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Evaluation of Antioxidant Capacity of Methanol Extracts of Leaf and Stem Bark of *Tinospora Cordifolia*

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ABSTRACT
Conventionally used herbs are the major sources of biologically active phytoconstituents that are extensively used for the management of several health complications particularly in developing countries. *Tinospora cordifolia*, locally known as *Gurjo*, is one of the most widely utilized medicinal plants in Nepal. This study was carried out on quantifying the total phenolic and flavonoid contents, and evaluating antioxidant potential of the methanolic extracts of stem barks and leaf of a wild plant collected from Rupa Village of Kaski District of Nepal. The Folin-Ciocalteu reagent (FCR) method showed a total phenolic content of 50.84 ± 0.38 and 41.39 ± 0.44 mg GAE/g in the extracts of stem barks and leaves respectively. The $AlCl_3$-based colorimetric method showed the total flavonoid contents of 27.44 ± 0.68 mg QE/g in stem barks and 18.44 ± 0.25 mg QE/g in the leaves extract. Besides, the antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. The stem bark exhibited higher antioxidant activity (IC_{50} = 36.50 ± 0.79 μg/mL) than that of the leaf extract (IC_{50} = 50.77 ± 1.29 μg/mL), as compared to the standard ascorbic acid (IC_{50} = 10.82 ± 0.04 μg/mL). These findings validate the traditional practice of using the plant, underscore the existence of a diverse range of bioactive compounds, and highlight the necessity for further investigations into its potential health benefits.

KEYWORDS: *Tinospora cordifolia*, phenolics and flavonoids content, antioxidant

INTRODUCTION

All over human history, the human beings have employed plants as their main solution for a variety of health complications. The use of plant-based remedies is linked to various aspects, including socio-economic status, level of knowledge, age, and the inadequacy of contemporary treatments. The adoption of herbal therapies is on the rise because of their minimal side effects, sustainable sourcing, and affordability (Bachtel & Israni-Winger, 2020). A plentiful evidence indicates that the use of plant-based substances has been a long-lasting tradition for more than 5,000 years in the regions such as China, the Indian subcontinent, Egypt, and Syria. The esteemed Hindu texts, including Ayurveda, Charak Samhita, and Sushrut Samhita, contain the descriptions of numerous plants, both toxic and edible, to animals (Prasathkumar et al., 2021).

Unlike animals, plants are sedentary organisms and have to tolerate different biotic and abiotic stresses in their natural habitat. To endure the compression of these burdens, they synthesize unique compounds in their leaves, stems, roots, and flowers, which are not essential for regular metabolisms including growth, reproduction, and development (Ozyigit et al., 2023). The secondary metabolites are more complex structured molecules developed from the primary metabolites through different biosynthesis variations, such as methylation, glycosylation, and hydroxylation. The major plant secondary metabolites are the phenolic compounds that consist of simple sugars and benzene rings, terpenes, and steroids consisting only of carbon and hydrogen, and compounds containing nitrogen (Twaij & Hasan, 2022). Various secondary compounds, such as alkaloids, flavonoids, polyphenols, terpenes, steroids, coumarins, and more, have displayed remarkable biological and pharmaceutical effects in both humans and animals (Pang et al., 2021).

In living cells, two major types of reactive free radicals are produced during the mitochondrial reactions. They include reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and reactive nitrogen species (RON) such as NO_2, N_2O_3, $-ONO_2^-$, and HNO_2 produced as metabolic byproducts (Pizzino et al., 2017; Wani & Tirumale, 2018). These chemical substances, when present in the correct concentrations, play crucial roles in several physiological processes like protein phosphorylation, activation of transcription factors, apoptosis, oocyte maturation, and cellular immunity. However, an excessive concentration leads to a state of oxidative and nitrosative stress that contributes to cellular damage by distracting cellular lipids, proteins, and DNA (Rajendran et al., 2014). The oxidative stress perplex essential physiological activities and cause cancer, strokes, myocardial infarction, diabetes, and neurodegenerative disorders (Sharifi-Rad et al., 2020). The human body possesses inherent mechanisms for regulating oxidative stress. These mechanisms include endogenous enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), along with non-enzymatic intracellular reducing agents like NADPH, coenzyme Q, glutathione (GSH), uric acid, albumin, and bilirubin, among others. Additionally, exogenous antioxidants derived from various plant-based sources including vitamin C, vitamin E, β-carotene, phenolic acids, zinc, selenium, flavonoids, isoflavones, kaempferol, etc. constitute the primary components, thus involving in the management of oxidative stress (Bouayed & Bohn, 2010).

The secondary metabolites found in fruits, seeds, and other parts of the plants including phenolic acids, flavonoids, tannins, anthocyanins, and stilbenes are regarded as antioxidant agents to fight against ROS-induced disorders (Yu et al., 2021). The promising antioxidant property of polyphenols is attributed to their ability of quenching singlet and triplet oxygen and degrading the peroxides. The flavonoids and phenolic which are abundant in most of the plants scavenge free radicals as well as hinder the activities of free radical producing enzymes (Stanković et al., 2016). Modern isolation and characterization techniques have led to the discovery of numerous noteworthy compounds derived from plants, which have been further developed into pharmaceutical drugs. The current obstacle lies in comprehending the precise mechanisms of these molecules, harnessing the traditional wisdom, which are associated with various plants from different communities worldwide, and improving the production of these compounds from natural sources (Wink, 2015).

With the growing research on natural products, *Tinospora cordifolia* is also one of the important bioactive plants. It is a deciduous shrub native to tropical areas of India, Nepal, Myanmar, and Sri Lanka which is known as *Giloya* in Hindi, and *Gurjo* in Nepali belonging to the family of Menispermaceae (Sinha et al., 2004). There is a long history of employing *T. cordifolia* successfully in Ayurvedic medicine (P. Sharma et al., 2019). The secondary metabolites isolated from various parts of the plant have been reported with a wide medicinal uses, including anti-arthritic, anti-cancer, anti-diabetic, anti-microbial, anti-inflammatory, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, and anti-neoplastic activities (Ahsan et al., 2023).

The plant has captured the interest of researchers from all over the world. Different communities residing at various villages of Nepal use *Tinospora cordifolia* against several health complications. The Raji people of western part of Nepal use the plant for gastrointestinal disorders, fever, and diarrhea. In Chitwan District of central part of Nepal, the plant is used in the treatment of asthma, jaundice, cough, gout, skin rashes, and uropathy (Modi, Shah, et al., 2021). This plant was extensively used against several health complications like asthma, cold, fever as well as against Covid-19 pandemic (Panneer selvam et al., 2023). The present study uses a methanolic extract of *T. cordifolia* stem barks and leaves collected from Rupa Village of Kaski to assess the total phenolic, flavonoid contents, and antioxidant activity. The study aims to validating the traditional use of the plant for the treatment of disease that is associated with free radicals.

MATERIALS AND METHODS

Chemicals and Tools

Chemicals and reagents of the highest purity and distilled water were used throughout the lab work. The Folin-Ciocalteu reagent (FCR), $AlCl_3$, CH_3COOK, and Na_2CO_3 were purchased from the Thermo-Fisher Scientific India, Pvt. Ltd. Secondly, gallic acid, ascorbic acid, and quercetin were purchased from the Himedia Laboratories Company Ltd., India. Similarly, dimethyl sulphoxide (DMSO), ethyl acetate, methanol, ethanol, chloroform, n-hexane, and dichloromethane were received from the Merk Life Science Ltd. Next, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Tokyo Chemical Industries Co. Ltd. All chemicals and solvents used during the study were of

analytical grade. The Thermo Fisher Scientific Multichannel pipettes, Bio Tek Synergy LX Multi-mode readers, IKA RV 10 Rotary evaporators, and Samsung refrigerators were some of the instruments used during the study.

Collection and Identification of Plant

Plant materials of *T. cordifolia* were collected from Rupa Village. The herbarium was deposited and the plant was identified by comparing with the specimen at the Department of Botany, Prithvi Narayan Campus, Pokhara. The stem barks and leaves from a single wild mature plant were also collected for the study.

Preparation of Plant Extracts

The plant's leaves and barks were washed, divided into the little pieces, and dried for four weeks at the shed. The dried parts were mechanically ground into a fine powder. The extracts of pulverized powder of stem barks and leaves were prepared separately by maceration with methanol to obtain the crude extracts. The methanol extracts of leaves and barks were concentrated by using a rotary evaporator and stored in a refrigerator at 4ºC until further analysis (S. Sharma & Joshi, 2011).

Phytochemical Screening

The methanol extracts of the plant barks and leaves were screened for several phytochemicals like polyphenols, alkaloids, flavonoids, reducing sugars, glycosides, tannins, carotene, terpenoids, phytosterols, coumarins, saponins, and anthracenes adopting standard protocols (Bora et al., 2019; Tiwari et al., 2020).

Determination of Total Phenolics Content (TPC)

The Folin-Ciocalteu method was adopted for the estimation of the TPC of plant (Adhikari, 2021; Lu et al., 2011). To 20 μL of the plant extracts/standard (Gallic acid) in 96 wells plate, 100 µL of FCR (1:10; v/v) was added with shaking, and initial absorbance was taken at 765 nm by using Synergy LX Multi-Mode Reader. Then, 80 μL of 1 M Na_2CO_3 solution was added to the above mixture making a final volume of 200 μL and incubated for 30 minutes and final absorbance was taken. The results were expressed as milligrams of gallic acid equivalent per gram of plant extract (mg GAE/g) using gallic acid as the standard (10-60 μg/mL).

Determination of Total Flavonoids Content (TFC)

The TFC was determined by following the $AlCl_3$ method adopted previously with slight modifications (Ahmed et al., 2015). In 96 wells plate, 130 μL of quercetin of different concentrations was loaded. To 20 μL of plant extracts, 110 μL of distilled water was added. The initial absorbance was taken at 415 nm by using Synergy LX Multi-Mode Reader. 60 μL of ethanol was added to both standard and extract-containing wells. Then, 5 μL of 10% $AlCl_3$ solution was added to the above mixture followed by 5 μL of 1M CH_3COOK making the final 200 μL volume, and incubated in dark for 30 minutes. The final absorbance was taken where quercetin (10–60 μg/mL) was used as standard and the results were expressed as milligrams of quercetin equivalent per gram of plant extract (mg QE/g).

Antioxidant Activity Assay

The In-vitro DPPH radical scavenging method was used to assess the antioxidant activity (Brand-Williams et al., 1995; Khanal et al., 2022). 100 μL of plant extracts and controls were loaded in 96 plate wells. Then, 100 μL of 0.1 mM DPPH radical prepared in methanol was added to each well and initial absorbance was measured at 515 nm. After incubating 30 minutes in dark, the final absorbance was measured. Only DPPH radical and 50% DMSO were used in controls. The percentage (%) of DPPH scavenging was calculated by the following formula:

$$\% \text{ DPPH Scavenging} = \left[\frac{\text{Ac} - \text{As}}{\text{Ac}}\right] \times 100$$

Where; Ac = Absorbance of control, As = Absorbance of sample

Data Analysis

Different computer software were used for the analysis of data as per the requirement. The GraphPad Prism was used for graphical plots and to determine the IC_{50} value (concentration required for 50% inhibition of a particular reaction). The results were presented as mean ± standard deviation (mean ± SD) of triplicate experiments.

RESULTS AND DISCUSSION

Phytochemical Screening, TPC, and TFC

The structurally diverse phytochemicals found in medicinal plants are a significant source of therapeutic benefits for treating a variety of diseases (Adhikari, 2021). Methanol extracts from leaves and barks of *Tinospora cordifolia* collected from Rupa Village were analyzed for various phytochemicals and the result is shown in Table 1. The similar results were shown in other research where phytochemical screening shows the presence of anthraquinone, flavonoids, phenols, and terpenoids in petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol, and water extracts of *T. cordifolia* stem. Additionally, alkaloids, cardiac glycosides, saponin, and tannin were also tested positive in different stem extracts of plant (Shrestha & Lamichhane, 2021).

Table 1:

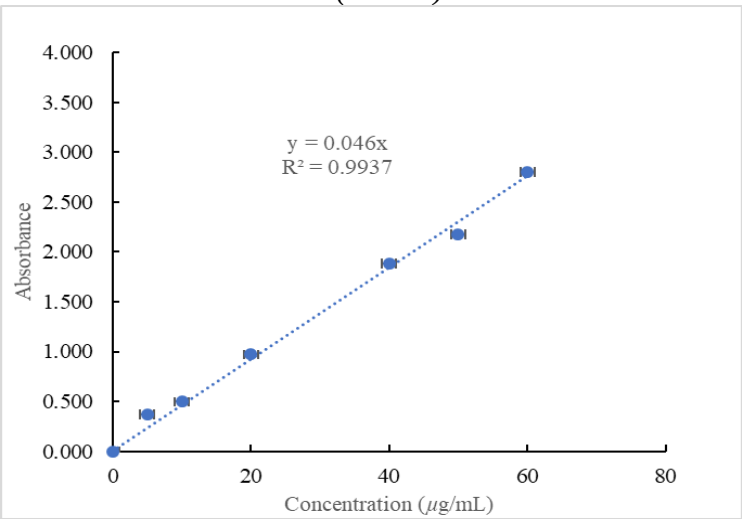
List of Phytochemicals Present in Leaf and Stem Bark Extracts of Tinospora cordifolia

S.N.	Secondary Metabolites	Methanol Extract of Leaves	Methanol Extract of Barks
1	Alkaloids	++	+
2	Anthracene	+	+
3	Carotene	-	-
4	Coumarins	+	+
5	Flavonoids	++	++
6	Glycosides	++	++
7	Polyphenols	++	++
8	Phytosterol	-	+
9	Reducing sugar	+	+
10	Saponins	++	+
11	Tannins	-	-
12	Terpenoids	++	++

A colorimetric method was used to estimate the TPC of crude methanolic plant extracts using FCR. During the experiment reduction of the molybdenum component in the phosphotungstic-phosphomolybdic complex reagent takes place. The content of phenols in the extracts was expressed in gallic acid equivalents (GAE) through the standard curve equation: y = 0.046x, R^2 = 0.9976 (Figure 1). Similarly, TFC was measured using the $AlCl_3$ method in which complexes are formed between $AlCl_3$ and flavonoids. The TFC of plant extracts was expressed in quercetin equivalents (QE) using the standard curve equation: y = 0.0239x, R^2 = 0.9964 (see Figure 2). The methanol extract of *T. cordifolia* bark showed higher phenol levels (50.84 ± 0.38 mg GAE/g) as compared to the leaf extract (41.39 ± 0.44 mg GAE/g). In addition, the bark extract has a higher flavonoid content (27.44 ± 0.68 mg QE/g) than the leaf extract (18.44 ± 0.25 mg QE/g). Phenols and flavonoids are responsible for bioactivities. Other research carried out in different parts of the world also shows the similar results. Different extracts of *T. cordifolia* purchased from the local market of Nanded City, India showed the phenolic content of 0.244 (Ethanol extract), 0.2167 (water extract), 0.176 (ethyl acetate extract), 0.125 (petroleum ether extract), 0.087 (acetone extract) and 0.042 mg/mL of GAE (chloroform extract) (Sonkamble & Kamble, 2015).

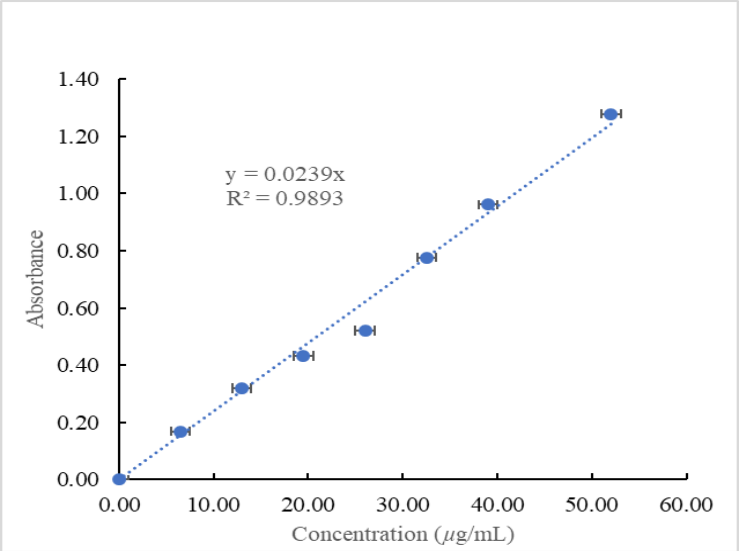
Figure 1

Gallic Acid Standard Curve (765 nm)



Similarly, in other study, petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol, and water extracts of plant stem showed the total phenolics of a range of 8.75-52.50 mg catechol equivalent per gram of the sample (mg CE/g) (Mishra et al., 2013). The methanol extract from the leaves and stem of *Tinospora cordifolia* from Ilam District of Nepal, exhibited elevated Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) as compared to the hexane extract. This suggests a greater efficiency in extracting polar compounds by using a polar solvent (Modi, Koirala, et al., 2021). The secondary metabolites present in the plant can vary even within the same plant due to differences in sampling methods, analysis of plant parts, solvents used for extraction, harvest time, storage, growing climate conditions, genetic factors, and many more (Aryal et al., 2021).

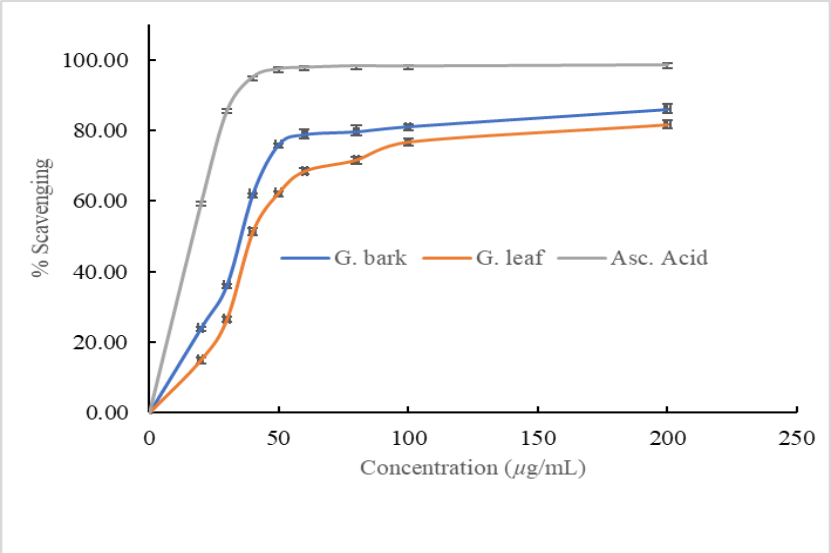
Figure 2
Quercetin Standard Curve (415 nm)



Antioxidant Activity

During the antioxidant assay, a stable DPPH radical that is initially violet in color fades out in presence of antioxidants and this color change was monitored to find the radical scavenging activity. The antioxidant ability of *T. cordifolia* barks and leaves were concentrations dependent and increased gradually with concentrations. Figure 3 shows the % scavenging of DPPH radical at different concentrations of plant extracts along with standard ascorbic acid.

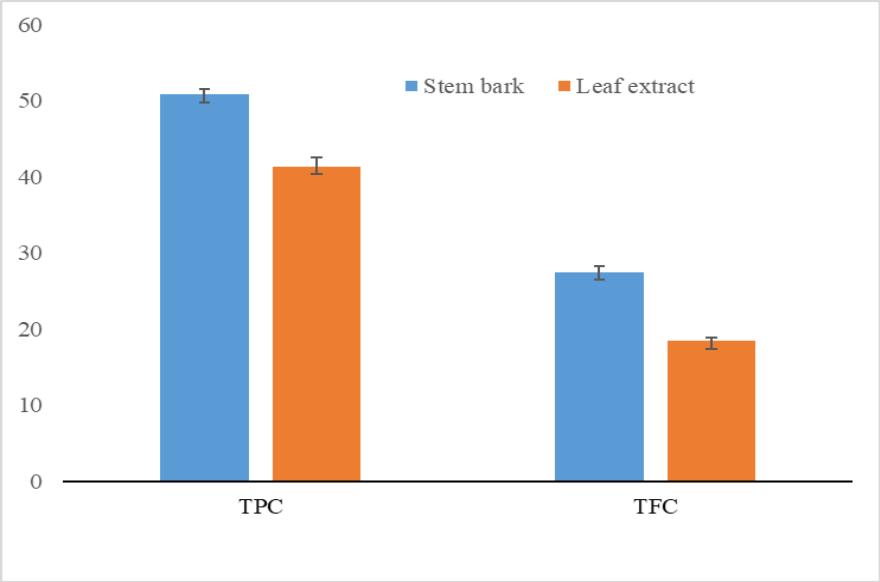
Figure 3
Dose-Dependent Variation of % Scavenging Capacity



The crude methanol extract of *T. cordifolia* barks and leaves showed a good DPPH radical scavenging activity with an IC_{50} value of 36.50 ± 0.79 and 50.77 ±1.29 μg/mL respectively, which was the least potent than the standard ascorbic acid (IC_{50} = 10.82 ± 0.04 μg/mL). The polarity-dependent extraction and antioxidant activity of extracts have been shown previously on *Phaseolus vulgaris* seeds (Nawaz et al., 2020). The high antioxidant activity of methanol extract of barks might be due to the rich in phenolic and flavonoid compounds as supported by TPC and TFC (Figure 4).

Figure 4

Comparison of TPC and TFC of Stem Bark and Leaf Extracts of Tinospora cordifolia



The living body constantly produces free radicals during metabolism because they are needed for a number of vital processes that are necessary for survival. Active oxygen species such as superoxide, hydroxyl radical, singlet oxygen, and alpha oxygen, which are responsible for cell damage or injury. A body with the weak defense system is unable to counteract these increased radicals, which ultimately leads to imbalance, and this condition of excess of free radicals than antioxidants is known as the oxidative stress. An excessive reactive oxygen species and free radicals have been linked to a number of human diseases such as the cardiovascular disease, nerve damage, blindness, nephropathy, arteriosclerosis, cancer, inflammatory disorders, aging, and spermatogenesis dysfunctions and damage oocytes (Abdel-Hameed, 2009; Noh et al., 2020). Antioxidants are substances that bind with free radicals and stabilize them preventing a damage caused to the body. In the body, antioxidants bind with freer radicals. They further emphasize the importance of structurally diverse secondary metabolites from the plants with antioxidants activity for the search of novel drug candidates against various diseases.

CONCLUSION

Various secondary metabolites from medicinal plants are useful for the management of different diseases. This study estimated the total phenolics and

flavonoids content, and antioxidant activity of a methanolic extract of *T. cordifolia* stem barks and leaves. The raw methanol extract from both the stem barks and leaves exhibited a significant antioxidant efficacy, with IC_{50} values of 36.50 ± 0.79 μg/mL and 50.77 ± 1.29 μg/mL, respectively. The antioxidant potential of the stem bark surpassed that of the leaves and revealed a positive correlation with the total phenolic and flavonoid compounds. To realize the therapeutic potential of the plant, further studies are needed to isolate pure compounds and validate them through *in vitro* and *in vivo* experiments.

ABBREVIATIONS

DNA: Deoxyribonucleic acid; DPPH: 2, 2-Diphenyl-1-picrylhydrazyl; FCR: Folin-Ciocalteu reagent; mg QE/g: Milligrams of quercetin equivalent per gram of plant extract; mg GAE/g: Milligrams of gallic acid equivalent per gram of plant extract; NADPH: Nicotinamide adenine dinucleotide phosphate, TFC: Total flavonoids content; TPC: Total phenolics content

CONFLICT OF INTERESTS

The author has no conflicts of interest to disclose.

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REFERENCES

Abdel-Hameed, E. S. S. (2009). Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. *Food Chemistry*, *114*(4), 1271–1277. https://doi.org/10.1016/j.foodchem.2008.11.005

Adhikari, B. (2021). Roles of alkaloids from medicinal plants in the management of diabetes mellitus. *Journal of Chemistry*, *2021*, 1–10. https://doi.org/10.1155/2021/2691525

Ahmed, I. A., Mikail, M. A., Bin Ibrahim, M., Bin Hazali, N., Rasad, M. S. B. A., Ghani, R. A., Wahab, R. A., Arief, S. J., & Yahya, M. N. A. (2015). Antioxidant activity and phenolic profile of various morphological parts of underutilised *Baccaurea angulata* fruit. *Food Chemistry*, *172*, 778–787. https://doi.org/10.1016/j.foodchem.2014.09.122

Ahsan, R., Mishra, A., Badar, B., Owais, M., & and Vijayshwari Mishra. (2023). Therapeutic application, phytoactives and pharmacology of *Tinospora cordifolia*: An evocative review. *Chinese Journal of Integrative Medicine*, *29*(6), 549–555.

Aryal, B., Adhikari, B., Aryal, N., Bhattarai, B. R., Khadayat, K., & Parajuli, N. (2021). LC-HRMS profiling and antidiabetic, antioxidant, and antibacterial activities of *Acacia catechu* (L.f.) Willd. *BioMed Research International*, *2021*, 1–16. https://doi.org/10.1155/2021/7588711

Bachtel, N., & Israni-Winger, K. (2020). Plant-based medicine and pharmacology. *Yale Journal of Biology and Medicine*, *93*(2), 227–228.

Bora, R., Khakhalary, S., & Dutta, T. (2019). Phytochemical profiling, assessment of total phenolic content, total flavonoid content, and antioxidant activity of ethnomedicinal plant, *Meyna spinosa* from Assam. *Asian Journal of Pharmaceutical and Clinical Research*, *12*(11), 61–63. https://doi.org/10.22159/ajpcr.2019.v12i11.34616

Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants - Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, *3*(4), 228–237. https://doi.org/10.4161/oxim.3.4.12858

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Khanal, L. N., Sharma, K. R., Pokharel, Y. R., & Kalauni, S. K. (2022). Phytochemical analysis and in vitro antioxidant and antibacterial activity of different solvent extracts of *Beilschmiedia roxburghiana* nees stem barks. *Scientific World Journal*, *2022*, 1–7. https://doi.org/10.1155/2022/6717012

Lu, X., Wang, J., Al-Qadiri, H. M., Ross, C. F., Powers, J. R., Tang, J., & Rasco, B. A. (2011). Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. *Food Chemistry*, *129*(2), 637–644. https://doi.org/10.1016/j.foodchem.2011.04.105

Mishra, A., Kumar, S., & Pandey, A. K. (2013). Scientific validation of the medicinal efficacy of *Tinospora cordifolia*. *The Scientific World Journal*, *2013*, 1–9. http://dx.doi.org/10.1155/2013/292934

Modi, B., Koirala, N., Aryal, S. P., Shrestha, J., Koirala, S., Jitendra, U., Basnyat, R. C., Nassan, M. A., Alqarni, M., & Batiha, G. E.-S. (2021). *Tinospora cordifolia* (Willd.) Miers: Phytochemical composition, cytotoxicity, proximate analysis and their biological activities. *Cellular and Molecular Biology*, *51*(1), 50–57.

Modi, B., Shah, K. K., Shrestha, J., Shrestha, P., Basnet, A., Tiwari, I., & Aryal, S. P. (2021). Morphology, biological activity, chemical composition, and medicinal value of *Tinospora cordifolia* (willd.) Miers. *Advanced Journal of Chemistry-Section B*, *3*(1), 36–53.

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*, 1–9. https://doi.org/10.1590/s2175-97902019000417129

Noh, S., Go, A., Kim, D. Bin, Park, M., Jeon, H. W., & Kim, B. (2020). Role of antioxidant natural products in management of infertility: A review of their medicinal potential. *Antioxidants*, *9*(10), 1–65. https://doi.org/10.3390/antiox9100957

Ozyigit, I. I., Dogan, I., Hocaoglu-Ozyigit, A., Yalcin, B., Erdogan, A., Yalcin, I. E., Cabi, E., & Kaya, Y. (2023). Production of secondary metabolites using tissue culture-based biotechnological applications. *Frontiers in Plant Science*, *14*, 1–28. https://doi.org/10.3389/fpls.2023.1132555

Pang, Z., Chen, J., Wang, T., Gao, C., Li, Z., Guo, L., Xu, J., & Cheng, Y. (2021).

Linking plant secondary metabolites and plant microbiomes: A review. *Frontiers in Plant Science*, *12*, 1–22. https://doi.org/10.3389/fpls.2021.621276

Panneer selvam, K., Payyappallimana, U., Ravikumar, K., & Venkatasubramanian, P. (2023). Can Guduchi (*Tinospora cordifolia*), a well-known ayurvedic hepato-protectant cause liver damage? *Journal of Ayurveda and Integrative Medicine*, *14*(1), 100658. https://doi.org/10.1016/j.jaim.2022.100658

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 1–13. https://doi.org/10.1155/2017/8416763

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., & Sadhasivam, S. (2021). Therapeutic and pharmacological efficacy of selective Indian medicinal plants – A review. *Phytomedicine Plus*, *1*(2), 100029. https://doi.org/10.1016/j.phyplu.2021.100029

Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R., Gnanadhas, E. N., Lakshminarasaiah, U., Gopas, J., & Nishigaki, I. (2014). Antioxidants and human diseases. *Clinica Chimica Acta*, *436*, 332–347. https://doi.org/10.1016/j.cca.2014.06.004

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., … Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, *11*, 1–21. https://doi.org/10.3389/fphys.2020.00694

Sharma, P., Dwivedee, B. P., Bisht, D., Dash, A. K., & Kumar, D. (2019). The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. *Heliyon*, *5*(9). https://doi.org/10.1016/j.heliyon.2019.e02437

Sharma, S., & Joshi, M. (2011). Comparison of anti-oxidant activity of *andrographis paniculata* and tinospora. *Journal of Current Chemical and Pharmaceutical Sciences*, *1*(1), 1–8.

Shrestha, T., & Lamichhane, J. (2021). Assessment of phytochemicals, antimicrobial, antioxidant and cytotoxicity activity of methanolic extract of *Tinospora cordifolia* (*Gurjo*). *Nepal Journal of Biotechnology*, *9*(1), 18–23. https://doi.org/https://doi.org/10.3126/njb.v9i1.38646

Sinha, K., Mishra, N. P., Singh, J., & Khanuja, S. P. S. (2004). *Tinospora cordifolia* (*Guduchi*), a reservoir plant for therapeutic applications: A review. *Indian Journal of Traditional Knowledge*, *3*(3), 257–270.

Sonkamble, V. V, & Kamble, L. H. (2015). Antidiabetic potential and identification of phytochemicals from *Tinospora cordifolia*. *American Journal of Phytomedicine and Clinical Therapeutics*, *3*(1), 1–14. http://www.ajpct.org/index.php/AJPCT/article/download/242/242

Stanković, N., Mihajilov-Krstev, T., Zlatković, B., Stankov-Jovanović, V., Mitić, V., Jović, J., Čomić, L., Kocić, B., & Bernstein, N. (2016). Antibacterial and antioxidant activity of traditional medicinal plants from the Balkan peninsula. *NJAS - Wageningen Journal of Life Sciences*, *78*, 21–28. https://doi.org/10.1016/j.njas.2015.12.006

Tiwari, S., Nepal, S., Sigdel, S., Bhattarai, S., Rokaya, R. K., Pandey, J., Khadka, R. B., Aryal, P., & Bhandari, R. (2020). Phytochemical screening , antibacterial - guided fractionation, and thin - layer chromatographic pattern of the extract obtained from *Diploknema butyracea*. *Pharmacognosy Research*, *12*(4), 437–443. https://doi.org/10.4103/pr.pr

Twaij, B. M., & Hasan, M. N. (2022). Bioactive secondary metabolits from plant sources: Types, synthesis, and their therapeutic uses. *International Journal of Plant Biology*, *13*(1), 4–14. https://doi.org/10.3390/ijpb13010003

Wani, N. A., & Tirumale, S. (2018). Evaluation of antioxidant properties of different extracts of *Chaetomium cupreum* SS02. *Bulletin of Faculty of Pharmacy, Cairo University*, *56*(2), 191–198. https://doi.org/10.1016/j.bfopcu.2018.08.001

Wink, M. (2015). Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, *2*(3), 251–286. https://doi.org/10.3390/medicines2030251

Yu, M., Gouvinhas, I., Rocha, J., & Barros, A. I. R. N. A. (2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific Reports*, *11*(1), 1–14. https://doi.org/10.1038/s41598-021-89437-4