ISSN: 1991-2951 (Print)
ISSN: 2091-2781 (Online)
Our Nature
Journal homepage: http://nepjol.info/index.php/ON

Evidence of Antimicrobial, Toxicity, and Antioxidant Properties of Selected Plants Based on Ethnomedicinal Practices in The Eastern Highlands of Nepal

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Abstract
Evidence-based ethnomedicine is the study of the comparison of traditional medicine based on bioactive compounds in plants. The current study aimed to record the medicinal plants used by the Tamang people of Udayapurgadhi Rural Municipality, Udayapur, Nepal, and performed some bioactivity to reveal the scientific basis of their use. Data were collected by observation of study areas and interviews (N=61) and group discussions (N=7) with local people. Of the plants using by ethnic communities, extracts of two species *Clerodendrum viscosum* and *Iresine herbestii* were investigated for antimicrobial tests, cytotoxicity, total phenolic content, total flavonoid content, and DPPH free radical assay for antioxidant properties. Altogether, 25 plant species (21 Family) as traditional medicine were found to be used for 19 human illnesses by the Tamang people. A very strong antioxidant activity of methanolic extracts was observed with *Clerodendrum viscosum* and *Iresine herbestii.* The antimicrobial test was slightly positive, while the cytotoxicity test was negative. Traditional medicine systems and their scientific evaluation are essential to create reliable alternative medicinal approaches to human welfare.

Keywords: Clerodendrum, DPPH, Ethnomedicine, Hindu-Kush Himalayas, Iresine

DOI: https://doi.org/10.3126/on.v24i1.97230
Manuscript details: Received: 07.05.2026 / Accepted: 28.07.2026
Citation: Katuwal, K., Badal, C., Pant, P., Ranjit, R., & Ghimire, T. R. (2026). Evidence of antimicrobial, toxicity, and antioxidant properties of selected plants based on ethnomedicinal practices in the eastern highlands of Nepal. *Our Nature*, *24*(1): 31-46. https://doi.org/10.3126/on.v24i1.97230

Introduction
Ethnomedicine is the study of traditional medicine practiced by different ethnic and indigenous communities. It is a multidisciplinary subject that comprises a set of empirical indigenous practices of using plants and animals accompanied by the use of spirituality with or without mantra to heal animals or humans (Ghimire et al., 2020a). Scientifically, it is a comparison of traditional

medicine based on bioactive compounds in plants (Deepak & Anshu, 2008). Knowledge, perception, and practices (KPP) of ethnomedicine depend on locally available plant species as well as plant-based products; thus, it is cosmopolitan (Awas & Demissew, 2009). For many years, plants have been central keys in treating and preventing many diseases and disorders in humans and domestic animals in many indigenous/ethnic communities in the Hindu-Kush Himalayan regions (Ghimire et al., 2020b; Ghimire et al., 2020a). The popularity of ethnomedicine in these communities is due to their cultural relationships with plants and the shared knowledge among people, and the inexpensiveness, accessibility, safety, and efficacy of plant-based drugs. Even secondary metabolites present in many ethnomedicine-based plants have been evidenced to be effective in antimicrobial therapy, including COVID-19 (Adeleye et al., 2021; Li et al., 2021; Mahapatra et al., 2019), suggesting a critical role of this branch in public health.

Nepal, the landlocked country bordered between India and China, and rich in the context of biodiversity, is a multi-ethnic, multicultural, multireligious, and multilingual HKH country. For example, it has 142 castes/ethnicities with 124 mother tongues and is living in a perfectly coherent society (National Statistics Office [NSO], 2022). Interestingly, Tamang (horse trader), as described by Bista (1967), is one of the major ethnic groups of Nepal comprising 5.62% of the total Nepalese population (NSO, 2022). They mostly inhabit hilly and mountainous areas with complex landscapes of culture, languages, geography, and environments, and biodiversities. Many of these ethnic communities are farmers engaged in agriculture and few of them usually practice traditional medicines, especially in the countryside**.** Thus, their relationship with local flora is crucial and their daily lives have been observed to include the use of various plants to treat and prevent different diseases in the country. However, the scientific basis of their ethnic knowledge and proper documentation is still lacking. Among these groups, highly useful knowledge required for the welfare of human and animal health is prevalent, which is being threatened in the form of fragmented knowledge due to the modern development of the medicinal system. Therefore, there is an urgent need for scientific evaluation and proper documentation of ethnomedicinal knowledge. Thus, the current study aimed to document the ethno-medicinal knowledge of the Tamang community in the rural municipality of Udayapurgadhi. Furthermore, the study aimed to examine the extracts of any two plants mentioned by those people for antimicrobial and antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), and cytotoxicity."

Materials and Methods

Study area

The current study area was Dumre ward located in the Udayapurgadhi Rural Municipality of Udayapur district, Nepal **(Figure 1)**. The district is surrounded by Mahabharata hills from north and Shiwalik from south and both hills meet together by west, which forms a valley called the Udayapur Valley. It has subtropical and temperate climatic conditions. The Dumre ward actually indicates ward number 4, where different ethnic communities such as Tamang, Magar, and Rai reside.

Collection of ethnomedicinal data

To collect ethnomedicinal data, structured questionnaire interviews, observation, and focal group discussion were used. A total of 61 local respondents of age 40 or more than 40 were purposively selected for the interview because these groups of people had a high awareness of ethnomedicinal contexts. There were 55.7% males and 44.3% among the age groups of the participants (40-80 years). There were 90.1% farmers, 3.3% healers, and 6.6% teachers among the respondents. FGDs with 7 different people were also carried out for three hours to prepare a consensus list of medicinal plants based on the agreement on the use of medicinal plants cited by the local people. Additionally, during the study, the researchers directly observed the treatment process of local healers and the tools needed to process medicine.

Collection and identification of samples

A total of 25 plants having medicinal value mentioned during the interview were collected in the field with the help of local people and traditional healers. Herbaria were prepared according to standard technique and were identified by experts in National Herbarium & Plant Laboratories (KATH), Government of

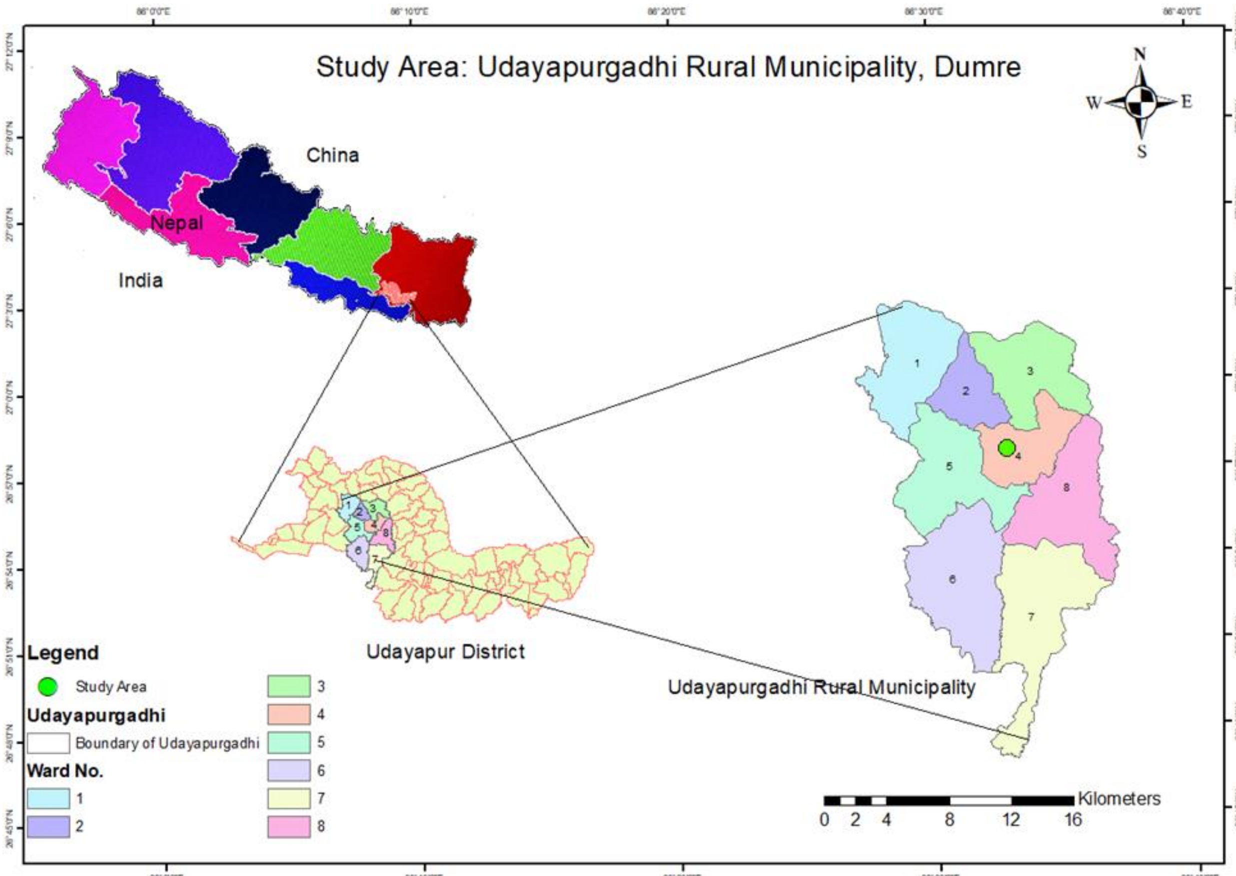

Figure 1. Map of the study area.

Nepal, Department of Plant Resources Ministry of Forest and Environment, Godavari, Lalitpur

Laboratory assays

Two plant species (*Clerodendrum viscosum* and *Iresine herbestii*) **(Figure 2)** were selected for the study of antimicrobial susceptibility, antioxidant properties, and cytotoxicity assays based on the respondents' belief (Paudel et al. 2020).

Chemicals & Reagents

All materials (Fresh leaf extractions of *Clerodendrum viscosum* and *Iresine herbestii),* including solvents (Methanol), distilled water, ferric chloride ($FeCl_3$), ferrocyanide, Gallic acid solution, and other reagents were analytical grade. Ascorbic acid was purchased from Hi-Media and DPPH (1, 1 -diphenyl-2-pycrylhydrazyl), manufactured by Sigma Chemical Co. Ltd. (St. Louis, MO, USA) was used.

Extraction

Plant samples were dried in the shade, powdered in a grinder, and soaked with methanol for three days. The solvent was filtered, and the filtrate was evaporated with the help of a rotatory evaporator to obtain concentrated extract. The residue left was again extracted with methanol, and the process was repeated three times. The extract was then left to air dry to completely evaporate the solvent. After being completely dried, the extract was weighed. To prepare the stock solution, each plant sample was weighed 0.1 g and dissolved in 1 ml of methanol to obtain 10mg/ml of stock solution.

Antimicrobial Susceptibility Test (AST)

The Mueller Hinton Agar (MHA) (38%w/v) and the Nutrient Broth (28% w / v) mixture was heated in distilled water to completely dissolve all components and autoclaved for approximately one hour. After cooling, it was poured (25 ml per MHA plate) on the sterilized Petri plates and left to solidify at room temperature.

The American Type Culture Collection (ATCC) culture of *Klebsiella pneumonia* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhi* (clinical isolate), *Enterococccus faecalis* (ATCC 29212) were maintained by subculture on nutrient agar and preserved at 4 ° C. Then, we inoculated in nutrient broth (NB) and incubated for about one hour. The NB became turbid before being removed from the incubator. Then, already prepared MHA plates were swabbed with microorganisms from the NB. When the plate was dried, a well was made in Agar with the help of sterilized cork-borer. Then 50 µl of Streptomycin as standard, DMSO as negative control, and extract as experiment were placed in different respective wells. The plates were allowed to dry and incubated at 37ºC for 24 hours. The next day, the size of the zone was measured. Each experiment was repeated three times.

Figure 2A

Figure 2B

Figure 2. A. *Clerodendrum viscosum* B. *Iresine herbesti*

Total phenolic content (TPC)

Phenolic compounds have redox properties, which allow them to act as antioxidants (Soobrattee,Neergheen et al. 2005). Therefore, the amount of total phenolic contents (TPC) in both plant extracts was determined using the Folin Ciocalteu (FC) assay previously described with a slight modification using gallic acid as standard (Zhang, Chen et al.

2010). 20µL plant extract was mixed with 100µL of Folin Ciocalteu reagent and 80µL of Na_2CO_3 (7.5%). The sample was then incubated at 45 ° C for 15 minutes until the blue color was observed. Absorbance was measured at 765 nm against methanol as a blank in a 96-well plate ELISA reader (BIOTEK, USA). All tests were carried out in triplicate and TPC was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method as previously described (Ahn, Kumazawa et al. 2004) with slight modification using quercetin as standard. Briefly, both 500l methanolic extracts of 500µl were mixed with 150µl of $NaNO_2$ (5%w/v). After 5 minutes, 250 µl of $AlCl_3$ (2% w/v) was added. The whole mixture was then mixed and incubated for 6 minutes. Then it was neutralized with 0.5 ml of 1M NaOH solution and the mixture was left for 10 min at room temperature. All experiments were performed in triplicate and absorbance was measured at 424 nm on a 96-well plate ELISA reader (BIOTEK, USA). TFC was expressed as the milligram of quercetin equivalent per gram of dry weight (mg QE/ g DW).

Antioxidant activity

The antioxidant activity of these plant extracts was examined based on the scavenging activity of the stable 1, 1 diphenyl 2-picrylhyorazyl (DPPH) free radical (Surinut, Kaewsutthi et al., 2003, Valko, Leibfritz et al., 2007, Gawron-Gzella, Dudek-Makuch et al., 2012, Ramakrishna, Murthy et al., 2012). One milliliter (ml) of 0.6 mM DPPH solution in methanol was mixed with 1mg/ml of plant extract solution of varying concentrations (10, 20, 30, 40, 50, 60, 70 and 80 µg/ml). Corresponding blank samples were prepared and L-ascorbic acid (1-100 µg/ml) was used as the reference standard. A Mixer of 1 ml of methanol and 1 ml of DPPH solution was used as a control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in the dark using a UV-vis spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{Inhibition} = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)\text{X } 100}{Absorbance\ of\ control}$$

Then, the linear plot of % inhibition versus concentration was obtained using the equation, y = mx + c, where, x represents the concentration of the measured substance and y denotes the % inhibition. The IC_{50} (50% inhibition concentration) values were obtained from the prepared inhibition curves. Thus, the IC_{50} value was calculated from the graph equation (y = mx+c) when y was equal to 50% and x = IC_{50}.

Cytotoxicity assay

To analyze the role of extracts in cytotoxicity, sea salt was prepared in a beaker in which the egg of brine shrimp was subjected to hatching and kept the required temperature. It was kept for almost 48-72 hours. After hatching, 10 shrimps transformed into another vial containing 5 ml of seawater with extract solution (100-1000mg/ml). These vials were then left over for 24 hours. After 24 hours, survived brine shrimp were counted and calculated by following method (Paudel et al., 2020).

$$\%\ \text{of death} = \frac{\text{Number of death of shrimpX 100}}{Initial\ number\ of\ shrimp}$$

Data analysis

Data were analyzed to know the number/ frequency of people who were still using the medicinal plants, many people who had been cured by them or who had faith in medicinal plants, and who had provided common suggestions for the enhancement of the use of medicinal plants. The data was expressed in tables and graphs. The IC_{50} values were calculated by plotting the graph between concentration and %RSA.

Results

Knowledge, perceptions, and practices (KPP) of medicinal plants

In the current study, the group discussion found that people, first of all, would prefer traditional healers and Vaidhya and would use medicinal plants in case of any disorder. After consulting traditional healers, they only go to local hospitals and use medicines. However, in case of serious illnesses, they used to visit the district or zonal hospital which was far from their place.

Of the 61 respondents, all would go to local medicals for any treatment procedures. 78.7% would go to the hospital, 62.3% would visit the doctors from Jhakri, Dhami and Witch, 54.1% would use medicinal plants and local herbs, and 8.2% would prefer Vaidhya **(Table 1)**. The questionnaire survey explored that out of 61 respondents, 44.3% stated that they have used medicinal plants for treatment.

Their views on the problems with the use of traditional medicines were also recorded. Most of them (90.2%) stated that people do not believe in herbal medicine. Other problems included lack of methodology, lack of evidence, lack of identification/taxonomy, and lack of exact herb **(Table 1)**.

Table 1: Knowledge, practices, and perceptions (KPPs) of local Tamang people to the medicinal plants.

Knowledge, practices, and perceptions		Numbers	%
Duration of the use of herbs	>10 years	10	16.4%
	don't remember	51	83.6%
Treatment procedures	Local Medicals	61	100%
	Hospitals	48	78.7%
	Jhakri/Dhami/Witch doctors	38	62.3%
	Use medicinal plants/local herbs	33	54.1%
	Vaidhya	5	8.2%
Use of medicinal plants for treatment	Yes	27	44.3%
	Don't remember	26	42.6%
	No	8	13.1%
Problems of treating diseases by using traditional methods	Don't know methodology	33	54.1%
	Don't know about the proof whether the herbs actually worked or not	29	47.5%
	Don't know much about the herbal identification/taxonomy	27	44.3%
	Cannot be found the exact herb	25	41.0%
	People don't believe on the herbal medicine	55	90.2%

Most of the respondents (37.7%) mentioned that awareness-raising programs should be brought to their places to preserve the knowledge of traditional practices. Similarly, 8.2% of the respondents suggested that training related to plant identification should be brought to recognize accurate medicinal species. They also demanded allocation of budgets to medicinal plant cultivation, seed and nursery distribution, establishment of herbal processing centers, proper market management, and provision of employment opportunities for the ethnic community **(supplementary 1)**.

In particular, a total of 25 plants crucial to medicinal uses for the local Tamang community were recorded in this study. Those 25 medicinal plants belonged to 21 different families with their different scientific, local, and English names, and their parts, methods of preparation, and for specific disorders, for example for 19 human ailments in the current surveys **(Table 2)**.

Table 2: List of medicinal plants of the study area

Scientific Name	English Name	Local Name	Family	Wild/ Cultivated/ Invasive	Used Parts	Illness/ Diseases	Preparation Methods
Acarus calamus Linnaeus, 1753	Sweet Flag	Bojo	Acaraceae	Agriculture	Rhizome	Throat pain	Chew it
Achyranthes bidentata Blume	Ox Knee	Kamle	Amaranthaceae	Wild	Leaves	Cut wounds	Leaves paste
Albizia lucida (Jacques) Benth. (1844)	Silk Trees	Tate Siris	Fabaceae	Wild	Bark	Dandruff	By grinding the bark and washing hair with it
Amaranthus spinosus Linnaeus	Spiny Amaranthum	Latteko Sag	Amaranthaceae	Agriculture	Leaves	Body ache	Cook as vegetables
Artemisia dubia var. *subdigitata* (Mattf.) Y.R. Ling	Mug-Wort	Titepati	Asteraceae	Wild	Leaves	Skin disease	Apply leaves paste
Astilbe rivularis Buchanan-Hamilton	Astilbe	Thulo Okhati	Saxifragaceae	Wild	Root	Relief pain	Grind the root in powder form and mix water and drink it
Azadirachta indica A. Juss., 1830	Neem	Neem	Meliaceae	Agriculture	Leaves	Skin diseases, pressure	Apply leaves paste or leaves juice
Berberis aristata de Candolle	Tree Turmeric	Chutro	Berberidaceae	Wild	Roots	Conjunctivitis, Jaundice	Grinding the root in powder form
Berginia pacumbis (Buch.-Ham. ex D. Don)	Pashanbheda	Pakhanbed	Saxifragaceae	Wild	Roots	Dysentery	Grind the root in powder form
Centella asiatica (L.) Urban	Gotukola	Ghodtapre	Umbelliferae	Wild	Leaves, Root	Fever	By grinding the used parts and filtering the liquid

							from muslin cloth
Clerodendrum viscosum Vent.	Hill Glory Bower	Bhati	Verbenaceae	Wild	Leaves	Ear pain	Sweat of leaves is put on the ear
Drepanostachyum sp. Keng f.	Khasia Bamboo	Nigalo	Poaceae	Wild	leaves	Shed umbilical cord	Grinding to make it in a powder form
Elsholtzia blanda (Benth.) Benth	Indian Squirrrels	Dhursil	Lamiaceae	Wild	Leaves	Headache	Grinding the leaves and resultant paste is put on the forehead
Iresine herbestii Hook.	Blood Leaf	Iodine Plants	Amaranthaceae	Wild	Leaves/Roots/Stem/	Wound/Cut parts	Grinding the leaves and use paste over wound
Justicia ahatoda Linnaeus	Malabar Nut	Asuro	Acanthaceae	Wild	Leaves	Fractured ligament	Paste of leaves is put on the broken parts
Martynia annua Linnaeus	Cat's Claw	Gahumukhi	Martyniaceae	Agriculture	fruits	Throat pain	Chewing the fruit
Mirabilis jalapa Linnaeus	Four O'clock Plant	Lankasan	Nyctagianaceae	Wild	Roots	Shed umbilical cord	Powder form of root
Momordica Linnaeus	Balsam Apple	Ban karela	Cucurbitaceae	Wild	Seeds	Jaundice	Eat it
Nephrolepis cordifolia (L.) K. Presl	Himalayan Ground Gooseberry	Pani Amala	Nephrolepidaceae	Wild	Fruit	Jaundice	Grind it in powder form and mix the water to make liquid
Oroxylum indicum (L.) Benth. ex Kurz	Buddhist Tree	Totola	Bignoniaceae	Wild	Bark	Throat pain	Dip the bark in water and drink it
Phyllanthus emblica Linnaeus	Emblica	Thulo Amala	Euphorbiaceae	Wild	leaves	Conjunctivitis	Leaves are boiled and its paste wr put on the eyes
Smallanthus	Ground	Bhui	Asteraceae	Agricultu	Fruits under	Increase	Bite it

sonchifolius (Poepp.) H. Rob.	Apple	Syau		re	soil	concentration of water	
Swertia chirayita (Roxb.) H. Karst.	Chiraito	Chiraito	Gentianaceae	Wild	Root, leaves	Fever, throat pain	By grinding the used parts and filtering the liquid via muslin cloth
Thalictrum foliolosum de Candolle	Meadow-rue	Mirmire Jhar	Ranuculaceae	Wild	Leaves	Stomachache	Roots and leaves are made paste and then taken
Vitis heyneana Roemer & Schult.	Vitis	Puruni Lahara	Vitaceae	Wild	Tendril	Typhoid, Conjunctivitis	Cut the climbing part and drink the water

Clerodendrum viscosum and *Iresine herbestii* extracts in ATCC of *Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Enterococccus faecalis* at different concentrations (10µg/ml, 20µg/ml, 50 µg/ml, & 100µg/ml) were negative. Thus, there were few antimicrobial properties in the extracted samples of *C. viscosum* at 20µg/ml **(Supplementary 2);** however, other concentrations did not have any impact on antimicrobial properties.

Total phenolic content (TPC)

The total phenolic content of the methanolic extract, calculated from the calibration curve ($R^2 = 0.994$), was 12.00 ± 0.56 mg equivalent gallic acid / g in *Clerodendrum viscosum* and 51.61 ± 4.38 mg equivalent / g in *Iresine herbestii* (Table 3).

Total flavonoid content (TFC)

Flavonoids are among the major groups of phenolic compounds with a broad spectrum of chemical and biological activities, particularly radical scavenging and antimicrobial activities (Ayele, Akele et al. 2022). The total flavonoid content ($R^2 = 0.969$) was 0.94 ± 0.05 mg equivalent quercetin / g found in *Clerodendrum viscosum* and 8.87± 0.43 mg equivalent quercetin equivalents/g *Iresine herbestii* (Table 3).

Table 3: Total phenolic content (TPC) (Mean±SD), total flavonoid content (TFC) (Mean±SD)

Sample	TPC (mg GAE/gm)	TFC (mg QE/gm)
Clerodendrum viscosum	12.00±0.56	0.94± 0.05
Iresine herbestii	51.61±4.38	8.87±0.43

Antioxidant activity assay

In the DPPH radical scavenging activity assay, 10-100µg/ml of methanol extracts produced moderate to high DPPH scavenging activity in both plant extracts. The DPPH scavenging activity was observed in the *Iresine herbesti* extract sample (86.37%), which was almost similar to standard ascorbic acid (86.093%). In contrast, it was 51.56% in *Clerodendrum viscosum.* Interestingly, the respective IC_{50} values of the methanolic extracts of *C. viscosum, I. herbesti* and standard ascorbic acid were 20.63µg/ml, 7.267µg/ml, and 6.726 µg/ml **(Figure 3A-3C)**.

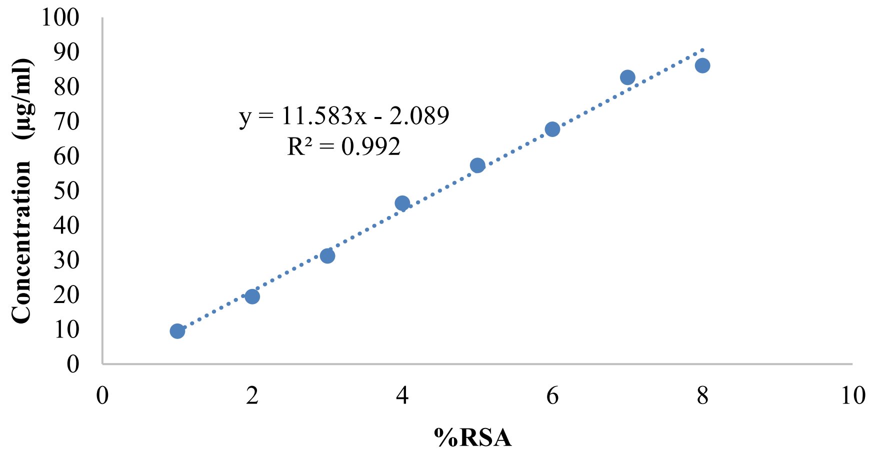

Figure 3A: Calibration curve of % RSA for Ascorbic Acid

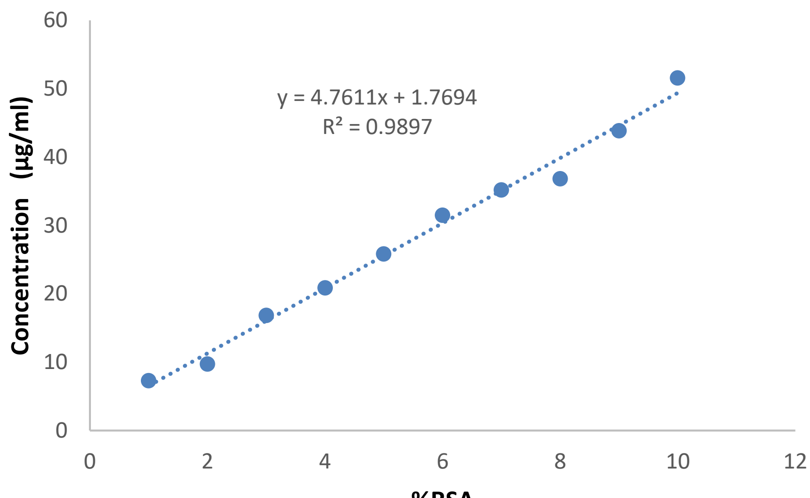

Figure 3B: Calibration curve %RSA of extracts of *C.viscosum*

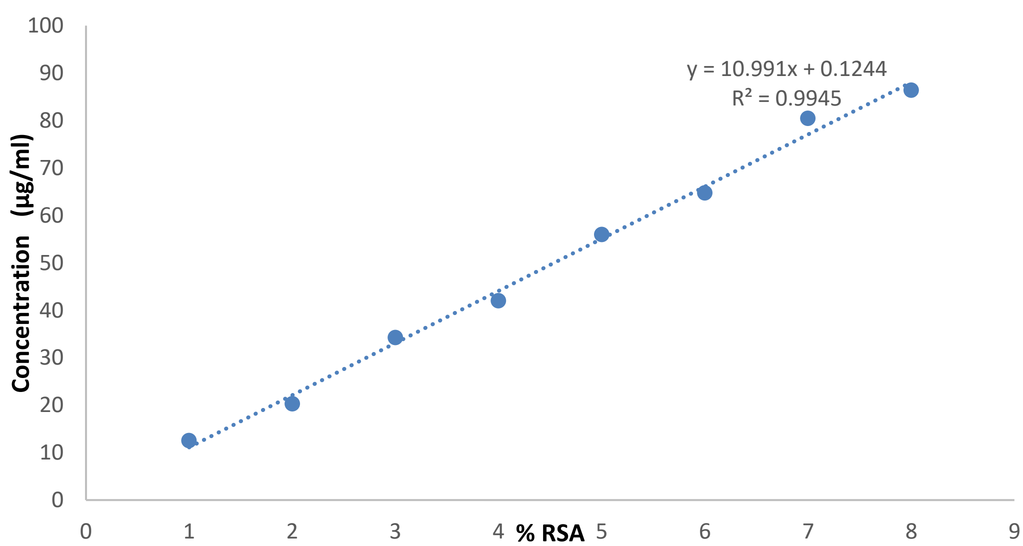

Figure 3C: Calibration Curve % RSA of extracts of *I.herbestii*

Cytotoxicity activity

None of the extracted plants were toxic, as the % mortality rate of the animals at different concentrations of Clerodendrum viscosum and Iresine herbestii extracts was 0.

Discussion

This study surveyed ethnomedicinal knowledge, perceptions and practices (KPP) of Tamang ethnic groups in remote areas of Nepal where people usually depend on available natural resources for food, feed, livelihoods, and medicines. Due to the lack of medical healthcare facilities and cultural factors, these people highly depended on traditional medicinal practices. The present study recognized 25 different plant species used as medicine by the Tamang people of Udayapurgadhi Rural Municipality of Udayapur, Nepal. It is found that the number of plant species used as medicine is lower than those studies conducted in Rasuwa district (46 species) (Prasai et al., 2014), in the Nuwakot district (44 species) (Tamang 2003), in Dolakha district (57 species) (Paudel et al., 2021) and in Makwanpur district (161 species) (Rokaya et al., 2014). However, the number of plants used by the Tamang community was higher than that reported in the same community (21 species) (Yadav & Rajbhandary 2016).

It has been recorded that all of these ethnic groups follow traditional mechanisms in preparing drugs at home, and, they are interested in learning and practicing identification, plantation, and harvesting of plants, and preparation of drugs. In addition, their demands for the establishment of herbal processing units and training indicate that ethnic people have a greater attachment to nature and natural medicines. Furthermore, KPPs are usually passed down generation by generation; however, current ethnic people are concerned about the modern generation. The modern generation does not want to follow alternative medicines due to lack of trust in them or because they think it would be difficult to earn money through the study of this branch (Pant et al., 2020).

In this study, we have tried to translate the beliefs of the Tamang people into laboratory results. These people believed that *Clerodendrum viscosum* (Family: Verbenaceae) and *Iresine herbestii* (Amaranthaceae) could act as wound healing. The role of *I. herbstii* leaves as antimicrobial agent (Khare 2008), wound healing and anticancer agent (Fronza et al., 2009) and externally against skin eczemas, sores and pimples (De Feo 2003) have been already reported. These plants have been shown to be anti-inflammatory, with cytotoxic and apoptotic properties, and also have very low antioxidant activity (Sun et al., 2003). The leaf juice of another plant, *Clerodendrum viscosum,* is used as a strong antihelminthic, emetic, mild laxative, and cholagogue. It is externally used for tumors, skin diseases, snake bites and scorpion stings (Ghani 2003). Thus, we evaluated their antimicrobial, antioxidant properties, and acute toxicity to confirm the ethnotherapeutic claim of wound healing by these plants. Chronic wounds contain *Bacillus* spp*., E. coli, Staphylococcus aureus, Pseudomonas auregenosa*, and *Corynebacterium* (Swogger et al., 2008). A study found that these bacteria delay the healing process by delaying the inflammatory phase (Mertz & Ovington 1993). However, current results lacked antimicrobial susceptibility at the ranges of 10 to 100 µg/ml concentrations of extracts and cytotoxicity; although few antimicrobial properties of *C. viscosum* were shown at 20 µg/ml. Therefore, current laboratory assays do not show the wound healing mechanisms of methanolic extracts from both plants.

Antioxidants are those chemicals that reduce the risks caused by free radicals in biological systems. Hydroxyl radicals are highly reactive to essential macromolecules, including proteins and nucleic acids, and to organisms as a whole (Hashim et al., 2016).

Therefore, the elimination of hydroxyl radicals is essential to protect the body, and understanding the antioxidant content of any plant is essential to evaluate the values of plants. This study showed that *I. herbestii* is a good source of phenolics and flavonoids (12.00 ± 0.56 mg GAE/g and 51.61 ± 4.38 mg QE / g) compared to *C. viscosum* (0.94 ± 0.05 mg GAE/g and 8.87 ± 0.43 mg QE/g). Phenol compounds possess the redox properties (Neergheen et al., 2005). Thus, TPC is an indicator of effectiveness as a free radical scavenging and antioxidant property that is mediated by their hydroxyl (-OH) group (Soobrattee, Neergheen et al. 2005). In particular, the presence of free–OH groups determines mechanisms of action of antioxidant properties of plant flavonoids, including flavonoids, flavones, flavanols, and tannins. Flavonoids have been shown to be antioxidant in action *in vitro* and *in vivo* (Masuda et al., 1996, Ram et al., 2003); therefore, based on strong flavonoid contents, *I. herbestii* currently studied is a strong antioxidant plant compared to *C. viscosum*.

While TPC and TFC are used to indirectly assess the antioxidant property of a plant, the DPPH test is applied to assess antioxidant activity based on the process through which antioxidants limit lipid oxidation, resulting in DPPH free-radical scavenging and thus determining free-radical scavenging potential (Mukherjee et al., 2022). It is one of the most widely used methods for assessing the antioxidant capacities of natural products (Sujarwo & Keim 2019). It normally evaluates IC_{50}, the concentration required for the sample to inhibit 50% of the radical, which is inversely proportional to the antioxidant activity of the given sample. In our study, the IC_{50} value of *Clerodendrum viscosum* was 20.63µg/ml (0.02063 mg/ml) and that of *Iresine herbestii* was 7.267µg/ml (0.007267mg/ml). The level of antioxidant activity has been classified into four levels (Ludwig 2000); for example, very strong (IC_{50} < 50 µg/mL), strong (IC_{50}: 50–100 µg/mL), moderate (IC_{50}: 101–150 µg/mL), and weak (IC_{50}: 250–500 µg/mL). This indicated that both plants in our study had very strong antioxidant activity. Even, *I. berbestii* had three times more antioxidant activity than *C. viscosum*. Therefore, the antioxidant activities of these plants can determine the wound healing mechanisms through their methanolic extracts, which the belief of ethnic groups seemed to be true in our study. As a result, reactive oxygen species (ROS) are generated. The increased free radical activity or low antioxidant activity may lead to a further delay of wound healing (Adly 2010).

Conclusion

The current study revealed that Tamang people in the current study area are known to a total of 25 medicinal plants, their preparation methods for treating and preventing a total of 19 human diseases. Although methanolic extracts from only two plants were used to analyze antibacterial, cytotoxicity, and antioxidant activities in the laboratory, both *Iresine herbestii* and *Clerodendrum viscosum* contained a very strong antioxidant activity, indicating that different assays should be used to reveal these properties as believed by ethnic groups. As inhabitants of the complex landscapes of geography, culture, knowledge, climates, and biodiversities, the KPPs of these ethnic groups should be updated and preserved by carrying out planning, policy, and programs by the government.

Acknowledgements

The authors would like to acknowledge local respondents who took part in the research, Nepal Academy of Science and Technology (NAST) for carrying out the laboratory assays, Ministry of Forest and Environment, Department of Forest & Soil Conservation, Kathmandu, Nepal for issuing the permission of the research (Letter: 075/076/33), Division Forest Office, Udayapur, Nepal for permitting the work (075/76/308), Udayapurgadhi Rural Municipality for permitting the research

(Letter: 157/075/76), Nepal Health Research Council for Ethical Approval (Letter: 1872/6 January, 2019), Nepal Health Research Laboratory, Teku, Kathmandu, for providing ATCC pathogenic bacteria, and Ms. Sonica Dawadi for assisting during laboratory works. We are very grateful to The World Academy of Science (TWAS) Trieste, Italy for providing ELISA Reader (Rosa Ranjit,, RGA No. 13-088RG/CHE/AS_G).

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Availability of Data and Materials
All data generated or analyzed during the research work are included in this research article and its supplementary information files.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The laboratory support was managed by the Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal.

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Supplementary 1: Suggestions to the Government

Suggestions	**Numbers**
Awareness raising program should be brought	23
Identification program should be brought in order to recognize true medicinal plants	5
People should be inspired for medicinal plant farming	5
Training should be given to the unskilled people	4
Herbal processing Center should be established in own local levels	3
Medicinal plant farming should be encouraged as a source of living then many more people would be involved in it	3
Providing employment opportunities by establishing herbal processing center	3
Providing seeds or plantlets of medicinal plants	2
Providing knowledge about the useful medicinal values	2
Process of using medicinal plants should be taught through trainings	2
Budget should be arranged for the importance and conservation of medicinal plants	2
Providing seeds of endangered medicinal plants	1
Publicity should be increased to make it as a source of living	1
Programs should be conducted for the conservation of medicinal plants	1
Skilled instructor should be sent to the local levels for the betterment of use of medicinal plants	1
Budget should be allocated for medicinal plant farming	1
Market arrangement should be done for different medicinal plants	1
Farmers should get the satisfied amount for the medicinal plants	1

Supplementary 2: Antimicrobial Susceptibility pattern of *Bacillus subtilis.* Positive Control (Streptomycin) and Extract of *Clerodendrum viscosum* (20µg/ml).