchlibrary. com. *Asian J Plant Sci Res*. 3:10-13.

Vivek MN, HC SS, Manasa M, Pallavi S, Kambar Y, Asha MM, Chaithra M, TR PK, Mallikarjun N, Onkarappa R. 2013. Antimicrobial and antioxidant activity of leaf and flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum. J Appl Pharma Sci* 3:064-71.

Zahoor MK, Zahoor MA, Mubarik MS, Rizvi H, Majeed HN, Zulhussnain M, Ranian K, Sultana K, Imran M, Qamer S. 2020. Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: Muscidae) to some indigenous weed plant extracts. *Saudi J Biol Sci* 27:106-116.

Zain M, Yasmin S, Hafeez FY. 2019. Isolation and characterization of plant growth promoting antagonistic bacteria from cotton and sugarcane plants for suppression of phytopathogenic fusarium species. *Iranian J. Biotech*. 17: e1974.

Zubasheva M, Ganushkina LA, Smirnova TA, Azizbekyan RR. 2010. Larvicidal activity of crystal-forming strains of *Brevibacillus laterosporus*. *Appl. Biochem. Microbiol.* 46: 69 755–762.