Evaluation of cytotoxicity and antidiabetic activities of plant extracts used in Triphala from Western Nepal using different solvents

A. Chataut[1,2], J. Maharjan[1], R. C. Poudel[1], R. Malla[2*], and D. Khadka[1*]

Received: 22, December 2024 Revised: 17, March 2025 Accepted: 19, May 2025 Published: 30, May 2025

The Triphala plants: *Phyllantus emblica*, *Terminalia chebula* and *Terminalia bellirica* have been traditionally used in the treatment of various aliments since prehistoric times. Their antidiabetic activity, particularly against α-amylase enzyme, has been reported in studies from different countries. However, limited research has evaluated the antidiabetic potential of Triphala plants originating from Nepal using solvents like hexane, ethylacetate and water. In this study, extraction of the plants was carried out using the Soxhlet method with the solvents. The antidiabetic activity was evaluated through an α-amylase enzyme inhibition assay, while cytotoxic effect was determined through the brine shrimp lethality assay. Among the extracts, the highest percentage yield was obtained from the aqueous extract of *T. chebula* (7.17%), while the lowest was from the hexane extract of *P. emblica* (1.28%). The aqueous extracts of *T. chebula* demonstrated the highest antidiabetic potential with an IC_{50} value of 97.86 ± 0.17 μg/mL, forming the smallest polygon in the radar diagram, whereas the least potential was exhibited by the hexane extract of *P. emblica* (IC_{50} = 810.85 ± 2.05 μg/mL; 0.81 mg/mL), forming the largest polygon in the radar diagram. Regarding safety, the cytotoxicity of these extracts was assessed using the brine shrimp lethality assay. The hexane extract of *P. emblica* exhibited the least toxicity (LC_{50} = 8.54 mg/mL). In contrast, the aqueous extract of *T. cheuba* showed the highest toxicity with an LC_{50} of 0.99 mg/mL.

Key words: Medicinal plants; Antidiabetic; Brine shrimp lethality; Triphala; Various solvents.

Plant products have played a vital role in traditional medicine for centuries due to the abundance of secondary metabolites like alkaloids, steroids, flavonoids, terpenoids and tannins. These bioactive compounds are beneficial in addressing various health issues (Chhetri et al., 2008). About 80% of the population in developing countries rely on traditional medicine, including medicinal plants, for primary healthcare needs (WHO, 2013). Although an estimated 250,000 to 500,000 plant species exist globally, only a small fraction has been scientifically investigated for their phytochemical composition and therapeutic potential (Prabhu et al., 2010). Nepal is rich in biodiversity, sheltering over 2,332 species of medicinal and aromatic plants that are extensively used in traditional healing practices (Baral & Kurmi, 2006). These plants are equally significant for both traditional medicine and modern pharmacological applications.

The safety of herbal products is often taken for granted, as many people believe that their long history of use without apparent side effects implies that they are inherently safe; however, this belief can be misleading. Just because something is natural or has a long history of use does not guarantee the

[1] Molecular Biotechnology Laboratory, Faculty of Science. Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal
[2] Central Department of Biotechnology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
*Email: rajanimalla200@gmail.com; deegendrakhadka@gmail.com; deegendra.khadka@nast.org.np

absence of harmful effects. Adulteration, improper formulation, and insufficient knowledge about plant-drug interaction can lead to severe adverse reactions. Numerous studies have linked herbal medicines to hepatotoxicity, while other effects such as harm to the kidneys, nervous system, blood, heart, and skin as well as risks of mutation and cancer are also documented (Saad & Said, 2011).

Herbal toxicity primarily arises from poor quality control during production, confusion arising from similar plant names and misidentification of plant species. The level of active compounds can vary depending on several factors; the parts of the plant used, the time of harvest, the growth stage and the region and climate where the plant is grown. Herb may also be contaminated with microorganism, fungal toxin (aflatoxins), pesticides, heavy metals, or synthetic drugs (Saad et al., 2006). The identification of the therapeutic and toxic compounds in natural products is cumbersome because of the contamination caused by different harvesting seasons and various extraction protocols used in herbal medication preparation (Opuni et al., 2023). Therefore, scientific validation and safety assessments are essential to ensure the efficacy and safety of herbal products for public health.

Diabetes mellitus (DM) is a condition in which blood glucose level exceeds its limit range. It develops when the pancreatic β-cells fail to produce sufficient insulin, when body cells become resilient to insulin or due to a combination of both factors (Khadka & Pandey, 2022). Diabetes is a major risk factor for several complications, including cardiovascular diseases, neuropathy, and retinopathy. According to the International Diabetes Federation (IDF, 2021), an estimated 537 million adults worldwide were living with diabetes in 2021, and this figure is projected to rise to 643 million by 2030 if current trend continues. IDF claims that a significant portion of this increase is occurring in developing countries.

In Nepal, diabetes is emerging as a growing public health problem, particularly in urban areas. This is largely attributed to lifestyle and environmental changes such as sedentary activities and dietary shifts. As of 2021, approximately 6.3% of Nepal's adult population was affected by diabetes (IDF, 2021). The rising prevalence of diabetes places increasing pressure on healthcare systems, necessitating the urgent need for prevention and early intervention strategies. Despite the availability of several synthetic antidiabetic drugs in the global markets, they are not free from severe side effects such as hypoglycemia (sulphonylureas), lactic acidosis and folate and B12 malabsorption (metformin), gastrointestinal symptom (acarbose), weight gain (sulphonylureas and thiazolidinediones), and edema (thiazolidinediones) (Campbell, 2007). Developing antidiabetic drugs without any side effects is still a challenge. Therefore, efforts to discover more secure and effective hypoglycemic agents are continually increasing.

Regarding the discovery of natural hypoglycemic agents, our research team focused on testing constituent plants of Triphala, collected from the western regions of Nepal. Triphala is a well-known polyherbal formulation in Ayurveda, composed of three medicinal fruits: *P. emblica*, *T. chebula* and *T. bellirica.* Triphala mixture is prepared by blending equal proportions of the powdered fruits (Venkateswarlu et al., 2019). Owing to its antioxidant, anti-inflammatory, and digestive benefits, *Triphala* has been widely studied in herbal medicine (Peterson et al., 2017). *P. emblica*, associated with the family Phyllanthaceae, is indigenous to the Indian subcontinent and its primary chemical constituents include vitamin C, tannins, flavonoid, and phenolic compounds (Prananda et al., 2023).

P. emblica has been used traditionally for treating digestive disorders, diabetes, inflammation and for enhancing immunity (Prananda et al., 2023). *T. bellirica*, a member of the family Combretaceae, is commonly found in Nepal and India. It contains tannins, gallic acid and ellagic acid as its major components. Traditionally, *T. bellirica* has been used for treating respiratory issues, supporting digestive health and as a mild laxative (Gupta et al., 2020b). Likewise, *T. chebula,* also from the family Combretaceae, is native to south Asia, particularly, India, Nepal and Sri Lanka.

The major chemical components are chebulinic acid, ellagic acid and gallic acid (Muhammad et al., 2012). *T. chebula* has long been used in traditional medicine for treating digestive issues, respiratory ailments, and promoting would healing (Bag et al., 2013). In this study, the fruit pulp of each plant was subjected to extraction using three different solvents; water, ethylacetate and hexane to identify the most effective extract based on its half-maximal inhibitory concentration (IC_{50}) against α-amylase for diabetes management. Detailed information about the plant samples is presented in Table 1. The choice of solvent plays a crucial role in determining the type and quantity of bioactive compounds extracted (Harborne, 1998). Non-polar solvents like hexane

extract fat-soluble compounds while polar solvents like water isolate polar compounds and moderately polar solvent such as ethyl acetate extracts a wide range of bioactive compounds including polyphenols, flavonoids, alkaloids due to its intermediate polarity (Cowan, 1999). This approach facilitates the exploration of diverse bioactive compounds in Triphala plants of Nepal origin.

Materials and methods

Collection and authentication of plant material

Plant samples were collected in April 2019 from Mahendranagar, Kanchapur, Nepal, situated at an altitude of 210 meters above sea level, to ensure seasonal consistency. The collected samples were identified by Dr. R. C. Poudel, Senior Scientist at the Nepal Academy of Science and Technology (NAST). The collected materials were air-dried until a consistent weight was achieved. All the samples were collected with the necessary permission from local authorities and in compliance with ethical guidelines to ensure sustainable and responsible sampling practices.

Preparation of plant extracts

The collected fruits were briefly cleaned with 70% ethanol to remove surface impurities and then shade-dried until a stable weight was achieved. The dried materials were ground into a fine powder using an electric grinder. Soxhlet extraction method was employed to isolate the bioactive compounds using three different solvents: hexane, ethyl acetate, and water. For each extraction, approximately 50 grams of powdered material was uniformly packed in a thimble and extracted separately with 350 mL of the respective solvents. The extraction was carried out until the solvent in the siphon tube appeared transparent. The resulting extracts were concentrated using a rotary evaporator to remove the bulk of the solvent and the obtained extract was then transferred to a beaker and heated on a hot plate at 30-40 °C until a semi-solid consistency was achieved. The semi solid extracts were stored at 4 °C for further analysis. The percentage yield of extracts obtained from each solvent is presented in Table 2.

Antidiabetic activity

The antidiabetic potential of the plant extracts was evaluated using the α-amylase inhibition assay, following a standard protocol with slight modifications (Tamil et al., 2010; Karki et al., 2021). The presence of undigested starch due to enzyme inhibition was identified by the formation of a blue starch-iodine complex measured at 630 nm. 100 µL of a 1% starch solution was pre-incubated at 37 °C for 5 minutes with 50 µL of different concentrations (20, 40, 80, 160 µg/mL) of each plant extract and the standard inhibitor acarbose. Subsequently, 50 µL of a 50 µg/mL α-amylase solution was introduced into each mixture and incubated at 37 °C for 15 minutes. The enzymatic reaction was halted by adding 200 µL of 0.1M hydrochloric acid (HCl), followed by 250 µL of iodine reagent to develop the color. The absorbance of the resulting blue starch-iodine complex was recorded at 630 nm using a UV-Visible spectrophotometer. All experiments were conducted in triplicate and the percentage inhibition of α-amylase activity was calculated using the following formula:

$$\% \text{ Inhibition} = [1-(Abs_2-Abs_1/Abs_4-Abs_3)] \times 100$$

where,

Abs_1 = Absorbance of a mixture incubated with plant extract, starch and amylase.

Abs_2 = Absorbance of a mixture incubated with

Table 1: Detailed information about the studied plants in the study

Scientific name	Vernacular name	Family	Collected sites	Parts used	Traditional usage
Phyllanthus emblica	Amala	Phyllanthaceae	Mahendrnagar, Kanchanpur, Nepal	Fruit	Rejuvenating agent, tonic for longevity, improve digestion, ailment like respiratory issues sore throat and fever (Prananda et al., 2023)
Terminalia chebula	Harro	Combretaceae	Mahendrnagar, Kanchanpur, Nepal	Fruit	Digestive disorder, wound healing, respiratory disorder. Oral health (Chopra et al., 2023)
Terminalia bellirica	Barro	Combretaceae	Mahendrnagar, Kanchanpur, Nepal	Fruit	Wound healing, liver health, digestive disorder (Gupta et al., 2021)

plant extracts and starch only.

Abs_3 = Absorbance of a mixture incubated with starch and amylase only.

Abs_4 = Absorbance of a mixture incubated with starch alone.

The IC_{50} value denotes the inhibitor concentration needed to reduce enzyme activity by 50%, A graph was mapped with the extract concentration on the x-axis and percentage inhibition on the y-axis to obtain a linear regression equation. IC_{50} was calculated through the linear regression by fitting straight line equation with variable slope using Microsoft excel 2019.

Brine shrimp lethality assay

A pinch of brine shrimp eggs was sprinkled into a beaker filled with artificial seawater and illuminated with a 60 W table lamp at 30 °C for 24 hours to hatch the shrimps. The extracts were initially dissolved in a 1% aqueous dimethyl sulfoxide (DMSO) and subsequently diluted with sea water to obtain test concentrations of 1000 ppm, 100 ppm and 10 ppm. An aliquot of 1 mL of each concentration was transferred into a cleaned sterile measuring cylinder and the volume was raised up to 5 mL using seawater. Twenty nauplii were transferred into each measuring cylinder. The test samples were incubated at room temperature for 24 hours, after which the number of survivors was counted using a pipette. The lethal concentration (LC_{50}) is defined as the concentration causing 50% mortality after 24 hours of exposure along with its 95% confidence intervals. LC_{50} was determined using the probit analysis method (Finney, 1971). According to Meyer's toxicity index, extracts with LC_{50} values below 1000 µg/ml (1 mg/mL) are considered toxic, while those with LC_{50} values above 1000/ µg/mL are considered as non-toxic (Meyer et al., 1982).

Results

The extraction yield for the three plants: *T. chebula*, *T. bellirica* and *P. emblica* varied depending on the solvents used (hexane, water and ethyl acetate). Among the solvents, water consistently produced the highest yield, except in the case of *T. bellirica*. The aqueous extract of *T. chebula* showed the highest percentage yield (7.16%) followed by *P. emblica* (7.11%). In comparison, the ethyl acetate extracts yielded moderate yields, with *P. emblica* yielding 3.69%, *T. bellirica* 3.62% and *T. chebula* 3.49%. In contrast, hexane extracts yielded the lowest percentages, ranging from 1.28% in *P. emblica* to 1.41% in *T. chebula,* as shown in Table 2.

The inhibitory effects of hexane, ethylacetate and water extracts of Triphala plants against α-amylase were evaluated. Acarbose was used as the reference standard, with an IC_{50} value of 86.49 ± 0.31 µg/mL. The results revealed differences in IC_{50} values among the various extracts. For *P. emblica*, the water extract exhibited the lowest IC_{50} value of 235.22 ± 0.64 µg/mL (0.235 mg/mL), compared to the hexane and ethyl acetate extracts, as shown in Table 2. Similarly, *T. bellirica* had IC_{50} values of 651.05 ± 10.75, 627.12 ± 4.49, and 180.69 ± 0.44 µg/mL for the hexane, ethylacetate and water extracts, respectively. In the case of *T. chebula,* the aqueous extract had the lowest IC_{50} value of 97.86 ± 0.17 µg/mL followed by the ethyl acetate extract (163.01 ± 1.8 µg/mL), while the hexane extract showed the highest IC_{50} value of 500.51 ± 4.33 µg/ml, indicating the least potency. The radar diagram in Figure 1 illustrates that *T. chebula* exhibited the highest antidiabetic potency of all three solvents, followed by *T. bellirica* and *P emblica,* as indicated by the area occupied by the respective polygons.

Table 2: Yield percentage and IC_{50} value of the studied plant in different solvents

Name of the plants	% yield				IC_{50} ± SEM (µg/mL)		
	Hexane	Water	Ethylacetate	Acarbose	Hexane	Ethylacetate	Water
Phyllanthus emblica L.	1.28	7.11	3.69		810.85 ± 2.058	656.37± 2.92	235.22 ± 0.64
Terminalia bellirica Retz.	1.32	1.43	3.62		651.05 ± 10.75	627.12±4.49	180.69± 0.44
Terminalia chebula (Gaertn.) Roxb.	1.41	7.16	3.49	86.49 ± 0.31	500.51 ± 4.33	163.0±1.8	97.86 ± 0.17

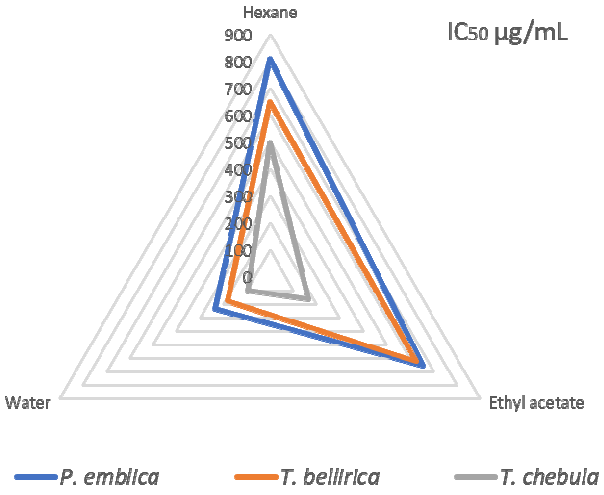

Figure 1: Radar diagram showing IC_{50} in µg/mL of the studied plants in different solvents

Cytotoxicity of the extracts was assessed using the brine shrimp lethality (BSL) assay. The LC_{50} values for different solvents extracts revealed noticeable variations (Table 3). For *P. emblica*, the aqueous extract exhibited the highest cytotoxicity, with an LC_{50} value of 1.970 mg/mL, while the ethyl acetate and hexane extracts showed LC_{50} values of 2.560 mg/mL and 8.54 mg/mL, respectively. *T. bellirica* displayed LC_{50} of 1.32 mg/mL in its aqueous extract. Notably, *T. chebula*'s aqueous extract exhibited the highest cytotoxicity among the tested extracts, with an LC_{50} of 0.99 mg/mL. According to Meyer's toxicity index, all extracts except the aqueous extract of *T. chebula* were considered non-toxic (LC_{50} > 1 mg/mL).

Discussion

The choice of solvent significantly influenced the yield percentage of Triphala plant extracts. Aqueous extracts demonstrated the highest yields, with *T. chebula* yielding 7.17% and *P. emblica* showing a comparable 7.11%. In contrast, the hexane extracts yielded the lowest, with *P. emblica* producing just 1.28%, shown in Table 2. These findings suggest that non-polar solvents are less effective in isolating water-soluble phytochemicals like tannins and flavonoids (Nawaz et al., 2020). The high yield observed in aqueous extract aligns with the fact that polar solvents enhance the extraction of hydrophilic bioactive compound (Xia et al., 2023). In comparison, hexane primarily isolates non-polar components like lipids, which are generally less abundant in medicinal plants used for antidiabetic purposes (Sutedja et al., 2020) The moderate yields obtained with ethyl acetate indicate that this solvent is effective for extracting compounds of intermediate polarity, such as phenolic acids and some flavonoids (Baehaki et al., 2020). These results emphasize the importance of solvent-specific extraction methods, as solvent polarity directly affects the composition and quantity of bioactive compounds extracted (Lee et al., 2024). Furthermore, the higher yields in aqueous extracts support their potential for cost-effective and scalable antidiabetic formulations considering the environmental and economic advantages of using water as a solvent (Castro-Puyana et al., 2017).

The present study focused on Triphala plants to evaluate their antidiabetic activities using hexane, ethyl acetate and water as extraction solvents. Although extensive research has been conducted on these three plants to evaluate their α-amylase inhibitory activity (antidiabetic) and cytotoxicity using brine shrimp lethality assay, studies specifically using these solvents on Nepal-originating Triphala plants remain scarce. The α-amylase inhibitory activity of *P. emblica* varies considerably across the literature, with IC_{50} values ranging from 85.92 µg/mL for seed extract (Dinesh et al., 2016) to 397.67 µg/mL for methanolic fruit extract (Poongunran et al., 2015), and ethanolic leaf extracts showing 61.12% inhibition under specific conditions (Singh & Kaur, 2015). In our study, the lowest IC_{50} value observed for *P. emblica* was 235.22 ± 0.64 µg/mL in the aqueous extract, as compared to its hexane and ethyl acetate extracts (Table 2).

Table 3: LC_{50}, slope and regression equation of the studied plants in different solvents

Plants	Solvent used	LC_{50} mg/mL	Slope	Regression Equation
Phyllanthus emblica	Hexane	8.54	R^2 = 0.9932	y = 0.0053x + 4.7222
	Ethylacetate	2.56	R^2 = 0.9745	y = 0.0188x + 1.3889
	Aqueous	1.90	R^2 = 0.8972	y = 0.0218x + 8.6111
Terminalia bellirica	Hexane	7.59	R^2 = 0.3243	y = 0.006x + 4.4444
	Ethylacetate	3.20	R^2 = 0.9382	y = 0.0135x + 6.6667
	Aqueous	1.32	R^2 = 0.8176	y = 0.0165x + 28.889
Terminalia chebula	Hexane	3.20	R^2= 0.9382	y = 0.0135x + 6.6667
	Ethylacetate	1.12	R^2 = 0.8176	y = 0.033x + 12.778
	Aqueous	0.99	R^2 = 0.9138	y = 0.0353x + 15.278

Gupta et al. (2020a) reported that the ethyl acetate extracts of *T. bellirica* exhibited stronger α-amylase inhibitory activity (IC_{50} = 43.5 µg/mL) than aqueous extract (IC_{50} = 74.8/ µg/mL). However, in our study, the hexane, ethyl acetate, and aqueous extracts of of *T. bellirica* exhibited IC_{50} value of 651.05 ± 10.75, 627.12 ± 4.49 and 180.69 ± 0.44 µg/mL respectively. Similarly, Mukherjee et al. (2010) reported that tannins from *T. chebula* fruits exhibited 52% inhibition of pancreatic amylase at 100 µg/mL. However, our study found the lowest IC_{50} in the aqueous extract *T. chebula* fruit pulp (97.86 ± 0.17 µg/mL), followed by the ethyl acetate extract (163.0 ± 1.8 µg/mL), while the hexane extract demonstrated the highest IC_{50} value of 500.51 ± 4.33 µg/mL indicating the least potency (Table 2). The radar diagram presented in Figure 1 visually illustrates the comparative potency of three plant extracts in different solvents for antidiabetic activity based on the size of the polygon. The small polygon for *T. chebula* indicates higher potency across all three solvents, followed by *T. bellirica* and then *P. emblica*.

Previous studies on the cytotoxicity of *P. emblica* using the brine shrimp lethality assay have demonstrated potent activity, with LC_{50} values ranging from 10.25 µg/mL for the chloroform fraction of a crude methanolic extract (Rahman et al., 2009) to 1.25 µg/mL for seed extract-capped nanoparticles (Dinesh et al., 2017). Similarly, Krishnaraju et al. (2005) reported an LC_{50} value of 58 µg/mL for the ethanol extract. In comparison, our study revealed that the aqueous extract of *P. emblica* exhibited the highest toxicity among the three solvents tested, with an LC_{50} value of 1.38 mg/mL (Table 3). This indicates that the three solvent extracts of *P. emblica* are non-toxic based on Meyer's toxicity index. Ali et al. (2013) reported high cytotoxicity for the methanolic bark extract of *T. bellirica* with an LC_{50} value of 3.21 mg/mL. In our findings, the aqueous extract of *T. bellirica* exhibited the highest cytotoxicity among the tested solvents, with an LC_{50} value of 1.378 mg/mL, which is still categorized as non-toxic.

Previous investigations have demonstrated that ethanol and methanol extracts of *T. chebula* fruit show cytotoxic activity with LC_{50} value of 107 µg/mL (Ved et al., 2010) and 97.36 µg/mL (Sarwar et al., 2013) respectively. However, our study found LC_{50} values of 0.99, 1.12 and 3.2 mg/mL for the aqueous, ethylacetate and hexane extracts respectively. The aqueous extract exhibited significantly higher toxicity than the other two solvents. The LC_{50} of the extracts in different solvents are shown in Table 3. According to Meyer's toxicity index, extracts with LC_{50} below 1000 µg/ml (1 mg/mL) are considered toxic, while those above 1 mg/mL are considered non-toxic (Meyer et al., 1982). Based on this classification, all three extracts of the Triphala plants prepared using hexane, ethylacetate, and water were found to be non-toxic, except for the aqueous extract of *T. chebula,* which had an LC_{50} value of 0.99 mg/mL Supplementary details are presented in Table 3.

Conclusion

This study demonstrates that Triphala plants as evidenced by their inhibitory effects on α-amylase enzyme, possess promising antidiabetic properties. Among the three Triphala constituent plants, *T. chebula* demonstrated the strongest activity followed by *T. bellirica* and *P. emblica*. While most plant extracts were found to be non-toxic, the aqueous extract of *T. chebula* showed toxicity, indicating the need for further investigation. Overall, the findings of this study highlight the potential of Nepal-originating *Triphala* plants as sources for developing natural antidiabetic remedies. The maximum percentage yield of *T. chebula* in aqueous extract was found. Further pharmacological and toxicological studies are recommended to validate these initial results and to ensure their safe application in clinical settings.

Acknowledgments

This study was conducted with the support of the Nepal Academy of Science and Technology (NAST). The authors gratefully acknowledge NAST for providing the necessary resources and institutional support to carry out this research.

Conflicts of Interest

The authors declare that there are no conflicts of interest concerning the research, authorship, and/or publication of this article.

Author's contribution statement

A. Chataut: Carried out laboratory experiments, data analysis and methodology. **R. Malla:** Methodology and Supervision. **D. Khadka:** Conceived the study, interpreted the data, supervised the work and critically reviewed the manuscript. **J. Maharjan:** Manuscript revision and data analysis. **R. C. Poudel:** Collection of plant samples, morphological identification and manuscript revision.

References

Ali, M. S., Faruq, K. O., Islam, A., Nurullah, A. M. M., Chowdhury, K. A. A., & Sayeed, M. A. (2013). Thrombolytic and cytotoxic activities of *Terminalia bellerica* Roxb. *Bangladesh Pharmaceutical Journal*, *16* (2), 131-135. https://doi.org/10.3329/bpj.v16i2.22293

Baehaki, A., Lestari, S., Hendri, M., & Ariska, F. (2020). Antidiabetic Activity with N-Hexane, Ethyl-Acetate and Ethanol Extract of *Halodule uninervis* Seagrass. *Pharmacognosy Journal, 12* (4), 805-8. http://dx.doi.org/10.5530/pj.2020.12.115

Bag, A., Bhattacharyya, S. K., & Chattopadhyay, R. R. (2013). The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. *Asian Pacific Journal of Tropical Biomedicine, 3* (3), 244–252. doi: https://doi.org/10.1016/S2221-1691(13)6005

Baral, S. R., & Kurmi, P. P. (2006). *A Compendium of Medicinal Plants in Nepal*. Mass Printing Press, Kathmandu.

Campbell, I. (2007). Oral antidiabetic drugs: their properties and recommended use. *Prescriber*, *18* (6), 56–74. https://doi.org/10.1002/psb.48

Castro-Puyana, M., Marina, M. L., & Plaza, M. (2017). Water as green extraction solvent: Principles and reasons for its use. *Current Opinion in Green and Sustainable Chemistry, 5,* 31–36. https://doi.org/10.1016/j.cogsc.2017.03.009

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Journal of Pharmacology, 1* (2), 564-582. https://doi.org/10.1128/cmr.12.4.564

Chhetri, H. P., Yogol, N. S., Sherchan, J., K. C., A., Mansoor, S., & Thapa, P. (2010). Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. *Kathmandu University Journal of Science, Engineering and Technology, 4* (1), 49–54. https://doi.org/10.3126/kuset.v4i1.2883

Chopra, M., Deswal, G., Chopra, B., Kriplani, P., Dass, R., Grewal, A. S., Dhingra A. K., & Rathi, V. (2024). Therapeutic and health promoting potential of *Terminalia*: an exploratory literature review. *Current Traditional Medicine*, *10* (7), 65-77. http://dx.doi.org/10.2174/2215083810666230815142547

Dinesh, M., Roopan, S. M., & Selvaraj, C. I. (2016). Photocatalytic degradation of nitro phenol using biologically active *Phyllanthus emblica* seed extract. *Journal of Photochemistry and Photobiology Biology*, *161*, 273-278**.** https://doi.org/10.1016/j.jphotobiol.2016.05.033

Dinesh, M., Roopan, S. M., Selvaraj, C. I., & Arunachalam, P. (2017). *Phyllanthus emblica* seed extract mediated synthesis of PdNPs against antibacterial, heamolytic and cytotoxic studies. *Journal of Photochemistry and Photobiology B: Biology, 167*, 64–71. https://doi.org/10.1016/j.jphotobiol.2016.12.

Finney, D. J. (Ed.) (1971). *Probit Analysis*. **(**3rd ed.). Cambridge University Press, Cambridge, UK. https://doi.org/10.1002/jps.2600600940.

Gupta, A., Kumar, R., Ganguly, R., Singh, A, K., Rana, H. K., & Pandey A. K. (2021). Antioxidant, anti-inflammatory and hepatoprotective activities of *Terminalia bellirica* and its bioactive component ellagic acid against diclofenac induced oxidative stress and hepatotoxicity. *Toxicology Reports, 24* (8), 44-52. https://doi.org/10.1016/j.toxrep.2020.12.010.

Gupta, A., Kumar, R., & Pandey, A. K. (2020a). Antioxidant and antidiabetic activities of *Terminalia bellirica* fruit in alloxan induced diabetic rats. *South African Journal of Botany, 130,* 308–315. https://doi.org/10.1016/j.sajb.2019.12.010

Gupta, A., Kumar, R., Bhattacharyya, P., Bishayee, A., & Pandey, A. K (2020b). *Terminalia bellirica* (Gaertn.) roxb. (Bahera) in health and disease: A systematic and comprehensive review. *Phytomedicine, 77*, 153278. https://doi.org/10.1016/j.phymed.2020.153278

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.

IDF (2021). *IDF Diabetes Atlas*, (10th Ed.) Brussels, Belgium: International Diabetes Federation; https://diabetesatlas.org

Karki, Y., Khadka, D., & Subba, B. (2021). Antioxidant, PTP1B and alpha amylase inhibition property and GC-MS analysis of methanolic leaves extract of *Achyranthes aspera* and *Catharanthus roseus* of Nepal. *International Journal of Pharmacy and Pharmaceutical Sciences, 13* (4), 49-55. http://dx.doi.org/10.22159/ijpps.2021v13i4.40490

Khadka, D., & Pandey, K. (2022). Exploring the crucial role of plant growth-promoting rhizobacteria (PGPR) in plant secondary metabolite production and diabetes management. In R. Sayyed, A. Singh, N. Ilyas (Eds.), Antifungal metabolites of rhizobacteria for sustainable agriculture (pp. 351-373). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-04805-0

Krishnaraju, A. V., Rao, T. V., Sundararaju, D., Vanisree, M., Tsay, H. S., & Subbaraju, G. V. (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science Engineering, 3* (2), 125-34.

Lee, J. E., Jayakody, J. T. M., Kim, J. I., Jeong, J. W, Choi, K. M., Kim, T. S., Seo, C., Azimi, I., Hyun, J., & Ryu, B. (2024). The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. *Foods, 13* (19), 3151. https://doi.org/10.3390/foods13193151

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica, 45* (5), 31-34. https://doi.org/10.1055/s-2007-971236

Mukherjee, S., Mitra, A., Dey, S., & Thakur, G. (2010). Alpha-amylase activity of tannin isolated from *Terminalia chebula*. *International Conference on Systems in Medicine and Biology,* 443-445. https://doi.org/10.1109/ICSMB.2010.5735421

Muhammad, S., Barkat, A. K., Naveed, A., Tariq, M., Akhtar, R., Irshad, H, Haroon, K., & Amir, B. (2012). The morphology, extractions, chemical constituents and uses of *Terminalia chebula*: A review. *Journal of Medicinal Plants Research*, *6* (33), 4772-4775 https://doi.org/10.5897/JMPR11.1339

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, *56*, e17129. https://doi.org/10.1590/s2175-97902019000417129

Opuni, K. F. M., Kretchy, J. P., Agyabeng, K., Boadu, J. A., Adanu, T., Ankamah, S., Appiah, A., Amoah, G. B., Baidoo, M., & Kretchy, I. A. (2023). Contamination of herbal medicinal products in low-and-middle-income countries: A systematic review. *Heliyon. 9*, e19370. https://doi.org/10.1016/j.heliyon.2023.e19370

Peterson, C. T., Denniston, K., & Chopra, D. (2017). Therapeutic uses of Triphala in Ayurvedic medicine. *Journal of Alternative and Complementary Medicine, 23* (8), 607-614. https://doi.org/10.1089/acm.2017.0083.

Poongunran, J., Perera, H. K. I., Fernando, W. I. T., Jayasinghe, L., & Sivakanesan, R. (2015). α-glucosidase and α-amylase inhibitory activities of nine Sri Lankan antidiabetic plants. *Journal of Pharmaceutical Research International*. *7* (5), 365-374. https://doi.org/10.9734/BJPR/2015/18645

Prabhu, N., Divya, T. R., & Yamuna, G. (2010). Synthesis of silver Phyto nanoparticles and their antibacterial efficacy. *Digest Journal of Nanomaterial and Biostructutres, 5* (1), 185-189.

Prananda, A. T., Dalimunthe, A, Harahap, U., Simanjuntak, Y., Peronika, E, Karosekali, N. E., Hasibuan, P. A. Z., Syahputra, R. A., Situmorang, P. C., & Nurkolis, F. (2023). *Phyllanthus emblica*: A comprehensive review of its phytochemical composition and pharmacological properties. *Frontiers in Pharmacology. 14*, 1288618. https://doi.org/10.3389/fphar.2023.1288618

Rahman, S., Akbor, M. M., Howlader, A., & Jabbar, A. (2009). Antimicrobial and cytotoxic activity of the alkaloids of Amlaki *(Emblica officinalis*). *Pakistan Journal of Biological Sciences, 12* (16), 1152. https://doi.org/10.3923/pjbs.2009.1152.1155

Saad, B., Azaizeh, H., Abu-Hijleh, G., & Said, O. (2006). Safety of traditional Arab herbal medicine. *Evidence-Based Complementary and Alternative Medicine, 3* (4), 433–439. https://doi.org/10.1093/ecam/nel058

Saad, B., & Said O. (2011). *Greco-Arab and Islamic herbal medicine: Traditional system, ethics, safety, efficacy, and regulatory issues***,** John Wiley & Sons. https://doi.org/10.1002/9780470944363

Sarwar, S., Akhter, A., Islam, S. N., Rahman, A., & Sultana, R. (2013). Antioxidant, cytotoxic and analgesic activities of the methanolic fruit extract of *Terminalia chebula* Retz. *Journal of Scientific Research, 5* (2), 393-399. https://doi.org/10.3329/jsr.v5i2.14751

Singh, J., & Kaur, S. (2015). *Phyllanthus emblica* leaves extract: A potential amylase enzyme inhibitor with antioxidant and antimicrobial activity. *International Journal of Pharmaceutical Research, 5* (9), 201-207. https://doi.org/10.7439/ijpr

Sutedja, A. M., Yanase, E., Batubara, I., Fardiaz, D., & Lioe, H. N. (2020). Antidiabetic components from the hexane extract of red kidney beans (*Phaseolus vulgaris* L.): Isolation and structure determination. *Bioscience, Biotechnology, and Biochemistry, 84* (3), 598–605. https://doi.org/10.1080/09168451.2019.1691911

Tamil, I. G., Dineshkumark, B., Nandhakumar, M, Senthilkumar, M., & Mitra, A. (2010). *In vitro* study on α-amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*. *Indian Journal of Pharmacology, 42* (5), 280-282. https://doi.org/10.4103/0253-7613.70107

Ved, C. H., More, N. S., Bharate, Sandip. B., & Bharate, S. B. (2010). Cytotoxicity screening of selected Indian medicinal plants using brine-shrimp lethality bioassay. *Advance National Applied Science, 4*, 389-395.

Venkateswarlu, G., Ganapaty, S., & Sudhakar, A. M. S. (2019). Preparation of Triphala churna using the ingredients obtained from local market and comparative standardization. *Pharmacognosy Journal*, *11* (1), 102-11. https://doi.org/10.5530/pj.2019.1.18

WHO (2013). *WHO traditional medicine strategy: 2014-2023*. World Health Organization.

Xia, P., Ahmmed, M. K., & Rashidinejad, A. (2023.) Exploring efficient extraction methods: Bioactive compounds and antioxidant properties from New Zealand damson plums. *Food Bioscience, 55* (2), 103057. https://doi.org/10.1016/j.fbio.2023.103057