International Journal of Secondary Metabolite
2025, Vol. 12, No. 1, 166–180
https://doi.org/10.21448/ijsm.1469608
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Molecular identification and phytochemical profiling of selected medicinal plants in Bongabon, Nueva Ecija, Philippines

Dana Theresa De Leon[1*], **Arwil Nathaniel Alfonso**[2], **Angeles De Leon**[1,3,4], **Jerwin Undan**[1,2,3]

[1]Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines
[2]Biotechnology and Analytical Laboratory, Central Luzon State University, Science City of Munoz Nueva Ecija, Philippines
[3]Tuklas Lunas Development Center, Central Luzon State University, Science City of Munoz Nueva Ecija, Philippines
[4]Ethnobiology Research Laboratory, College of Science, Central Luzon State University, Science City of Munoz Nueva Ecija Philippines

ARTICLE HISTORY
Received: Apr. 19, 2024
Accepted: Sep. 19, 2024

KEYWORDS
Pharmacology,
Plant-based medicine,
Toxicity,
Phytochemicals,
Radical scavenging activity.

Abstract: The study focuses on the five medicinal plants used by the local people residing in Calaanan, Bongabon Nueva Ecija Philippines. The study aimed to investigate using DNA-based identification, phytochemical screening, and antioxidant analysis of the plant's ethanolic extract. The selected five plants were initially identified by a taxonomist and molecularly identified using the *rbc*L gene marker. These plants were identified as *Scoparia dulcis, Vachellia fernasiana, Centella asiatica, Sapindus saponaria,* and *Ocimum tenuiflorum*. The extracts of the plants underwent Fourier Transform Infrared spectroscopy (FTIR) analysis to determine the functional group present in each plant and further analysis led to Thin Layer Chromatography (TLC) to unveil the presence and absence of the plant's secondary metabolites. The phytochemical profiles revealed the presence of essential oils, phenols, fatty acids, anthraquinones, anthrones, coumarins, flavonoids, and tannins. The results from the phytochemical analysis demonstrated the chemical diversity of the plant, prompting further investigations into its various bioactive properties. Further, the plants were subjected to 2,2'diphenyl-1-1picrylhydrazyl (DPPH) radical scavenging assay using a 1000ppm concentration of crude extracts, results revealed a range from 22.71% to 79.01% radical scavenging activity compared to the control which is at 83.56%. Collectively, this study reveals the accurate identity, phytochemical profile, and antioxidant activity of the medicinal plants.

1. INTRODUCTION

Plants were long recognized as vital sources of novel pharmacologically active compounds, which played a crucial role in drug development within the pharmaceutical industry. Their historical significance in treating various diseases underscored their importance in medical science (Boy *et al*., 2018; Veeresham *et al*., 2012) Plant-based traditional medicines remained

*CONTACT: Jerwin UNDAN jerwinundan@clsu.edu.ph Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

e-ISSN: 2148-6905

widespread due to their affordability, efficacy, and minimal side effects in treating common ailments. In addition, plant extracts emerged as a valuable resource for the discovery of new compounds and the development of new pharmaceutical products.

In the Philippines, medicines and health services from physicians and other healthcare professionals are difficult to obtain in a geographically distant plunge location where people are physically or economically constrained (Lazarte, 2020). More than 12,000 different plant species may be found in the Philippines, of which, 1500 are used in traditional medicine by the indigenous peoples (Dela Cruz & Ramos 2006; Tantengco *et al*., 2018). Indigenous communities in the Philippines have used plants to cure a variety of illnesses, from less serious to potentially fatal ones (Balangcod & Balangcod, 2015).

The utilization of plants for medicinal purposes has been an important part of the cultural heritage of Philippine provinces for centuries. Through generations, indigenous and local communities have passed down invaluable knowledge about the practical use of local flora, offering effective remedies for common ailments. Depending on the location, the inaccessibility of the people communities adopted a deep dependence on natural resources, highlighting the importance of being familiar with the plants and their deep understanding of the efficacy and safety of the plants. The study identified five medicinal plants, *namely Scoparia dulcis, Vachellia fernasiana, Centella asiatica, Sapindus saponaria,* and *Ocimum tenuiflorum* confirmed by its DNA with matched sequences on the GenBank database. Before the conduct of this study, an initial survey for the knowledge and practices of the locals and collection of medicinal plants was provided by the research under the project of the Department of Science and Technology - Regional Office III of the Philippines.

The Barangay Calaanan in Bongabon, Nueva Ecija Philippines was surrounded by agricultural lands, rivers, and mountains. The residents live close to a mountainous region abundant with a diverse range of plants suitable for medicinal purposes. The distant location of the local residents from the community prompted them to make use of medicinal plants. However, the utilization of medicinal plants carries potential risks, as excessive dosages or the presence of harmful effects can lead to serious health issues shortly after consumption. The danger arises from the challenge some locals face in accurately discerning the safe quantity of medicinal plants to use, thus running the risk of encountering toxic or even life-threatening effects later on. In the interest of providing proper information for the locals, the goal of this research was to identify plants using molecular techniques, particularly those unknown to them. Despite their existing knowledge about the medicinal applications of the plants, understanding their bioactive constituents was similarly important. To validate their medicinal uses, the study also determined the antioxidant potential of the collected medicinal plants.

2. MATERIAL and METHODS

2.1. Molecular Identification

2.1.1. *DNA extraction*

The plant leaves were obtained in February 2022, from Barangay Calaanan in Bongabon, Philippines. Samples and a photograph voucher were initially identified by a taxonomist at Central Luzon State University. The extraction of DNA was carried out using Cetyltrimethylammonium bromide (CTAB) method followed by the published paper of Abdel-Latif and Osman (2017).

2.1.2. *PCR amplification*

Polymerase Chain Reaction (PCR) amplification of the nuclear ribosomal DNA was carried out using a primer pair of ribulose 1,5-biphosphate carboxylase *rbc*L forward (5′-ATGTCACCACAAACAGAGACTAAAGC-3′) and *rbc*L reverse (5′-GTAAAATCAAGTCC ACCRCG-3′) (CBOL Plant Working Group *et al*., 2009). The gene *rbc*L had the strongest characterization among plastid genes. It was now simple to retrieve across land plants owing to advances in primer design, and it was ideal for recovering high-quality bidirectional sequences

(Fazekas *et al*., 2008; CBOL Plant Working Group *et al*., 2009). The PCR reaction was composed of a 25µL mixture (manufacturer's protocol) containing the following: 1µL of genomic DNA, 1µL of each rbcL primer, 12.5µL of 1X GoTaq® Green Master Mix (Promega Corporation, USA), and 9.5µL of nuclease-free water. Samples were run into Veriflex™ 96-Well Thermal Cycler (Applied Biosystems, California, USA), and the conditions were programmed as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 51.2°C for 30 sec, extension at 72°C for 60 sec, and a final cycle of extension at 72°C for 5 min. The quality of PCR amplicons was checked following the same method described in the preceding section. The PCR product was quantified using Qubit 4 Fluorometer (Invitrogen Thermo Fisher Scientific, USA) and PCR purified before sequencing.

2.1.3. *DNA sequencing and sequence alignment*

Unpurified PCR products were sent to Apical Scientific in Selangor Malaysia for PCR Product Cleaning and DNA sequencing. The validated sequence data generated using the forward primer and reverse primer were edited and aligned using a pairwise alignment tool, and consensus sequences were conducted using the BioEdit 7.2 (Hall, 1999) application. The nucleotide sequence comparisons were performed using the standard nucleotide Basic Local Alignment Search Tool (BLASTN) against the National Center for Biotechnology Information (NCBI) GenBank database.

2.2. Phytochemical Analysis

2.2.1. *Preparation of extracts*

The air-dried samples were ground and sieved into a powder form using an automated grinder. Ten grams (10 g) of powdered sample were soaked in 90% ethanol for three days with frequent agitation. After three days, the soaked samples were filtered using a No. 2 Whatman filter. The filtrates were concentrated to dryness using a digital rotary evaporator (DLAB™, Flinn Scientific, Canada). The resulting extracts were used for various analyses and assays.

2.3. Phytochemical Analysis using FTIR Spectroscopy

To confirm the presence of secondary metabolites determined in phytochemical analysis using the TLC method, the ethanolic extract of selected medicinal plants was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) at the Central Instrumentation Facility, De La Salle University, Laguna Campus.

2.4. Phytochemical Analysis using Thin Layer Chromatography

The phytochemical screening of the ethanolic extract of collected medicinal plants was determined at the Chemistry Laboratory of the Center for Natural Sciences at St. Mary's University, Bayombong, Nueva Vizcaya. Phytochemical screening was performed to detect the secondary metabolites of the extract. The extract was spotted on a 7 x 4 cm marked and labeled TLC (thin layer chromatography) plate. This was done in the developing chamber using an acetate-methanol (7:3) mixture. To test the separation of the different substances, the spots for specific metabolites were observed using TLC plates that were subjected to UV light and a hot plate. Vanillin-sulfuric acid reagents were used to detect the presence of phenols, steroids, triterpenes, and essential oils. A methanolic potassium hydroxide was used to detect secondary metabolites such as anthraquinones, coumarins, and anthrones. The phenolic compounds and tannins were determined using the potassium ferricyanide-ferric chloride reagent. Lastly, Dragendorff's reagent was used to identify the presence of flavonoids (Guevara, 2005).

2.5. Screening of the Antioxidant Property

The 2,2'diphenyl-1-1picrylhydrazyl (DPPH) radical scavenging activity and the total phenolic content of the collected medicinal plant ethanolic extracts were also performed at the Chemistry Laboratory of the Center for Natural Sciences at St. Mary's University, Bayombong, Nueva Vizcaya.

The radical scavenging activity of bioactive chemicals extracted from medicinal plants was determined using the DPPH assay adapted from the methods of Kolak *et al*., 2006. Concentrated extract was used as a stock solution, and aliquots were taken to create 1000 ppm dilutions and 1000 ppm Catechin (1mg/mL) as controls. In a separate plastic cuvette, 4mL of 0.1 mM DPPH solution was combined with 1mL of prepared stock solution. The reactions were carried out in triplicate. The prepared mixtures were incubated in the dark for 30 minutes at 37°C. The absorbance measurements were checked at 517 nm using a UV-VIS spectrophotometer. Lower absorbance of the reaction mixture determined higher free radical scavenging activity. The radical scavenging activities were compared to Catechin activity, and the following equation was used to calculate the ability to scavenge the DPPH radical:

$$\%\ Radical\ Scavenging\ Effect = [\ (A_{control} - A_{sample}) / A_{control}\ ]\ x\ 100$$

Where $A_{control}$ is the absorbance of the control which is the DPPH without the sample. A_{sample} is the absorbance of the test sample containing the DPPH sample. Catechin was used as the positive control.

3. FINDINGS

3.1 Molecular Identification and Plant Assessment

In the present study, the five plants (Figure 1) declared by the local people of Bongabon Nueva Ecija, Philippines as medicinal plants were molecularly identified. The BLAST (NCBI-Genbank) tool was employed to determine the homology of the plant samples (see Table 1). According to the Consortium for the Barcode of Life (2009), an optimal DNA barcode marker should be easily amplifiable, exhibit sufficient variability for species identification, and offer maximum discrimination among species. Hence, PCR amplification of *rbc*L forward and reverse primer pairs was used to establish and produce a single band of approximately 570 base pairs.

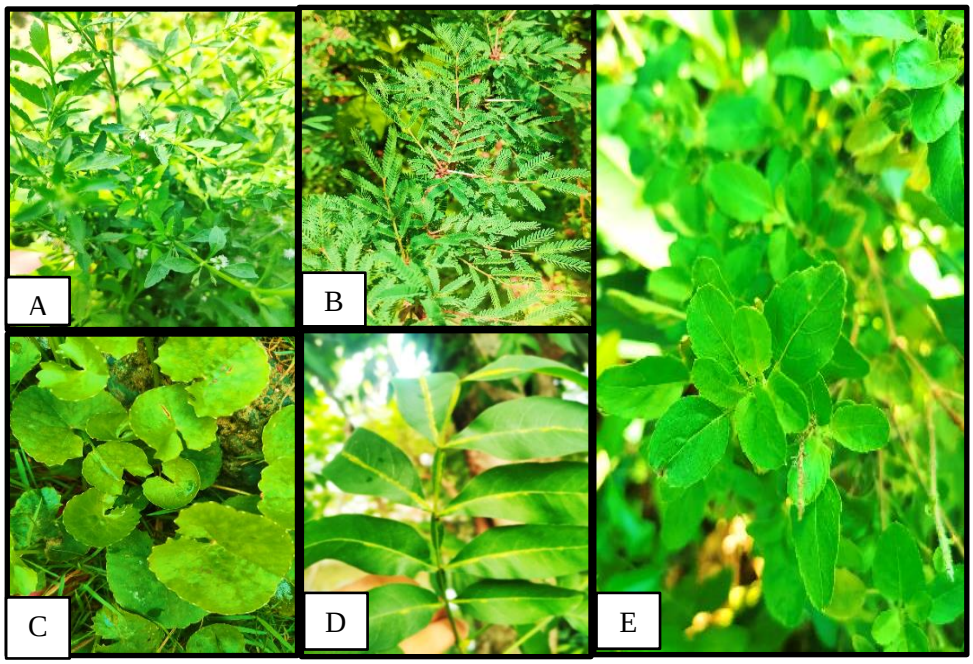

Figure 1. Photograph image of the five collected plants (A. *Scoparia dulcis* B. *Vachellia farnesiana* C. *Centella asiatica* D. *Sapindus saponaria* E. *Ocimum tenuiflorum*)

Table 1. BLAST analysis results or ribosomal DNA sequences of the five collected plants.

Sample Code	BLASTn Identity	% Identity	Query Cover	Accession Code
CB2	*Scoparia dulcis*	99.82	100	MZ958832.1
CB3	*Vachellia farnesiana*	99.82	100	MN592492.1
CB4	*Centella asiatica*	99.82	100	MN854377.
CB9	*Sapindus saponaria*	99.64	99	AY724366.1
CB10	*Ocimum tenuiflorum*	99.82	100	NC.043873.1

Based on the gathered data from the interview conducted by the Central Luzon Health and Research Department (CLHRDC) team, it was reported that the listed plants had been declared by locals to have been used as remedies for various diseases. The collected plants comprised primarily shrubs and trees; however, according to available literature, not all parts of these plants were utilized for medicinal purposes. To support, the consolidation of data from books and online sources was also cited to support these findings (see Table 2).

The *S. dulcis* is a compact shrub, with its roots and leaves historically utilized for a diverse range of remedies, including addressing dysentery and fever. On the other hand, the bark, roots, and leaves of *V. farnesiana* shrub were employed for treating sore throats and treats skin diseases. Similarly, *C. asiatica*, another shrub in the collection, was consumed for dysentery and colds, focusing specifically on the use of its leaves. *Ocimum tenuiflorum,* another shrub, had its roots and leaves applied for the treatment of conditions such as coughs, bronchitis, fungal infections, and malaria. In contrast, the lone tree in the assortment, *S. saponaria*, boasts a comprehensive use of its bark, fruit, roots, and leaves as remedies for external wounds, lesions caused by fungi, and ulcers.

The assortment of these five plants showcased a diverse selection of applications in addressing various diseases. Remarkably, many of these plants were commonly found in close proximity to households, highlighting the richness of natural resources in the study area and the accessibility of those plants for the local population to use as cost-effective remedies. This underscored the importance of traditional knowledge and the potential of local flora in providing healthcare solutions.

Table 2. List of the Medicinal Plants Species Collected in Calaanan, Bongabon Nueva Ecija.

Species	**Medicinal Value**	**Parts Used**	**Medicinal References**
Local Name: Culantro Family: Plantaginaceae Scientific name: *Scoparia dulcis* Leaf shape: Lanceolate Leaf margin: Serrate Leaf venation: Pinnate Habit: Shrub	gastralgia, diarrhea, and dysentery, fever, cough	root, leaves	1
Local Name: Aroma Family: Acacieae Scientific name: *Vachellia farnesiana* Leaf shape: Compound Leaf margin: Entire Leaf venation: Pinnate Habit: Shrub	astringent, sore throats, antispasmodic, skin diseases	bark, leaves, roots	1, 2

Local Name: Takip kuhol Family: Scientific name: *Centella asiatica* Leaf shape: Reniform Leaf margin: Serrate Leaf venation: Palmate Habit: Shrub	skin diseases, sclerotic wounds, fever, colds, dysentery	leaves	1, 2
Local Name: *Unknown* Family: Sapindaceae Scientific name: *Sapindus saponaria* Leaf shape: Compound Leaf margin: Entire Leaf venation: Pinnate Habit: Tree	ulcers, external wounds, skin lesions caused by fungi	bark, fruit, roots, leaves	1, 2
Local Name: Biday Family: Sapindaceae Scientific name: *Ocimum tenuiflorum* Leaf shape: Elliptical Leaf margin: Serrate Leaf venation: Pinnate Habit: Shrub	cough, bronchitis, asthma, malaria, dysentery, stress situations, worm infestations, superficial fungal infections, and as diuretic	bark, fruit, roots, leaves	1

Note: Plant families, followed by simple description, habit (T = tree, S = shrub, H = herb, V = vine, E = epiphyte) and medicinal references (1 = www.stuartxchange.org/CompleteList.html, 2 = www.tkdlph.com, 3 = other sources)

3.2. Phytochemical Analysis (Fourier Transform Infrared Spectroscopy)

Infrared (IR) spectroscopy serves as a valuable spectroscopic technique when combined with High Performance Thin Layer Chromatography (HP-TLC). Its notable advantages include the minimal or absence of sample preparation, making it an environmentally friendly and reagent-free tool that delivers results within a few minutes (Dytkiewitz & Morlock, 2008; Agatonovic-Kustrin, & Morton, 2020). The chemical bonds and functional groups present in the crude extracts of the leaves were predicted using the FTIR. Figures 1-5 represent the FTIR spectrum and Table 2 is the interpretation of the chemical bonds in the 5 collected plant extracts. Each functional group possesses a unique assigned wave number (cm^{-1}), which the FTIR spectrometer reads. The presence of a peak at specific wavelengths signifies the presence of the corresponding functional group. The FTIR spectrum confirmed the presence of the 8 functional groups (see Table 3). These were the alcohols or phenols group, alkanes or methyl group, carbonyl group, aromatic compounds, methyl or methylene group, alkanes group, ethers or esters group and amines or amides group.

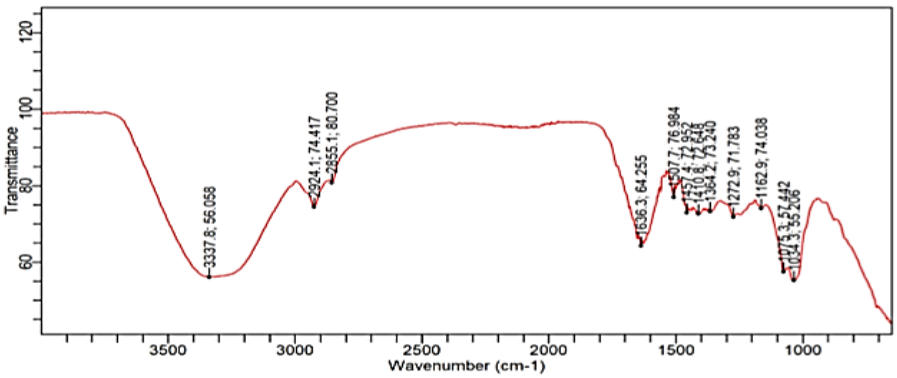

Figure 2. FTIR Spectrum of *Scoparia dulcis* leaves ethanolic crude extract.

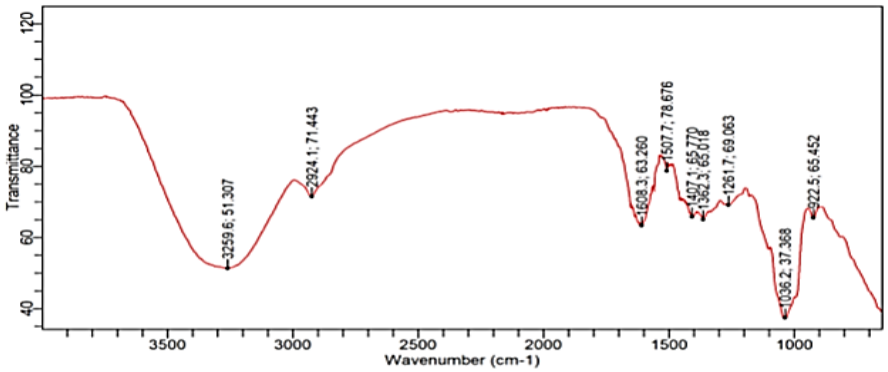

Figure 3. FTIR Spectrum of *Vachellia fernasiana* leaves ethanolic crude extract.

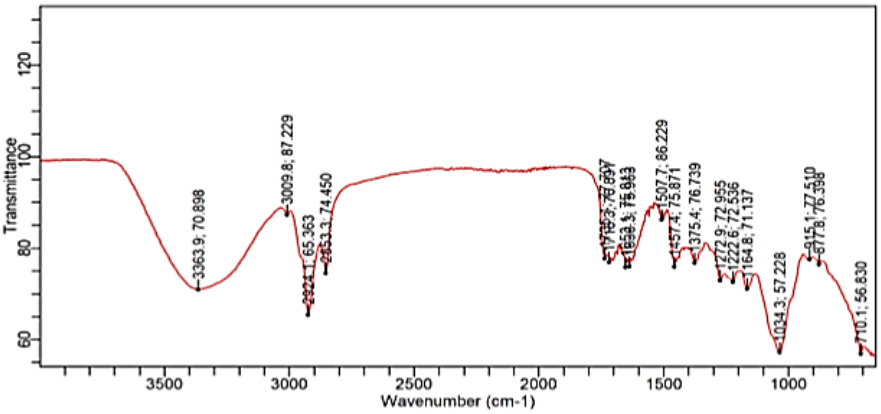

Figure 4. FTIR Spectrum of *Centella asiatica* leaves ethanolic crude extract.

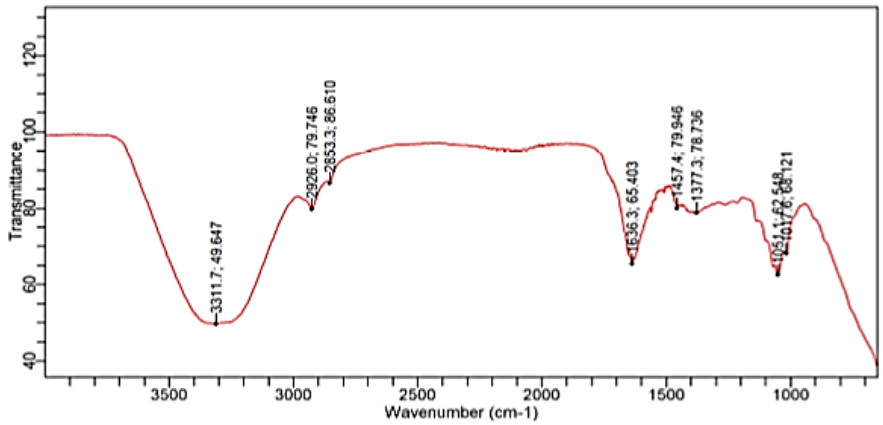

Figure 5. FTIR Spectrum of *Sapindus saponaria* leaves ethanolic crude extract

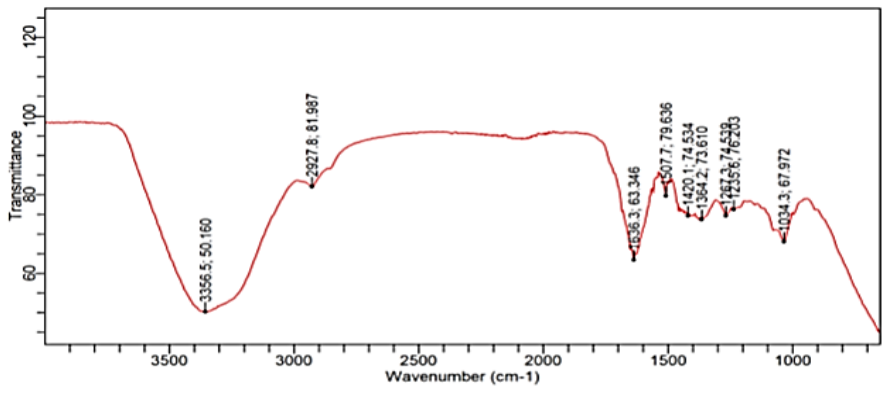

Figure 6. FTIR Spectrum of *Ocimum tenuiflorum* leaves ethanolic crude extract

Table 3. FTIR spectral peak values, functional groups and the possible presence of secondary metabolites of the five plants' ethanolic leaf extract.

Plant Samples	Peaks (cm-1)	Molecular Vibration	Functional Group	Possible Presence	References
Scoparia dulcis	3337.8	O-H stretching	Alcohol or Phenols group	Phenols, Tannins, Fatty Acids	1, 5
	2924.7	C-H stretching	Alkanes or Methyl group	Alkaloids	2
	1636.4	C=O stretching	Carbonyl group	Anthraquinones, Tannins, Flavonoids	2, 5
	1507.7	C=C stretching	Aromatic compounds	Flavonoids, Alkaloids, Saponins	2
	1457.4	C-H bending	Methyl or Methylene group	Flavonoids, Tannins	3
	1162.9	C-O stretching	Ethers or Esters group	Flavonoids, Coumarins	2
	1075.3	C-N stretching	Amines or Amides group	Alkaloids	4
	1034.3	C-H bending	Aromatic compounds	Essential oils	5
Vachellia fernasiana	3259.6	O-H stretching	Alcohol or Phenols group	Phenols, Tannins, Fatty Acids	1, 5
	2924.1	C-H stretching	Alkanes or Methyl group	Alkaloids	2
	1608.3	C=C stretching	Aromatic compounds	Flavonoids, Alkaloids, Saponins	2
	1362.3	C-H bending	Methyl or Methylene group	Flavonoids, Tannins	3
	1036.2	C-H bending	Aromatic compounds	Essential oils	5
Centella Asiatica	3363.9	O-H stretching	Alcohol or Phenols group	Phenols, Tannins, Fatty Acids	1,5
	3009.8	C-H stretching	Alkanes or Methyl group	Alkaloids	2
	1718.3	C=O stretching	Carbonyl group	Anthraquinones, Tannins, Flavonoids	2, 5
	1507.7	C=C stretching	Aromatic compounds	Flavonoids, Alkaloids, Saponins	2
	1457.4	C-H bending	Methyl or Methylene group	Flavonoids, Tannins	3
	1162.9	C-O stretching	Ethers or Esters	Coumarins, Flavonoids	2
	1222.6	C-N stretching	Amines or Amides group	Alkaloids	4
	1034.3	C-H bending	Aromatic compounds	Essential oils	5
	710.1	C-H out-of-plane bending	Aromatic compounds	Essential oils	6
Sapindus saponaria	3311.7	O-H stretching	Alcohol or Phenols group	Phenols, Tannins, Fatty Acids	1, 5
	2926.0	C-H stretching	Alkanes or Methyl group	Alkaloids	2
	1636.3	C=O stretching	Carbonyl group	Anthraquinones, Tannins, Flavonoids	2, 5
	1457.4	C-H bending	Methyl or Methylene group	Flavonoids, Tannins	3
	1272.9	C-H bending	Alkanes	Alkaloids	4
	1051.1	C-O stretching	Ethers or Esters	Coumarins, Flavonoids	2
	1017.6	C-N stretching	Amines or Amides group	Alkaloids	4
Ocimum tenuiflorum	3356.5	O-H stretching	Alcohol or Phenols group	Phenols, Tannins, Fatty Acids	1, 5
	2927.8	C-H stretching	Alkanes or Methyl group	Alkaloids	2
	1636.3	C=O stretching	Carbonyl group	Anthraquinones, Tannins, Flavonoids	2, 5
	1507.7	C=C stretching	Aromatic compounds	Flavonoids, Alkaloids	2
	1420.1	C-H bending	Methyl or Methylene group	Flavonoids, Tannins	3
	1267.3	C-N stretching	Amines or Amides group	Alkaloids	4
	1034.3	C-H bending	Aromatic compounds	Essential oils	5

**References used:* 1. Shahidi, F., & Ambigaipalan, P. (2015); 2. Megawati *et al*., (2023); 3. Sahayaraj *et al*., (2015) 4. Mohanapriya *et al*., (2019); 5. Mwangi *et al*., (2024) 6. Topala L. & Ducu T. (2017)

3.3 Phytochemical Analysis (Thin Layer Chromatography)

In the study, samples were subjected to thin-layer chromatography, revealing the presence and absence of the 14 plant constituents tested in each plant leaf crude ethanolic extract (see Table 4). The preliminary FTIR analysis conforms with the qualitative phytochemical screening using TLC. The detected phytochemical compounds were recognized for their valuable significance in both industrial and medicinal fields. The results from the phytochemical analysis demonstrated the chemical diversity of the plant, prompting further investigations into its various bioactive properties. Based on the analysis, each plants have 7-9 phytochemicals present. Tons of records showed the benefits of these compounds detected from the five plants.

Table 4. Secondary metabolites present in the five medicinal plants.

Plant Constituents	*S. dulcis*	*V. farnesiana*	*C. asiatica*	*S. saponaria*	*O. tenuiflorum*
Essential Oils	+	+	+	+	+
Triterpenes	-	-	-	-	-
Sterols	-	-	-	-	-
Phenols	+	-	+	+	+
Fatty Acids	+	+	-	-	+
Sugars	-	-	-	+	-
Anthraquinones	+	+	+	+	+
Coumarins	-	-	-	+	+
Anthrones	-	+	+	+	-
Tannins	+	+	+	+	+
Flavonoids	+	+	-	+	-
Steroids	+	+	+	+	+
Alkaloids	+	+	-	-	-
Amino acids	-	+	+	-	+

**(+) Present (-) Absent*

3.4 Antioxidant Activity

The plants were subjected to a DPPH radical scavenging assay using a 1000ppm concentration of crude extracts. From the 5 samples tested (see Table 5), results showed that *O. tenuiflorum* had the highest percentage of radical activity at 79.01% that were comparable to the positive control Catechin which was at 83.56%. These samples were followed by *V. farnesiana* at 76.55%, *C. asiatica* at 73.38%, *S. dulcis* at 42.33%, and lastly *S. saponaria* at 22.71%.

Table 5. Percentage radical activity of the plant samples in comparison with the control.

Plant Samples	% Radical Scavenging Activity
Scoparia dulcis	42.33
Vachellia farnesiana	76.55
Centella asiatica	73.38
Sapindus saponaria	22.71
Ocimum tenuiflorum	79.01
Catechin (Control)	83.56

4. DISCUSSION and CONCLUSION

Traditional approaches for identifying plants involve using our senses (taste, sight, smell, touch), as well as examining their physical characteristics (shape, color, texture) through macroscopic and microscopic methods. Moreover, chemical profiling techniques such as TLC, HPLC-UV, and HPLC-MS are employed. The accuracy of chemical profiles can be influenced by physiological and storage conditions. Thus, authentication at the DNA level offers enhanced

reliability. Subsequently, the development of DNA-based markers plays a significant role in verifying the authenticity of medicinal plants (Techen *et al*., 2014). Due to inadequate morphological identification, unfamiliarity, confusion surrounding species identity due to similar local names, and the absence of local name confirmation, molecular identification of the plants is conducted.

For the purpose of mainly identification, the present study only used the rbcL primer pair since the CBOL Plant Working Group chose *rbc*L as the core barcode because of its significant advantage in terms of PCR success scores (China Plant BO; Li *et al*., 2011; Olivar *et al*., 2014). Results showed that the plant has 99% above identity and 99% - 100% query cover using the primer pair which indicates the sequence divergence between the collected plants and related species in the Genbank repository of NCBI. According to the study of Pearson (2013), identifying homologous sequences through sequence similarity searching stands as a pivotal and highly informative initial step in analyzing newly acquired sequences. The vast comprehensiveness of modern protein sequence databases ensures that over 80% of metagenomic sequence samples commonly exhibit substantial similarity with proteins cataloged within these databases.

Following proper identification, the plants underwent phytochemical analyses. Plants serve as the most potent sources of drugs, with many pharmaceutical products originating from traditional ethnomedicine practices. The indigenous wisdom concerning medicinal plants held by local communities represents a vital source of information that consistently contributes to contemporary herbal remedies (Balangcod *et al*., 2012). Plants can produce a large number of diverse bioactive compounds. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Clemens-Pascual *et al*., 2022). With the progress of phytochemical research, more and more plant constituents have been isolated and identified (De Leon *et al*., 2018).

Based on the peaks of the five plants, the FTIR spectrum confirmed the presence of the 8 functional groups. The molecular vibration of O-H stretching at a range of 3259.6-3363.9 cm^{-1} and CH stretching at the range of 2853.3-3009.3 cm^{-1} (Oliveira *et al*., 2016) was found to be present uniformly in the five plant samples. The O-H and C-H were common and noticeable in diverse chemical environments within secondary metabolites, encompassing alcohols, phenols, aldehydes, ketones, carboxylic acids, and ethers. This broad spectrum of functional groups allows plants to produce secondary metabolites containing a diverse array of chemical structures and biological activities. These activities cover antioxidant, antimicrobial, antiviral, anticancer, and anti-inflammatory properties (de Sousa *et al*., 2023).

In addition, the carbonyl group of C=O stretching (George *et al*., 2022) was also found in the plants with the exception of *V. fernasiana*. With the possible presence of fatty acids, anthraquinones, tannins, flavonoids, and steroids, according to the study of Mamari and Hamad (2021) compounds containing carbonyl groups frequently exhibit pharmacological properties beneficial for medicinal use. Specifically, these groups can contribute to antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. Another important functional group is the aromatic compound of C=C stretching at the peak range of 1507.7-1608.3 cm^{-1} (Teclegeorgish *et al*., 2021) was also present in the plants except for *S. saponaria;* the presence of phenols, unsaturated fatty acids, anthraquinones, flavonoids, alkaloids in this group may exhibit antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, analgesic, and neuroprotective activities, making them valuable candidates for drug discovery and development (Gu *et al*., 2014).

The methylene functional group of C-H stretching with a peak range of 1362.3-1457.4 cm^{-1} (Md Salim *et al*., 2021) was again found to be present in all the plants that have the possible presence of tannins and flavonoids. A review from the study of Atanasov *et al*., (2021) mentioned that methyl groups are prevalent in natural products obtained from plants, and play a significant role in the extensive array of bioactive compounds utilized for medicinal purposes.

Traditionally employed in alternative medicine, these compounds persist as crucial reservoirs of potential drug leads and pharmaceutical agents. Furthermore, the alkanes group of C-H bending with a peak of 1272.9 was only detected in *S. dulcis* and *C. asiatica*. Within this group, it is expected to have the possible presence of terpenoids, alkaloids, and steroids. According to Hussein *et al*., (2021), many traditional medicinal plants contain alkane-rich secondary metabolites that have been used for centuries in herbal remedies and traditional medicine systems worldwide.

Moreover, ether groups of C-O stretching at the peak range of 1051.1-1164.8 cm^{-1} (Devi *et al*., 2019) have been detected in *S. dulcis, C. asiatica*, and *S. saponaria*. It is expected to have a presence of coumarins and essential oils in this functional group. Many plant-derived ethers exhibit valuable biological activities relevant to medicinal applications. These activities may include antioxidant, antimicrobial, anti-inflammatory, analgesic, and sedative properties, among others. Ethers can also serve as bioactive components in traditional herbal remedies (Oluwapelumi *et al*., 2023). Another important functional groups were the amines or amides with C-N stretching at the peak ranges of 1017.6-1235.6 cm^{-1} (Devi *et al*., 2019) a presence of alkaloids was expected in this group and it was observed in *S. dulcis* and *C. asiatica.* These plants were used by a huge number of population in the Philippines and were very well known to be consumed for different medicinal applications supporting that the plants had various of study conducted. Amines are organic compounds characterized by the presence of a nitrogen atom bonded to one or more alkyl or aryl groups (Ertl *et al*., 2020). Many drugs contain amine functional groups, which can participate in hydrogen bonding, receptor interactions, and enzymatic reactions (Ishtiaq *et al*., 2020).

Lastly, the lowest peak recorded at 710.1 cm^{-1} represents the functional group of the aromatic compound with a vibration of C-H out of plane bending (Nortije *et al*., 2024). The possible presence of essential oils is expected to be found in the plant containing this peak. The presence of these functional groups provides valuable insight into the chemical properties and potential biological activities of the plant samples.

Moreover, plant samples were also subjected to thin-layer chromatography, revealing the presence and absence of the 14 plant constituents tested in each plant leaf crude ethanolic extract. Based on the analysis, each plants have 7-9 phytochemicals present. The phytochemicals showed diverse compounds like essential oils, phenols, flavonoids, anthraquinones, coumarins, tannins and alkaloids. Tons of records showed the benefits of these compounds detected from the five plants.

The results from the phytochemical analysis demonstrated the chemical diversity of the plant, prompting further investigations into its various bioactive properties. Hence, the conduct of antioxidant activity was also initiated and confirmed high levels of radical scavenging activity in all five plants. Plants contain various natural compounds like polyphenols, flavonoids, glutathione, vitamin E (α-tocopherol), vitamin C, and other metabolites. These molecules have the ability to scavenge free radicals, neutralize singlet and triplet oxygen, decompose peroxides, inhibit enzymes, and work together to prevent and treat diseases (Latayada and Uy, 2016). The build-up of harmful free radicals in the body can lead to various health conditions such as ischemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, Down syndrome, the aging process, and potentially dementia (Mundhe *et al*., 2011; Kaur & Mondal 2014).

To further understand the specific chemical components of plants, researchers are carrying out assays such as antioxidant screenings. These tests help determine the true efficacy of traditional remedies passed down through folklore (Uy & Villazorda, 2015). Traditionally, antioxidant activity was linked to the presence of phenolic compounds in plants, which were considered secondary metabolites along with flavonoids. While phenolic compounds were directly associated with antioxidant activity, flavonoids also played a role in this effect. Their mechanism involved hydrogen donation and scavenging of free radicals. This scavenging

ability enabled them to interact with reactive oxygen species (ROS), which could cause oxidative stress and tissue damage. By reacting with ROS, phenolic compounds and flavonoids prevented oxidative stress, thereby promoting wound healing. Additionally, other plant metabolites besides phenols and flavonoids could contribute to antioxidant activity and wound healing (da Silva *et al*., 2006; Sharma *et al*., 2012; Oliveira *et al*., 2016). The results found in the present work could be related to phenols, flavonoids and other metabolites. Thus, this study provides necessary information for the medicinal plants usually consumed and used by the locals for their safety and awareness.

Acknowledgments

The main author would like to gratefully acknowledge the Department of Science and Technology - Science Education Institute (DOST – SEI) Accelerated Science and Technology Human Resource Development Program (ASTHRDP) for the scholarship to pursue the Master of Science in Biology program in Central Luzon State University. The authors would also like to express gratitude to the residents of Calaanan, Bongabon Nueva Ecija Philippines.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number**: Central Luzon State University Ethical Clearance Code: 2023-5192.

Authorship Contribution Statement

Dana Theresa De Leon: Investigation, resources, visualization, software, formal analysis, and writing - original draft. **Arwil Nathaniel Alfonso**: Investigation, resources, visualization. **Angeles De Leon**: Supervision, and validation. **Jerwin Undan**: Supervision, and validation.

Orcid

Dana Theresa De Leon https://orcid.org/0000-0001-6109-1530
Arwil Nathaniel Alfonso https://orcid.org/0009-0006-9741-8551
Angeles De Leon https://orcid.org/0000-0002-4954-8427
Jerwin Undan https://orcid.org/0000-0002-3657-1732

REFERENCES

Abdel-Latif, A., & Osman, G. (2017). Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods*, *13*, 1-9.

Agatonovic-Kustrin, S., Ristivojevic, P., Gegechkori, V., Litvinova, T.M., & Morton, D.W. (2020). Essential oil quality and purity evaluation via ft-ir spectroscopy and pattern recognition techniques. *Applied Sciences*, *10*(20), 7294.

Balangcod, T.D., Vallejo, V.L., Patacsil, M., Apostol, O., Laruan, L.M.V.A., Manuel, J., & Gutierrez, R.M. (2012). Phytochemical screening and antibacterial activity of selected medicinal plants of Bayabas, Sablan, Benguet Province, Cordillera administrative region, Luzon, Philippines. *Indian Journal of Traditional Knowledge, 11*(4), 580-585.

Balangcod, T., & Balangcod, K.D. (2015). Ethnomedicinal plants in Bayabas, Sablan, Benguet Province, Luzon, Philippines. *Electronic Journal of Biology*, *11*(3), 63-73.

Boy, H.I.A., Rutilla, A.J.H., Santos, K.A., Ty, A.M.T., Alicia, I.Y., Mahboob, T. & Nissapatorn, V. (2018). Recommended medicinal plants as source of natural products: a review. *Digital Chinese Medicine*, *1*(2), 131-142.

CBOL Plant Working Group 1, Hollingsworth, P.M., Forrest, L.L., Spouge, J.L., Hajibabaei, M., Ratnasingham, S. & Little, D.P. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, *106*(31), 12794-12797.

China Plant, B.O.L.G., Li, D.Z., Gao, L.M., Li, H.T., Wang, H., Ge, X.J., & Duan, G.W. (2011). Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS)

should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(49), 19641-19646.

Clemen-Pascual, L.M., Macahig, R.A.S., and Rojas, N.R.L. (2022). Comparative toxicity, phytochemistry, and use of 53 Philippine medicinal plants. *Toxicology Reports*, *9*, 22-35.

da Silva, J.F.M., de Souza, M.C., Matta, S.R., de Andrade, M.R., & Vidal, F.V.N. (2006). Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities. *Food Chemistry*, *99*(3), 431-435.

De Leon, D.T.C., Aquino, J.D.C., Valentino, M.J.G., & Undan, J.R. (2018). Molecular identification and phytochemical profiling of kamiling (wild toxic plant) using thin layer chromatography. *International Journal of Secondary Metabolite*, *5*(3), 217-223.

de Sousa, D.P., Damasceno, R.O.S., Amorati, R., Elshabrawy, H.A., de Castro, R.D., Bezerra, D.P., & Lima, T.C. (2023). Essential oils: Chemistry and pharmacological activities. *Biomolecules*, *13*(7), 1144.

Dela Cruz, P., & Ramos, A.G. (2006). *Indigenous health knowledge systems in the Philippines: a literature survey*. In 13th *CONSAL Conference*, Manila, Philippines (Vol. 1).

Devi, D.R., & Battu, G.R. (2019). Qualitative phytochemical screening and FTIR spectroscopic analysis of *Grewia tilifolia* (vahl) leaf extracts. *International Journal of Current Pharmaceutical Research*, *11*(4), 100-107.

Dytkiewitz, E., & Morlock, G.E. (2008). Analytical strategy for rapid identification and quantification of lubricant additives in mineral oil by high-performance thin-layer chromatography with UV absorption and fluorescence detection combined with mass spectrometry and infrared spectroscopy. *Journal of AOAC International*, *91*(5), 1237-1244.

Ertl, P., Altmann, E., & McKenna, J.M. (2020). The most common functional groups in bioactive molecules and how their popularity has evolved over time. *Journal of Medicinal Chemistry*, *63*(15), 8408-8418.

Fazekas, A.J., Burgess, K.S., Kesanakurti, P.R., Graham, S.W., Newmaster, S.G., Husband, B. C., & Barrett, S.C. (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PloS One*, *3*(7), e2802.

George, J., Dhavan, P., Jadhav, B., Meshram, G., Patil, V. (2023). FT-IR coupled secondary metabolites profiling and biological activities of *Neolamarckia cadamba* leaves. *Natural Resources for Human Health*, *3*(1), 94-100.

Gu, R., Wang, Y., Long, B., Kennelly, E., Wu, S., Liu, B., & Long, C. (2014). Prospecting for bioactive constituents from traditional medicinal plants through ethnobotanical approaches. *Biological and Pharmaceutical Bulletin*, *37*(6), 903-915.

Guevara, B.Q. (2005). A guidebook to plant screening: Phytochemical and Biological. *University of Santo Tomas Publishing House*.

Hussein, R.A., & El-Anssary, A.A. (2019). *Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants*. In I.A.M. Saeed (Ed.), Herbal Medicine (pp. 11–30). IntechOpen. https://doi.org/10.5772/intechopen.76139

Ishtiaq, S., Hanif, U., Shaheen, S., Bahadur, S., Liaqat, I., Awan, U. F., & Meo, M. (2020). Antioxidant potential and chemical characterization of bioactive compounds from a medicinal plant *Colebrokea oppositifolia* Sm. *Anais da Academia Brasileira de Ciências*, 92.

Kaur, S., & Mondal, P. (2014). Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. *Journal of Microbiology & Experimentation*, *1*(1), 00005.

Kolak, U., Osturk, M., Ozgokce, F., & Ulubelen, A.(2006). Norditerpene alkaloids from *Delphenium linearilobolum* and antioxidant activity. *Phytochemistry*, *67*, 2170-2175.

Latayada, F.S., & Uy, M.M. (2016). Screening of the antioxidant properties of the leaf extracts of Philippine medicinal plants *Ficus nota* (Blanco) Merr., *Metroxylon sagu* Rottb., *Mussaenda philippica* A.Rich., *Inocarpus fagifer*, and *Cinnamomum mercadoi* Vidal. Bull Environ. *Pharmacology and Life Sciences, 5*(3), 18-24.

Lazarte, C. (2020). Benefits of mainstreaming herbal medicine in the philippine healthcare system. Acta Medica Philippina, *The National Science Health Journal*, *54*(1), 3-4.

Mamari, H., & Hamad, H. (2021). *Phenolic compounds - chemistry, synthesis, diversity, non-conventional industrial, pharmaceutical and therapeutic applications*. (2022). In F.A. Badria (Ed.), Biochemistry. IntechOpen. https://doi.org/10.5772/intechopen.94825

Md Salim, R., Asik, J., & Sarjadi, M.S. (2021). Chemical functional groups of extractives, cellulose and lignin extracted from native *Leucaena leucocephala* bark. *Wood Science and Technology*, *55*, 295-313.

Megawati, E.R., Bangun, H., Putra, I.B., Rusda, M., Syahrizal, D., Jusuf, N.K., & Amin, M.M. (2023). Phytochemical analysis by FTIR of zanthoxylum acanthopodium, DC fruit ethanol extract, N-hexan, ethyl acetate and water fraction. *Medical Archives*, *77*(3), 183.

Mohanapriya, C., Uma, S., Nithyalakshmi, V., Rajmohan, K.S., Vijay, P., Pulla, R.H., & Gopinath, M. (2019). In vitro evaluation of secondary metabolites: characterization and antimicrobial activity of Manilkara zapota L. seed extract. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *89*, 729-738.

Mundhe, K.S., Kale, A.A., Gaikwad, S.A., Deshpande, N.R., & Kashalkar, R.V. (2011). Evaluation of phenol, flavonoid contents and antioxidant activity of *Polyalthia longifolia*. *Journal of Chemical and Pharmaceutical Research*, *3*(1), 764-769.

Mwangi, W.C., Waudo, W., Shigwenya, M.E., & Gichuki, J. (2024). Phytochemical characterization, antimicrobial and antioxidant activities of *Terminalia catappa* methanol and aqueous extracts. *BMC Complementary Medicine and Therapies*, *24*(1), 137.

Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P.(2024). Assessing the efficiency of antimicrobial plant extracts from *Artemisia afra* and *Eucalyptus globulus* as coatings for textiles. *Plants*, *13*(4), 514.

Olivar, J.E.C., Brillantes, R.Y., Rubite, R.R., & Alejandro, G.J.D. (2014). Evaluation of three candidate DNA barcoding loci in selected *Ficus L. (*Moraceae). *International Journal of Scientific and Technology Research*, *3*(9), 43-48.

Oliveira, R.N., Mancini, M.C., Oliveira, F.C.S.D., Passos, T.M., Quilty, B., Thiré, R.M.D.S. M., & McGuinness, G.B. (2016). FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. *Matéria (Rio de Janeiro)*, *21*, 767-779.

Oluwapelumi, A.E., Iweala, E.J., Otike, J.O., Dike, E.D., Uche, M.E., Owanta, J.I., & Ugbogu, E.A. (2023). Ethnomedicinal uses, phytochemistry, pharmacological activities and toxicological effects of *Mimosa pudica*-A review. *Pharmacological Research-Modern Chinese Medicine*, 100241.

Pearson, W.R. (2013). An introduction to sequence similarity ("homology") searching. *Current Protocols in Bioinformatics*, *42*(1), 3-1.

Sahayaraj, P.A., Gowri, J., Dharmalingam, V., Shobana, R., & Prema, A.A. (2015). Phytochemical screening by FTIR spectroscopic analysis of leaf and stem extracts of *Wedelia biflora*. *International Journal of Nano Corrosion Science and Engineering, 2*(5), 322-334.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, *18*, 820-897.

Sharma, P., Jha, A.B., Dubey, R.S., & Pessarakli, M.(2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, *1*, Article ID 217037, 1-26. https://doi.org/10.1155/2012/217037

Tantengco, O.A.G., Condes, M.L.C., Estadilla, H.H.T., & Ragragio, E.M.(2018). Ethnobotanical survey of medicinal plants used by Ayta communities in Dinalupihan, Bataan, Philippines. *Pharmacognosy Journal*, *10*(5), 859-870.

Teclegeorgish, Z. W., Aphane, Y. M., Mokgalaka, N. S., Steenkamp, P., & Tembu, V. J. (2021). Nutrients, secondary metabolites and anti-oxidant activity of *Moringa oleifera* leaves and Moringa-based commercial products. *South African Journal of Botany*, *142*, 409-420.

Topala, L., & Ducu, T. (2017). ATR-FTIR spectra fingerprinting of medicinal herbs extracts prepared using microwave extraction. *Arabian Journal of Medicinal and Aromatic Plants*, *3*(1), 1-9.

Uy, M.M., & Villazorda, M.G. (2015). The antioxidant properties of the Philippine medicinal plants *Cassia sophera* Linn., *Derris elliptica* Benth, *Ficus minahassea* Tesym. and De Vr., *Leea aculeata* Blume and *Leucosyke capitellata* Wedd. *Advances in Agriculture and Botanics*, *7*(3), 150-156.

Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *Journal of Advanced Pharmaceutical Technology & Research*, *3*(4), 200-201.