J. Biodivers. Conservation 9(2): 87-107 ISSN: 2457-0761 (online)
2025 http://www.aprfjournal.in

Review Article

Methods for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for pharmacological evaluation of medicinal plants

Nibedita Jena[1], Vimala[2], Balkar Singh[3], Arjun Patra[4], Bhagwati Prashad Sharma[5], Emdad Hossain[6] and Sanjeet Kumar[1*]

[1]Ambika Prasad Research Foundation, Odisha, India

[2]Department of Botany, Multanmal Bhikchand Chajjer, Government Girls College, Barmer, Rajasthan, India

[3]Department of Botany, Arya PG College, Panipat, Haryana, India

[4]Department of Botany, P.K. College, Contai, West Bengal, India

[5]Department of Botany, Sidharth Government College, Nadaun, Himachal Pradesh, India

[6]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India

*E-mail: sanjeetaprf@gmail.com; ORCID: https://orcid.org/0000-0001-9538-397X

DOI: https://doi.org/10.5281/zenodo.15368734

Article Details: Received: 2025-03-25 | Accepted:2025-05-05 | Available online: 2025-05-08

Abstract: The present paper outlines a comprehensive methodology for the evaluation of medicinal plants, encompassing ethnobotanical data collection, phytochemical analysis, and pharmacological screening for antioxidant, anthelmintic, and antimicrobial activities. The methods described include ethnobotanical surveys, herbarium preparation, extraction and fractionation of plant extracts, and various bioassays for evaluating pharmacological activities. The paper also provides detailed protocols for qualitative and quantitative phytochemical analysis, including thin-layer chromatography (TLC) and spectroscopic techniques. The antimicrobial activity of plant extracts is evaluated using agar well diffusion, disc diffusion, and broth dilution assays. This methodology paper serves as a valuable resource for researchers, scientists, and students working in the fields of pharmacognosy, phytochemistry, and pharmacology, providing a comprehensive framework for the evaluation of medicinal plants.

Keywords: Bioassay, medicinal plants, methodology, plant extraction

Introduction

Medicinal plants have been an integral part of human healthcare for centuries, providing a rich source of bioactive compounds with therapeutic potential (Kumar and Satapathy, 2011; Das et al., 2020; Devi et al., 2022; Das et al., 2025; Mukherjee et al., 2025). The World Health Organization (WHO) estimated that approximately 80% of the world's population relies on traditional medicine, which is largely based on plant-derived remedies (Kumar and Dash, 2012; Oyebode et al., 2016; Rout et al., 2024). However, the increasing demand for medicinal plants, coupled with habitat destruction, over-exploitation, and climate change, has led to a significant decline in their populations (Sethi et al., 2024a; Mykhailenko et al., 2025). As a result, there is an urgent need to document and evaluate the medicinal properties of these plants to ensure their conservation and sustainable use (Chen et al., 2016; Das et al., 2022). Ethnobotanical research plays a crucial role in the discovery and development of new drugs from medicinal plants (Devi et al., 2025; Sahu et al., 2025). Ethnobotanical data collection involves documenting the traditional knowledge and uses of medicinal plants by local communities (Kumar et al., 2018; Mekonnen et al., 2022). Ethnobotanical information serves as a valuable starting point for further research, including phytochemical analysis, pharmacological evaluation, and clinical trials (McClatchey et al., 2009; Agarwal et al., 2023). Phytochemical analysis involves the identification and quantification of bioactive compounds present in medicinal plants, while pharmacological evaluation assesses their therapeutic potential (Altemimi et al., 2017). Antioxidant, anthelmintic, and antimicrobial activities are important pharmacological parameters that are commonly evaluated in medicinal plants (Swargiary et al., 2016). Present review aims to provide a comprehensive overview of the methods used for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for the pharmacological evaluation of medicinal plants. Present article aims to serve as a valuable resource for researchers and students in medicinal plant research by providing a detailed overview of relevant methods.

Ethnobotanical Data Collection

Study area: For the collection of ethnobotanical data, study area should be considered and selected as per the availability of maximum number of local communities belonging to the tribal race (Asigbaase et al., 2023). As they are the indigenous people who have large-scale knowledge about the native medicinal plants, their common names, food, and medicinal values with traditional healing practices to fulfil their survival needs and combat health ailments as well. They generally prefer to reside in villages far from civilization, which are endowed with lush green vegetation and floral diversity. In these specific regions, the ethnobotanical data collection on traditional medicinal plants will be more authentic (Jena et al., 2025; Murmu et al., 2016).

Data collection of informants: During field survey the participants belonging to tribal communities namely Santhal, Kolha, Munda, Kondh Kharia, Gond, Kudmi, Bhumij, Gaddi, Gujjar etc., age group between 35-75 years and traditional healers should be prioritized for the interviews. The important step is to make them (participants) at ease and comfortable primarily for proper sharing of information not

only about the medicinal plants but also regarding their personal details and livelihood using native plants, to make it a good interactive discussion.

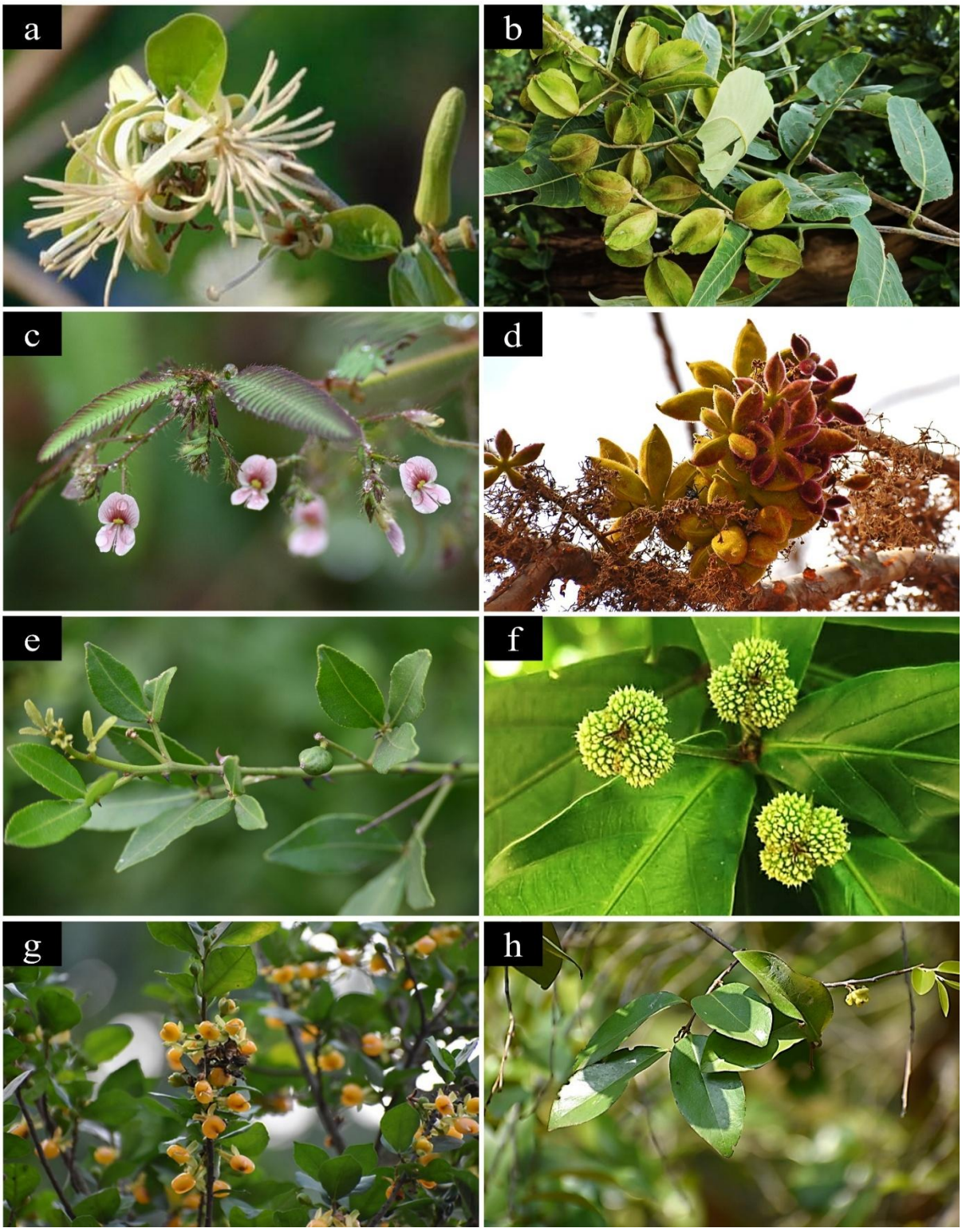

Plate 1: Common medicinal plants of India, a) *Alangium salviifolium*, b) *Terminalia arjuna*, c) *Aeschynomene americana*, d) *Sterculia urens*, e) *Zanthoxylum asiaticum*, f) *Lasiococca comberi*, g) *Streblus asper* and h) *Alphonsea lutea*

A questionnaire format should be prepared in which the personal details of informants like name, age group, race, gender, occupation, and consent must be enlisted for collection of information about the informants (Patra et al., 2017; Maity et al., 2018; Devi et al., 2022; Basole et al., 2025).

Data collection of ethnobotanical uses: Ethnomedicinal surveys must be carried out in the selected study areas. The traditional healing practices using native medicinal plants by tribal people must be recorded from the informants through group discussions, semi-structured interviews, note-making and open-ended conversations (Kumar et al., 2018; Saha et al., 2022; Saradar et al., 2023).

Herbarium preparation

Field visit and specimen collection: During field visits, it is necessary to obtain information from the locals or published literature at every growth and reproductive stages of a plant species. The plant species should be correctly identified by a field guide or taxonomist. Matured plant parts (like stem with leaves, flowers, and fruits) should be selected for the herbarium preparation. When collecting a specimen, the ideal length should be enough to represent the entire plant habit, including leaves, flowers, and fruits, if present. The ideal length is typically around 11.5 inches by 16.5 inches. To avoid damage during transportation and preservation at least 5-G (Gather at least five individual plants of the same species) specimens of a plant species should be collected. The collected specimens should be transported in a vasculum. Specimen must be tagged with a field number and necessary information should be recorded in a field data book (Jain and Rao, 1976; Sethi et al., 2024b; Sharma et al., 2024).

Tools for plant collection: Plant press, plastic or cloth or nylon bags, garden secateurs, towel, small note book, tags, pen, pencil, camera, GPS, and altimeter should be carried for plant specimen collection (Jain and Rao, 1976; Sharma et al., 2024).

Pressing and drying: The specimens should be spread between the folds of old newspapers or blotting sheets avoiding overlapping of plant parts. The larger specimen may be folded in ‘N’ or ‘W’ shapes. The blotting sheets with plant specimen should be placed in the plant press for drying. After 24-48 hours the press is opened for the change of newspapers or blotting sheets (Jain and Rao, 1976; Sharma et al., 2024).

Mounting: The dried specimens should be mounted on herbarium sheets of standard size (41 × 29 cm). Mounting is done with the help of glue, adhesive or cello tape. The bulky plant parts like dry fruits, seeds, cones, etc. should be dried without pressing and put in small envelopes called fragment packets (Jain and Rao, 1976; Sharma et al., 2024).

Preservation: The mounted specimens are sprayed with fungicides like 2% solution of mercuric chloride to prevent insects or fungus (Jain and Rao, 1976; Sharma et al., 2024).

Labelling: A label should be pasted or printed on the lower right-hand corner. The label must indicate the information about the locality, altitude, habit, date and time of collection, name of collector, common name, scientific name, and medicinal uses (Figure 1; Jain and Rao, 1976; Sharma et al., 2024).

Figure 1: An herbarium specimen of *Terminalia arjuna*

Figure 2: Soxhlet extraction of experimental plant sample

Storage: Properly dried, pressed and identified plant specimens should be placed in species, genus or family cover and arranged according to a well-known system of classification. In India, Bentham and Hooker's system of classification is used for this purpose, but APG system should be used (Jain and Rao, 1976; Sharma et al., 2024).

Qualitative Phytochemical Screening (Primary & Secondary metabolites)

Collection of experimental plant samples: The experimental plant samples could be identified by a taxonomist, followed by published literature on the flora of collection areas, then collected and kept in cloth bags tagged with the botanical name and sorted out as per standard sampling procedure and passport description (Plate 1). Samples should be preserved as voucher specimens and deposited in the herbarium unit of Botanical Survey of India or any other organisations having herbarium unit (Figure 1). The information gathered from the interviews should be compiled and analysed (Shirai et al., 2022). The experimental plant parts, their uses, mode of consumption, preparation techniques, and therapeutic uses should be noted (Patra et al., 2019; Basole et al., 2024; Das et al., 2024).

Preparation of extracts: The experimental plant parts should be left for air drying in shade areas and after the samples are completely dry, they should be powdered using mechanical devices and kept in closed container. The powdered material of the experimental plant should be taken in the thimble and extraction should be carried out using the Soxhlet apparatus. The residues should be collected and left for air drying and dried crude extracts should be stored in refrigerator for further experimental work (Figure 2; Mishra et al., 2012; Kumar et al., 2013; Mohapatra et al., 2020).

Detection of primary metabolites

Test for carbohydrates: About 1 ml of extract is mixed with 2-5 drops of Fehling's solution A and B and kept in the water bath for 30 minutes. A reducing sugar reacts with Fehling's reagent in an alkaline medium to form orange-to-red precipitate. The appearance of this orange or red precipitate indicates the presence of carbohydrates.

Test for protein: About 1 ml of extract is mixed with 2-5 drops of Millon's reagent and kept in the water bath for 30 minutes. The pink coloration indicates the presence of protein.

Test for starch: To 1 ml of extracts, 2-3 drops of concentrated HCl are added and heated for 1-2 minutes. NaOH following Benedict's reagent is added to this solution and kept in the water bath. The change in coloration from bluish-green to red-orange indicates the presence of starch.

Test for sucrose: 1 ml of extract is mixed with 2-3 drops of iodine solution. The appearance of a blue-black colour indicates the presence of sucrose.

Test for lipid: 1 ml of extract is mixed with 2-5 drops of Sudan-III. The appearance of pink droplets indicates the presence of lipids.

Plate 2: Qualitative screening of bioactive compounds presents e.g., (a) amino acid, (b) saponin, (c) reducing sugar & (d) phenol, tannin, and alkaloids in (5^{th}, 1^{st} and 8^{th} number) test tubes

Test for amino acids: 1 ml of extract is mixed with 5 drops of Ninhydrin reagent and kept in the water bath for 30 minutes. Ninhydrin is a powerful oxidizing agent to give a purple-coloured product (diketohydrin) termed Rhuemann's purple. The appearance of purple or bluish colour indicates the presence of amino acids (Plate 2).

Detection of secondary metabolites

Test for tannin: 1 ml of plant extract is mixed with 4-5 drops of 5% lead acetate solution. The formation of a light-yellow precipitate confirms the presence of tannins.

Test for saponin: 1 ml of plant extract is boiled in 8 ml of distilled water, filtered through Whatman 42 filter paper, and then 4 ml of the filtrate is mixed with 2.5 ml of distilled water. After vigorous shaking, the formation of a stable, persistent froth confirms the presence of saponins.

Test for flavonoids: To 2 ml of plant extract, a few drops of dilute sodium hydroxide (NaOH) are added. The appearance of an intense yellow colour indicates the presence of flavonoids. Subsequent addition of 5 drops of 70% dilute acid causes the colour to fade, further confirming the presence of flavonoids.

Test for terpenoids: A mixture of 1 ml of plant extract and 2 ml of chloroform is prepared, and an equal volume of concentrated sulphuric acid is added. The appearance of a reddish-brown coloration at the interface confirms the presence of terpenoids.

Test for phenolic compounds: 1 ml of plant extract is treated with 6 drops of 2% ferric chloride solution, resulting in the formation of a bluish-black coloration, which indicates the presence of phenolic compounds.

Test for reducing sugars: 1 ml of plant extract is dissolved in distilled water, filtered, and then boiled with 3 drops of Fehling's solution A and B for 6 minutes. The formation of an orange-red precipitate confirms the presence of reducing sugars.

Test for steroids: 1 ml of plant extract is mixed with 4 ml of chloroform, followed by the addition of 4 ml of concentrated sulphuric acid. The resulting formation of two distinct phases, characterized by an upper red layer and a lower yellow layer with green fluorescence, confirms the presence of steroids.

Test for alkaloids: 1 ml of plant extract is mixed with 2 ml of aqueous HCl and heated on a water bath. After filtration, 0.5 ml of Dragendorff's reagent is added to the filtrate. The formation of an orange-red precipitate confirms the presence of alkaloids in the sample extract.

Test for anthraquinone glycosides (Modified Borntrager's test): To 1 g of the powdered drug, 5 ml of diluted hydrochloric acid and 5 ml of ferric chloride solution (5% w/v) is added. The mixture is then boiled on a water bath for 10 minutes. After boiling, the solution is cooled, and filtered. The filtrate is extracted with benzene or carbon tetrachloride. To the organic layer, an equal volume of ammonia solution is added. A colour change from pink to red is observed, which indicates the presence of anthraquinone moiety (Tiwari et al., 2020).

Test for cardiac glycosides (Keller-Kiliani test): 5 ml of crude extract is treated with 3 ml of glacial acetic acid. Then, 3-5 drops of 1% ferric chloride solution are added followed by 2 ml of concentrated sulphuric acid. Formation of brown colour confirms the presence of cardiac glycosides (Tiwari et al., 2020).

Quantitative estimation of secondary metabolites

Estimation of tannin (Sadasivam and Manickam, 2019)

200 mg of tannic acid is mixed with 20 ml of distilled water. From this solution, 5, 15, 25, 35 and 50 µl are taken in different test tubes. The volume is made up to 1ml. Then 0.5 ml of Folin reagent and 2.5 ml of 20% sodium carbonate were added to each test tube. The mixed solutions are shaken for 5 minutes in dark. The solutions of each test tube are left for 40 minutes. After 40 minutes readings are taken at 720 nm for making standard graph.

Extraction of tannins

200 mg of sample is transferred into a 100 ml of conical flask to which 75 ml of distilled water is added and boiled for 30 minutes. The whole solution is centrifuged at 2000rpm for 20 minutes. Supernatant is then taken in a 100 ml volumetric flask and made up to the volume. 1 ml of sample is taken from 100 ml volumetric flask and 75 ml of distilled water, 5 ml of Folin reagent and 10 ml of 20% sodium carbonate are added. The volume is made up to 100 ml. After shaking 5 minutes, the readings are taken at 720 nm. The amount of tannin is calculated from the standard graph.

Estimation of saponins

The determination of total saponin is done by the method of Obadoni and Ochuko, (2001) with minor modifications. 2 g of sample is added to 30 ml of 20% aqueous ethanol and kept in a flask on stirrer for half an hour. It is heated for 4 hours at 45^{o}C. The mixture is filtered by using Whatman filter paper-1 and the residue is again extracted with another 20 ml of 25% aqueous ethanol. The combined extracts are made concentrated. The concentrate is transferred into separator funnel and is extracted twice with 10 ml diethyl ether. The ether layer is discarded while the aqueous layer is kept and re-extracted with 15 ml n-butanol. The n-butanol extract is washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution is evaporated. After evaporation, the sample is dried in the hot air oven at 40^{o}C to a constant weight. The saponin content is calculated.

% of saponins = (Final weight of sample/ Initial weight of extracts) × 100

Estimation of total phenol (Swain and Hillis, 1959)

Extraction of phenol

Three replicas of 0.5 g of sample are taken and crushed with 60% methanol in mortar and pestle. Samples are centrifuged 5 times at 5000 rpm for 20 minutes.

Estimation of phenols

Seven test tubes are taken including blank and 2 test tubes for each replica. 0.1 & 0.2 ml of sample is taken in each test tube except blank. 60% methanol is added to each test tube to make volume up to 1 ml. 1 ml of 0.1 N HCl is added and allowed to stand for a few minutes. 1 ml of sodium nitrite molybdate mixture is added, shaken well, and allowed to stand for a few minutes, diluted with 5 ml of distilled water. After dilution, 2 ml of 1N NaOH is added and allowed to stand for 20 minutes. Readings are taken at 515 nm. The amount of phenol present in the sample is calculated from the standard graph.

Estimation of flavonoids

A sample of 100 µl of the extract is mixed with 100 µl of 5% sodium nitrite and allowed to stand for 5 minutes. Next, 100 µl of aluminium trichloride is added and incubated for 6 minutes, followed by addition of 1 ml of 1 M sodium hydroxide. Total volume of the mixture was made up to 5 ml with distilled water and incubated for 15 minutes at room temperature in dark for taking absorbance at 510 nm. The amount of flavonoid present in the sample is calculated from the standard graph. The standard graph was prepared for quercetin (Chang et al., 2002; Raina and Misra, 2023).

Toxicity analysis

For toxicity analysis, hatching of brine cysts is the initial process. Standardization of the optimum is done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of brine shrimp (*Artemia salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration, room temperature or between 28-35^{o}C and light for 48 hours. Proper hatching can be observed within 24-48 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations of the sample extract are taken and 3.5% saline water is used to make the volume 2 ml in each test tube. 1% DMSO is used to dissolve the crude extracts. Ten nauplii are selected and introduced to five test tubes of the extracts. Positive and negative controls are prepared using vincristine sulphate (5 mg/ml) or potassium dichromate and 3.5% saline water respectively with a volume of 2 ml. The live larvae are highly motile and thus differentiate from the unhatched cysts. The survivor nauplii are counted and the death% are analysed. (Kumar et al., 2012; Dash et al., 2022; Devi et al., 2024; Jena et al., 2024).

TLC (Thin Layer Chromatography) analysis

Chromatographic analysis is carried out using standard methods to evaluate the secondary metabolites.

Preparative TLC: TLC plates are prepared on 6 cm glass slides using silica gel G powder (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd.). Slides are washed with clinical laboratory detergent and dried. Clean and dried slides are wiped with ethyl acetate for the removal of surface adherents. 3 g of silica gel G is taken in 20 ml of distilled water and slurry is prepared from constant stirring, then poured

over the slides and left undisturbed till the drying of silica layer (Kumar et al., 2013). Then the slides are activated by heating on the hot plate at 110-120 ^{o}C for 30-60 minutes (Pyka et al., 2005; Rout et al., 2024).

Mobile phase: The mobile phases are taken as per polarity index in single, double, and triple combining solvent systems such as n-Hexane, Chloroform: Methanol (CM), Ethyl acetate: Chloroform (EC), Chloroform: Ethyl acetate: Formic acid (CEF), Ethyl acetate: Water: Acetic acid (EWA) and Ethyl acetate: Methanol: Water (EMW).

R_f (Retention factor) values: The behaviour of an individual compound in TLC is characterized by R_f and is expressed as a decimal fraction. The R_f is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position (Plate 3).

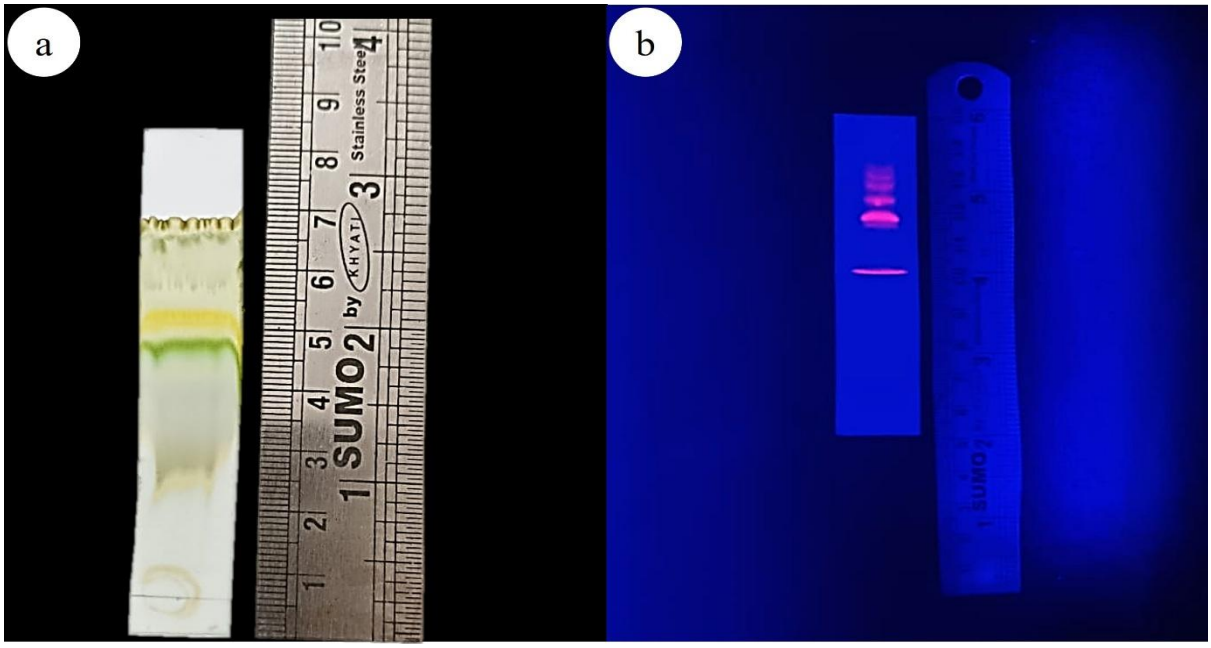

Plate 3: Thin Layer chromatography analysis showing multiple bands (a) without UV and (b) within UV chamber

Fractionation: A combination of preparative TLC and column chromatography are used for the initial fractionation of the crude extracts and isolation of the active compounds. The extracts are dissolved in respective solvents. 10 µl of the extract is applied at the origin of the preparative TLC plate with pre-determined mobile phase. The active well visualized bands are confirmed. The bands to be analysed are marked and the silica containing the compounds is scraped off repeatedly and collected in closed container. The collected samples are centrifuged with respective solvents. The supernatant is collected and kept for further experiments. Normal column chromatography is performed with the use of silica gel powder on a 45 cm glass column with 1.4 cm diameter. The previously collected supernatant is loaded in the packed column. A gradient mobile phase, composed of different ratios of chloroform and methanol, is used to elute the compounds over the range of polarities. The used mobile phase for active

visualized bands on TLC is also used in column. Fractions are collected and monitored on TLC again with respective mobile phase and the spots are confirmed at same R_f. The confirmed fraction is made concentrated. The concentrated fractions are dissolved in a small volume of respective solvent and subjected to FTIR and NMR analysis.

Qualitative antioxidant activity

To detect antioxidant activity, qualitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is carried out (Eloff et al., 2008; Bhatnagar et al., 2013). The plates are first air dried and then the chromatograms are sprayed with 0.2% 2,2-diphenyl-2-picryl-hydrazyl in methanol as an indicator. The presence of antioxidant compounds is detected by yellow spots against a purple background on the TLC plates sprayed with 0.2% DPPH in methanol. About 5µl of each sample is loaded on the TLC sheet and the chromatograms are developed in following solvent systems:

• Ethyl acetate/methanol/water (40:5.4:4) [EMW] (polar neutral);

• Chloroform/ ethyl acetate/ formic acid (5:4:1) [CEF] (intermediate polarity/acidic);

• Benzene/ethanol/ammonium hydroxide (90:10:1) [BEA] (non-polar/basic)

Anthelmintic activity

The anthelmintic activity is screened using Indian earthworm, *Eisenia fetida*. The plant samples are powdered and extracts are prepared using solvents by cold maceration. Vacuum distillation is used to concentrate extract. Then the total extract is fractionated using solvents in separating funnel. Fractions are concentrated and obtained. *E. fetida* of size 4-7 cm in length and 0.1-0.2 cm in width are considered for the study. Albendazole is used as standard. Then the fractions show paralysis of earthworm within less time indicated anthelmintic activity (Figure 3). For better result helminth like *Paramphistomum cervi* or *Fasciola gigantica* from buffalo or goat may be used (Hossain et al., 2012; Hossain et al., 2013).

Antimicrobial activity

Preparation of the stock solution of plant extract: The plant extract was obtained using Soxhlet method of plant extraction are diluted using 10% dimethyl sulfoxide (DMSO) to make a stock solution of 100 mg/ml. Different concentrations of the plant extracts are prepared from the stock solution (Mohanty et al., 2021).

Microorganisms: The extracts of experimental plant samples should be analysed for antibacterial activity against selected or desired pathogenic microbes collected from MTCC (Microbial Type Culture Collection) or Institute of Microbial Technology (IMTECH), Chandigarh, India, or other known organisations.

Media used: Nutrient broth is used to maintain broth cultures. The constituents of the nutrient broth include 0.5 g NaCl, 0.5 g peptone and 0.3 g beef per 100 ml. An additional 1.5 g of agar is used to make the nutrient agar medium.

Figure 3: Anthelmintic screening using *Eisenia fetida*

Broth preparation: Colonies of prepared slants of MTCC 442, MTCC 497, MTCC 1457 and MTCC 2672 are picked off using sterile loop and inoculated in sterile conditions in autoclaved cool liquid broth medium containing 0.3% of beef extract and 0.5% peptone. The broth is incubated for 24 h at 37±1.0ºC until there is visible growth indicated from the turbidity standard.

Preparation of inoculum: The fresh inoculum is prepared from 24 hours growth culture each time for antibacterial activity analysis. Stock culture of MTCC 442, MTCC 497, MTCC 1457 and MTCC 2672 are maintained at 4ºC on slants of semi-solid media containing 1.5% of agar-agar, 0.3% beef extract and 0.5% peptone. Active working culture for experiments are prepared by transferring a loopful of culture mass from the stock. Slants are incubated for 24 h at 37±1.0ºC.

Swabbing and inoculation of drugs: Swabbing with autoclaved cotton swab is done using broth strain on petri plates. Wells (6 mm) are made using sterile borer for agar well method. Stock solutions of samples are prepared in 100% DMSO and two-fold serial dilutions were made in amount of 100 µl well ranging from 0.5, 1.0 and 2.0 mg/ml. 100 µl of samples are added by sterile syringes into the wells in three above mentioned concentrations and allowed to diffuse at room temperature for 2 hours. Only the solvent (1% DMSO) is poured into the wells in another set of plates as part of negative control. The positive control set consists of standard antibiotics Kanamycin. For the disc diffusion assay, only

swabbing was done using sterile swab. Then discs of respective aforesaid concentration are placed on media. Both petri dishes (for agar well diffusion & disc diffusion method) are incubated at 37±1.0°C for 18 hours. Zones of inhibition for microbial growth appear around each well and disc in the form of clear rings which confirms the antibacterial activity of the respective samples. Those samples which do not have any inhibitory effect on the microbe did not form any clear ring. In this way, the antibacterial activity of the samples is confirmed. Triplicates are maintained and the experiment is repeated thrice. For each replication the readings (zone of inhibition) are taken and the mean values are recorded.

Data analysis: Mean and SD (standard deviation) are calculated taking triplicate values of zone of inhibition (cm for agar well diffusion assay; mm for disc diffusion) of samples using Excel, Microsoft Corporation.

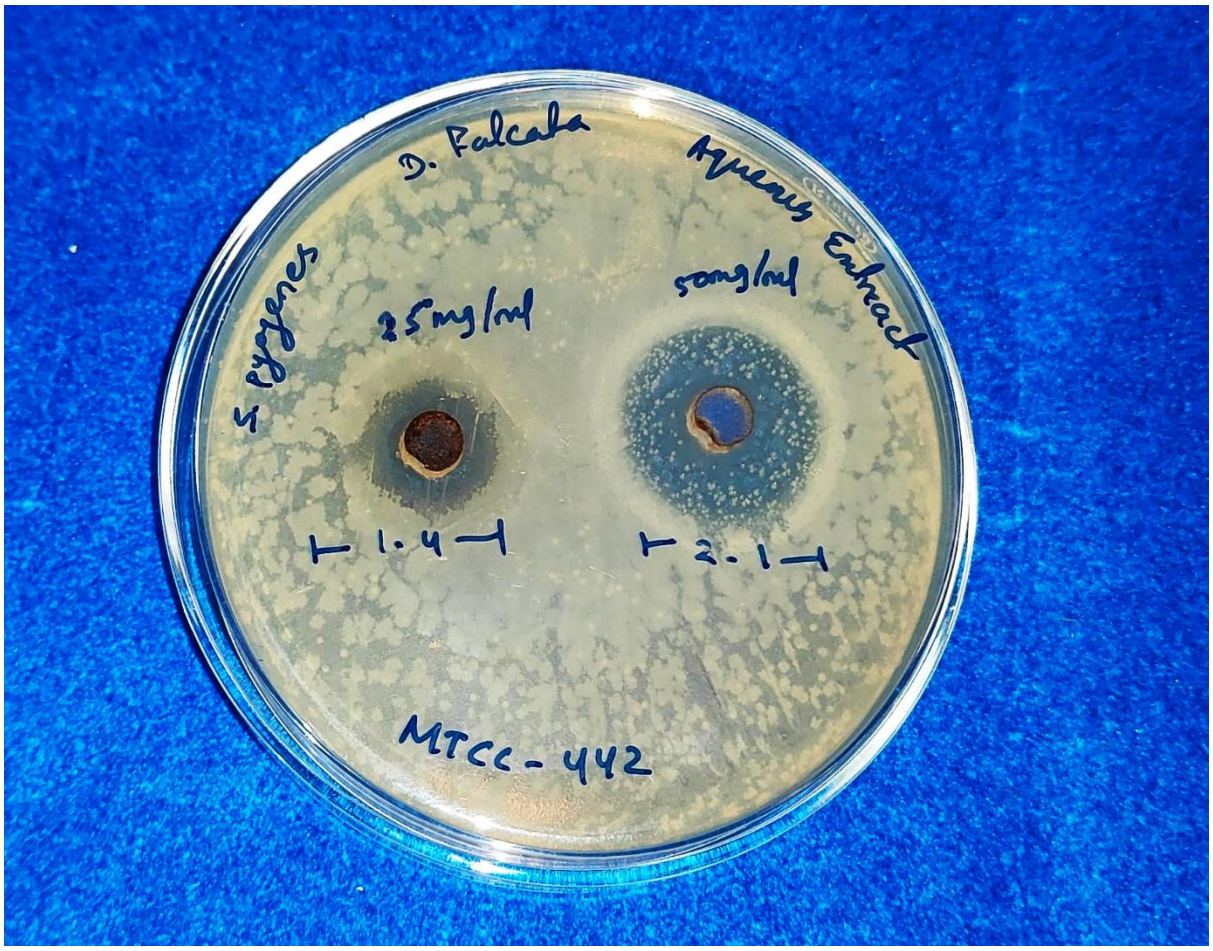

Figure 4: Agar well diffusion assay against *Streptococcus pyogenes*

Agar well diffusion (AWD) assay: Agar well diffusion assay of the plant extracts using different solvents e.g., aqueous, methanol and ethanol are carried out according to the National Committee for Clinical Laboratory Standards (NCCLS). Inoculum to be tested are spread on the nutrient agar petri plates using sterile swabs of bacterial suspension. Then 6 mm wells are punctured in the agar. Different concentrations of the plant extracts are prepared from the stock solution of the different solvents of plant extracts respectively. The volume of 100 µl of the different concentration of the plant extracts are filled

in the different wells and ampicillin of 5 mg/ml concentration is added as a positive control. It is then allowed to diffuse for 30 mins in a refrigerator. The plates are then incubated at 37°C for 24 hours. After 24 hours, the zone of inhibition is measured and compared with the positive control. Then the results are expressed with mean standard deviation (Figure 4; Kumar et al., 2017; Sarangi et al., 2021).

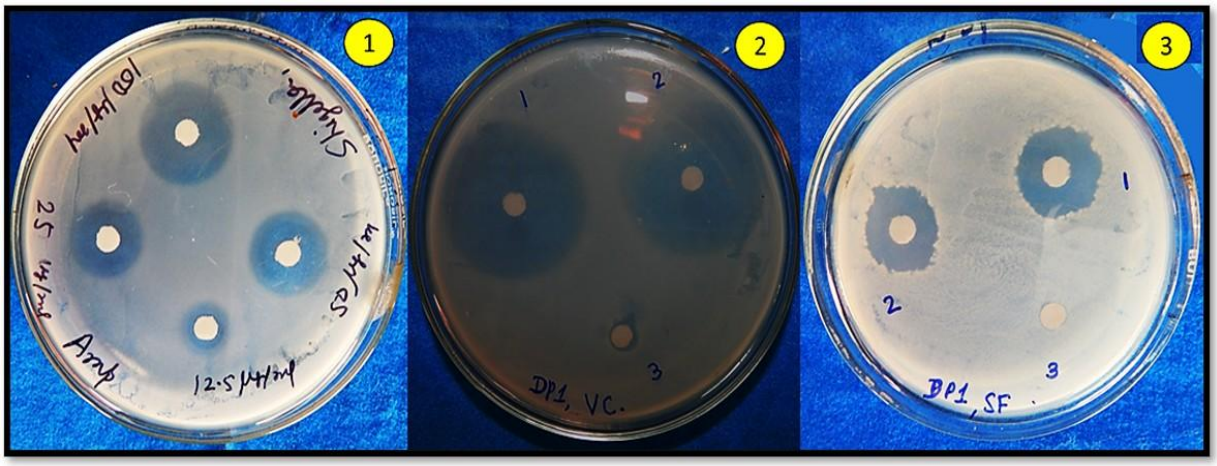

Figure 5: Disc diffusion assay against gram-negative bacteria *Shigella flexneri*

Disc diffusion (DD) assay: Disc diffusion assay of plant extracts is carried out according to the National Committee for Clinical Laboratory standards (NCCLS). Likewise in agar well diffusion, inoculum is spread on the sterile nutrient agar plates by using sterile swabs of bacterial suspension. Different concentrations of the plant extracts are prepared from the stock solution of the different extracts. Whatman filter paper No. 1 is cut into 6 mm disc pieces and allowed to suspend in different concentrations of the solvent extracts placed on the nutrient agar (NA) plate with test organism using sterile forceps on the petri-plates with approximately equal distance. It is then placed for incubation at 37°C for 24 hours. Ampicillin discs are suspended in 5 mg/ml ampicillin solution is also placed as a positive control. The results are expressed with mean standard deviation (Figure 5; Kumar et al. 2017; Naik et al., 2022).

Broth dilution assay for MIC (Minimum Inhibitory Concentration): Serial dilutions are made from the selected solvents of the plant extracts or stock solution. Different concentrations of the plant extracts are prepared from the stock solution of the different solvents of plant extracts respectively. 500 µl of the bacterial inoculum and 500 µl of the plant extract are added to 2 ml of the sterile nutrient broth. The inoculation is carried out for different concentration of the plant extracts for the selected solvents. For positive control, inoculum is taken in sterile nutrient broth and as negative control, sterile nutrient broth is taken and all are inoculated at 37°C for 24 hours. After 24 hours, turbidity is checked, compared, and recorded. The results are expressed with mean standard deviation (Figure 6; Nayak et al., 2015; Kumar et al. 2017; Soren et al., 2018; Sarangi et al. 2021).

Data interpretation: After the incubation, the tubes showing no visible growth after 24 hours show inhibition of bacteria which represent MIC values of a respective concentration. Inoculum control shows visible growth due to no antimicrobial agents, whereas the broth control shows no growth due to

absence of bacteria. Triplicates are maintained and the experiment is repeated thrice, for each replicate. The readings are taken as foresaid.

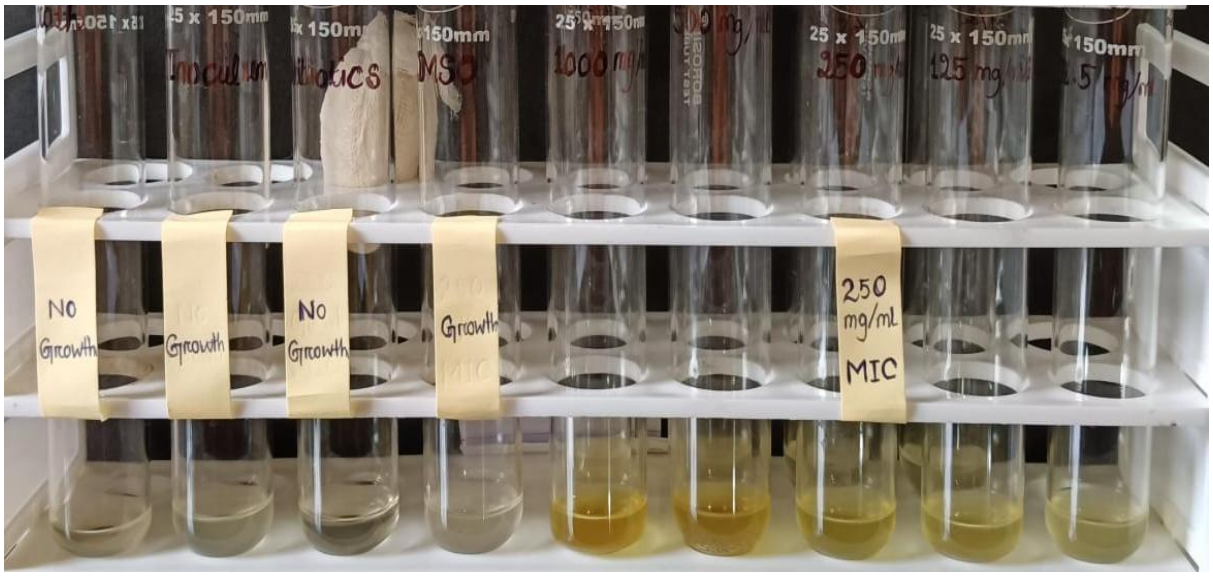

Figure 6: Minimum inhibitory concentration against *Streptococcus pyogenes*

Conclusion

In the present communication, the methods outlined in this review provide a comprehensive framework for the ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for the pharmacological evaluation of medicinal plants. These methods can be applied to various medicinal plants, providing valuable insights into their therapeutic potential. The importance of conserving and sustainably using medicinal plants cannot be overstated, and this paper aims to contribute to this effort by providing a detailed account of the methods used in medicinal plants research. By employing these methods, researchers can work together to discover new drugs, promote sustainable development, and improve human health.

References

Agarwal B, Rathore S, Krishnan R, Jaiswal A, Panda A, Hegde L and Kumar S. (2023). A check list on wild nutraceutical tuberous plants of India. Indian Forester. 149(5): 587-590.

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG and Lightfoot DA. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 6(4): 42. DOI: 10.3390/plants6040042

Asigbaase M, Adusu D, Musah AA, Anaba L, Nsor CA, Abugre S and Derkyi M. (2023). Ethnobotanical and ethnopharmacological survey of medicinal tree species used in the treatment of diseases by forest-fringe communities of Southwestern Ghana. Heliyon. 10(1): e23645. DOI: 10.1016/j.heliyon.2023

Basole S, Jena N and Kumar S. (2024). Indian Medicinal Plants, Volume 1. Ambika Prasad Research Foundation, Odisha, India.

Bhatnagar S, Panda P and Behera DR. (2013). Phytochemical analysis and evaluation of cytotoxic and antioxidant activity of fruit extracts of *Terminalia racemosa.* International Journal of Pharmacognosy and Phytochemical Research. 5(3): 151-154.

Chang CC, Yang MH, Wen HM and Chern JC. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 10(3): 3-5.

Chen SL, Yu H, Luo HM, Wu Q, Li CF and Steinmetz A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese Medicine. 11: 37. DOI: 10.1186/s13020-016-0108-7

Das K, Basole SG, Neeraj, Shivakumar P, Rout S, Kumar S and Saradar B. (2024). Phytochemical screening of *Cucumis melo* L., fruits (Cucurbitaceae). In: Plants & Secondary Metabolites, Volume 3. Ambika Prasad Research Foundation, Odisha, India.

Das K, Mahendru N, Sharma BP, Shivakumar P, Kumar N, and Kumar S. (2025). Ornamental plants of Bignoniaceae family: source of bioactive compounds with therapeutic applications and ecological services. Asian Journal of Environment & Ecology. 24(1): 79-86.

Das L, Mishra S, Das A, Dimri R and Kumar S. (2022). Some common flora of temple city of Odisha, India: source for ethno-medico-cultural values. Indian Forester. 148(2): 207-212.

Das MK, Das PK, Kumar S and Singh NR. (2020). Flora of Khandagiri & Udayagiri, an urban heritage of Odisha. Indian Forester. 146(6): 509-518.

Dash HB, Kumar S, Saha M, Devi RS and Biswal SK. (2022). Estimation of secondary metabolites and toxicity to *Artemia salina* L. of selected plants of family Combretaceae: an approach for screening of herbal preventive agents. International Journal of Pharmaceutical Sciences and Research. 13(2): 969-976.

Devi RS, Manjula BL, Mukherjee S, Watakhere DN, Satpathy KB and Kumar S. (2024). Cytotoxicity analysis of selected plants using *Artemia salina*. Annals of Agro-Bio Research. 29(1): 45-49.

Devi RS, Satapathy KB and Kumar S. (2022). Ethnomedicinal plants of Phumdi, Loktak Lake, Manipur, India. Asian Pacific Journal of Health Sciences. 9(4): 77-80.

Devi RS, Satapathy KB and Kumar S. (2025). Determining antibacterial activity of ethanol extracts through chromatography of *Oenanthe javanica* (Blume) DC., collected from Loktak lake, Manipur, India. Pleione.19(1): 133-141.

Eloff JN, Katrere D and Mc Gaw LJ. (2008). The biological activity and chemistry of the Southern African Combretaceae. Journal of Ethnopharmacology. 119(3): 686-699.

Hossain E, Chandra G, Nandy AP, Gupta JK and Mandal SC. (2012). Possible fasciocidal activity of methanol extract of *Dregea volubilis* leaves. Experimental Parasitology. 135(2): 183-187.

Hossain E, Chandra G, Nandy AP, Mandal SC and Gupta JK. (2012). Anthelmintic effect of a methanol extract of leaves of *Dregea volubilis* on *Paramphistomum explanatum*. Parasitology Research. 110(2): 809-814.

Jain SK and Rao RR. (1976). A handbook of field and herbarium methods. Today and Tomorrow's Printers and Publishers, New Delhi, India.

Jena N, Rote S, Devi RS and Kumar S. (2024). Phytochemical and cytotoxicity analysis of fruits of *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J. Wen: a minor fruit plant of Odisha, India. e-planet. 22(1): 62-70.

Jena N, Rout S, Mishra S and Kumar S. (2025). Evaluation of quantitative ethnobotanical uses in Mayurbhanj district, Odisha. Ambika Prasad Research Foundation, Odisha, India.

Kumar S and Dash D. (2012). Flora of Nandan Kanan Sanctuary: Medicinal plants with their role in health care. International Journal of Pharmacy & Life Sciences. 3(4): 1631-1642.

Kumar S and Jena PK. (2014). Chromatographic, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. Plant Science Research. 36(1 & 2): 83-90.

Kumar S and Maggirwar R. (2021). Phytochemical screening and antibacterial activity of *Dioscorea bulbifera* L. fruits. In: Plant Archives, Volume 1. Ambika Prasad Research Foundation, Odisha, India.

Kumar S and Satapathy MK. (2011). Medicinal plants in an urban environment; herbaceous medicinal flora from the campus of Regional Institute of Education, Bhubaneswar, Odisha. International Journal of Pharmacy & Life Sciences. 2(11): 1206-1210.

Kumar S, Das G, Shin HS, Kumar P and Patra JK. (2018). Diversity of plant species in the Steel City of Odisha, India: ethnobotany and implications for conservation of urban bio resources. Brazilian Archives of Biology and Technology. DOI:10.190/1678-4324-2018160650

Kumar S, Jyotirmayee K and Sarangi M. (2013). Thin Layer Chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. International Journal of Pharmaceutical Sciences Review and Research. 18(1): 126-132.

Kumar S, Mahanti P, Singh NR, Rath SK, Jena PK and Patra JK. (2017). Antioxidant activity, antibacterial potential and characterization of active fraction of *Dioscorea pentaphylla* L. tuber extract collected from Similipal Biosphere Reserve, Odisha, India. Brazilian Journal of Pharmaceutical Sciences. 53(4): e17006. DOI: 10.1590/s2175-97902017000417006

Kumar S, Tripathy PK and Jena PK. (2012). Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and its toxicity to *Artemia salina*. Plant Science Research. 34(1&2): 93-96.

Maity SK, Patra A, Pal B and Malakar A. (2018). Palyno-taxonomic study of some members of Malvaceae in Purba Medinipur, West Bengal. Annals of Plant Sciences 7(2): 2069-2072.

McClatchey WC, Mahady GB, Bennett BC, Shiels L and Savo V. (2009). Ethnobotany as a pharmacological research tool and recent developments in CNS-active natural products from ethnobotanical sources. Pharmacology & Therapeutics. 123(2): 239-254.

Mekonnen AB, Mohammed AS and Tefera AK. (2022). Ethnobotanical study of traditional medicinal plants used to treat human and animal diseases in Sedie Muja district, South Gondar, Ethiopia. Evidence-based Complementary and Alternative Medicine. 2022:7328613. DOI: 10.1155/2022/7328613

Mishra RC, Kumar S, Rani DR and Bhandari DC. (2012). Empirical tribal claims and correlation with bioactive compounds: a study on *Celastrus paniculata* Willd., a vulnerable medicinal plant of Odisha. Indian Journal of Traditional Knowledge. 11(4): 615-622.

Mohanty M, Choudhury R, Kumar S and Maggirwar R. (2021). Phytochemical screening and antibacterial activity of *Dioscorea bulbifera* L. fruits. Plant Archives. 21(1): 862-866.

Mohapatra S, Agasti S and Devi RS. (2020). Phytochemistry of food plants of *Columba punicea* Tickell 1842. Journal of Biodiversity and Conservation. 4(1): 293-293.

Mukherjee S, Dagawal MJ, Dhal A, Sreedevi A, Marndi S, Kumar S and Sahu JK. (2025). Ecological services and Indigenous Traditional Knowledge (ITK) of *Terminalia* L., species of India: a conservation aspect. Indian Forester. 151(2): 173-176.

Murmu P, Kumar S, Patra JK, Singh NR and Rath SK. (2016). Ethnobotanical, nutritional, phytochemical and antimicrobial studies of *Garcinia xanthochymus* fruit extracts. Biotechnology Journal International. 13(2): 1-11.

Mykhailenko O, Jalil B, McGaw LJ, Echeverría J, Takubessi M and Heinrich M. (2025). Climate change and the sustainable use of medicinal plants: a call for "new" research strategies. Frontiers in Pharmacology. 15:1496792. DOI: 10.3389/fphar.2024.1496792

Naik SK, Manjula BL, Balaji MV, Marndi S, Kumar S and Devi RS. (2022). Antibacterial activity of *Justicia betonica* Linn. Asian Pacific Journal of Health Sciences. 9(4S): 87-90.

Nayak S, Chakroborty S, Bhakta S, Panda P, Mohapatra S, Kumar S, Jena PK and Purohit C. (2015). Design and synthesis of (E)-4-(2-Phenyl-2H-Chromine-3-yl)-but-3-en-2-ones and evaluation of their in vitro antimicrobial activity. Letters in Organic Chemistry. 12: 1-7.

Obadoni BO and Ochuko PO. (2001) Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences. 8: 203-208.

Oyebode O, Kandala NB, Chilton PJ and Lilford RJ. (2016). Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 31(8): 984-991.

Patra A, Mondal AK and Banerjee D. (2017). Traditional phytotherapeutic uses in Purba Medinipur, West Bengal, India. International Journal of Pharmaceutical Sciences and Research. 8(9): 3904-3910.

Patra JK, Das G, Kumar S and Thatoi H. (2019). Ethnopharmacology and Biodiversity of Medicinal Plants. Apple Academic Press, New York. DOI: 10.1201/9780429398193

Pyka A, Dołowy M and Gurak D. (2005). Separation of selected bile acids by TLC. V. influence of temperature on the separation, Journal of Liquid Chromatography & Related Technologies. 28(4): 631-640.

Raina AP and Misra RC. (2023). Phytochemical evaluation of *Costus speciosus* (Koen) J. E. Sm. germplasm from Eastern India-an important antidiabetic plant. Medicinal Plants. 15(4): 723-731.

Rout S, Devi RS and Kumar S. (2024). Phytochemical, cytotoxicity and thin layer chromatography analysis of *Ophioglossum reticulatum* L.: a wild fern species. e-planet. 22(2): 181-187.

Sadasivam S and Manickam A. (2009). Biochemical methods. 3rd Edition. New Age International Publishers, New Delhi.

Saha M, Roy AB, Kumar S and Datta BK. (2022). Ethnobotanical diversity of some wetland plants of North-east India: implications for conservation. The Indian Forester. 148(11). DOI: 10.36808/if/2022/v148i11/158704

Sahu JK, Kumar S, Mishra S, Marndi S, Nayak NK and Malwal M. (2025). Diversity and significance of medicinal Araceae species in India: a review of their food, medicinal and ecological aspects. Asian Journal of Environment & Ecology. 24(5): 68-75.

Saradar B, Sampatrao TS, Nimbalkar VS, Rani A, Gupta A and Kumar S. (2023). Ethnobotanical survey of medicinal plants used in different health problems. In: Indigenous Traditional Knowledge and Ethnopharmacology. Ambika Prasad Research Foundation, Odisha, India. DOI: 10.5281/zenodo.7827896

Sarangi A, Rote K, Kumar A, Manjramkar H and Devarakonda R. (2021). Antibacterial activity of *Viscum articulatum*. In: Medico-Biowealth of India, Volume 3. Ambika Prasad Research Foundation, Odisha, India.

Sethi J, Devi RS, Jena N and Kumar S. (2024b). New distributional record of *Alysicarpus rugosus* (Willd.) DC. from the state Odisha, India. Asian Plant Research Journal. 12(1): 8-12.

Sethi J, Jena N and Kumar S. (2024a). Medicinal Plants of Rourkela Forest Division, Odisha, India. Asian Journal of Environment & Ecology. 23(12): 69-84. DOI:10.9734/ajee/2024/v23i12635

Sharma BP, Das K, Sinha MK, Yadav R, Kaminee, Lal S, Devi RS and Jain D. (2024). Preserving biodiversity: the critical role of herbaria in plant conservation. Journal of Biodiversity and Conservation. 8(4): 140-151.

Shirai M, Takano A, Kurosawa T, Inoue M, Tagane S, Tanimoto T, Koganeyama T, Sato H, Terasawa T, Horie T, Mandai I and Akihiro T. (2022). Development of a system for the automated identification of herbarium specimens with high accuracy. Scientific Reports. 12(1): 8066. DOI: 10.1038/s41598-022-11450-y

Soren S, Kumar S, Mishra S, Jena PK, Verma SK and Parhi P. (2018). Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. Microbial Pathogenesis. 119: 145-151. DOI: 10.1016/jmicpath.2018.03.048

Swain T and Hillis WE. (1959). The phenolic constituents of *Prunus domestica*. I.-the quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 10: 63-68.

Swargiary A, Daimari A, Daimari M, Basumatary N and Narzary E. (2016). Phytochemicals, antioxidant, and anthelmintic activity of selected traditional wild edible plants of lower Assam. Indian Journal of Pharmacology. 48(4): 418-423.

Tiwari SD, Tyagi CK, Budholiya P. (2020). Preliminary phytochemical evaluation and HPTLC profile of *Celastrus paniculatus* seed. Saudi Journal of Medical and Pharmaceutical Sciences. 6(11): 692-697.