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# Antibacterial Activity of Selected Ethnomedicinal Plants Popular in Magar Ethnic Community of Palpa District, Western Nepal

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### Abstract

The main objective of this research was to explore the potential antibacterial activity of 25 selected medicinal plant extracts against four strains of bacteria. The ethnomedicinal knowledge was documented using semi-structured, open-ended questionnaires, informal interviews, and group discussions with traditional healers and knowledgeable persons about plants and plant-based remedies. The evaluation of antibacterial activities of twenty-five extracts of different plants was carried out by adopting the disc diffusion method for four bacterial strains, namely - *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The results were reported by observing the inhibition zones. The results indicated that among 25 plant extracts examined, 16 (64%) plant extracts showed antibacterial property against *Escherichia coli* followed by 15 (60%) plant extracts against *Pseudomonas aeruginosa*, and 19 (76%) extracts each against *Staphylococcus aureus* and *Bacillus subtilis*. Four plant extracts were not able to produce the zone of inhibition with any of the tested bacteria. Gram-positive bacteria are found to show more positive effects as compared to Gram-negative. Present findings of this study indicate that ethnomedicinal plant extracts have antibacterial activity against the different strains of tested bacteria. This activity supports their use in the treatment of infections caused by such resistant bacteria.

## 1. Introduction

The history of human civilizations and the development of economic systems and thoughts are all inherently and intricately interwoven with the biological resources (Ravi & Pusphagadan, 1997). Plant resources are naturally precious for the synthesis of medicinal compound and provide great help in discovery in the area of the pharmaceutical field because of the unknown availability either as a standardized extract or as a pure compound (Hassan & Ullaha, 2019). Approximately 85,000 plant species are known to be medicinally useful in all over the world (Liu & Wang, 2008). Medicinal plants have been used for many centuries not only in rural areas but also increasingly by urban citizens in both developing and developed countries. Plants based primary healthcare customs have a long history for their uses in various human ailments. Being comparatively harmless, the naturally occurring plant species and their products have attracted the huge attention of modern researchers in the treatment of various challenging diseases (Guna, 2018). Use of herbal medicines in Nepal accumulates a long history of human interactions with the surrounding environment. Plants and their products-based, traditional medicine system continues to contribute to the role of an

important part in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Raja et al., 2011; Abraham and Thomas, 2012).

Over the last decades, significant amount of evidences have emerged indicating that chemically diverse classes of plant secondary metabolites are of potential interest for therapeutic interventions in several human diseases (Napagoda et al., 2020). Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further investigated (Kamaraj et al., 2012). Initially, the development of novel drugs was primarily through the extraction of biologically active compounds from plants which were identified through medicinal use or a variety of bioactivity screening tests (Hunter, 2001). Most of the drugs currently used to treat bacterial and other infections were first isolated from natural sources including ethnomedicinal plants (Coe & Anderson, 1996; Bhattarai & Basukala, 2016). The medicinal values of plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. Various herbal species have

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been known to display antimicrobial properties by acting against foodborne pathogens and spoilage bacteria and be used as sources of natural antimicrobial substances for the treatment of infectious diseases (Nabaviet et al., 2015). The documentation of several ethnomedicinal uses of plants and indigenous knowledge has been carried out at different corners of Nepal.

However, in Nepal, the investigation of ethnomedicinal plants used by various indigenous and local communities to correlate with antibacterial activities, the works are still on the way of exploring and only a few research work have been demonstrated by the researchers (Taylor & Towers, 1998; Parajuliet al., 2001; Sharma et al., 2002; Taylor et al., 2002; Vaidya et al., 2006; Bhattarai et al., 2008; Shakya et al., 2008; Bhattarai & Basukala, 2016). The current investigation aims to evaluate the *in-vitro* antibacterial activity of some selected ethnomedicinal plants explored from different regions of Purbakhola Rural Municipality of Palpa District for the first time to assess their potential antibacterial properties.

To answer the research questions for our work, whether all the medicinal plants used by indigenous (including Magar ethnic community) and local people show bioactivity against pathogenic bacteria or not? We focus to access the *in-vitro* antibacterial activity of selected ethnomedicinal plant species in the Palpa District in province number five of Nepal. In the present research, a total of 25 selected ethnomedicinal plants were examined for their antibacterial properties *in-vitro* using the disc diffusion method.

## 2. Material and methods

### 2.1 Collection and Processing of ethnomedicinal plant species

Ethnomedicinal plant species with the use of their particular parts were collected and their indigenous knowledge has been documented from different villages of Siluwa-1 and Ringneraha-3 of the Purbakhola Rural Municipality of Palpa District in Province Number five of Nepal (Figure 1). The villages surveyed were Arkhaldanda, Koranga, and Nandedanda of Ringneraha ward no. 3 and five villages in Siluwa ward no. 1 were Dhakrebash, Arghichaur, Gundanda, Hattilek, and Tarepahad.

The collected voucher herbarium specimens were identified and authenticated with the help of Standard literatures (Bailey, 1969; Hooker, 1872-1897; Polunin & Stainton, 1987, Stainton, 1988; Grierson & Long, 1983-2001). A set of voucher herbarium specimens was made for each collection and their numbers are listed in Table 1, and were deposited at the Tribhuvan University, Central Herbarium (TUCH), Nepal. Selected samples were based on the use of local people that were repeatedly used to treat the same illness by several traditional healers, villagers, and traders. The plants were dried at room temperature for two weeks.

Information about the ethnomedicinal utilization of plants and their products was gathered by interviewing knowledgeable persons and local faith healers according to previous works (Bhattarai et al., 2009; Pangeni, 2009; Bhattarai et al., 2010).

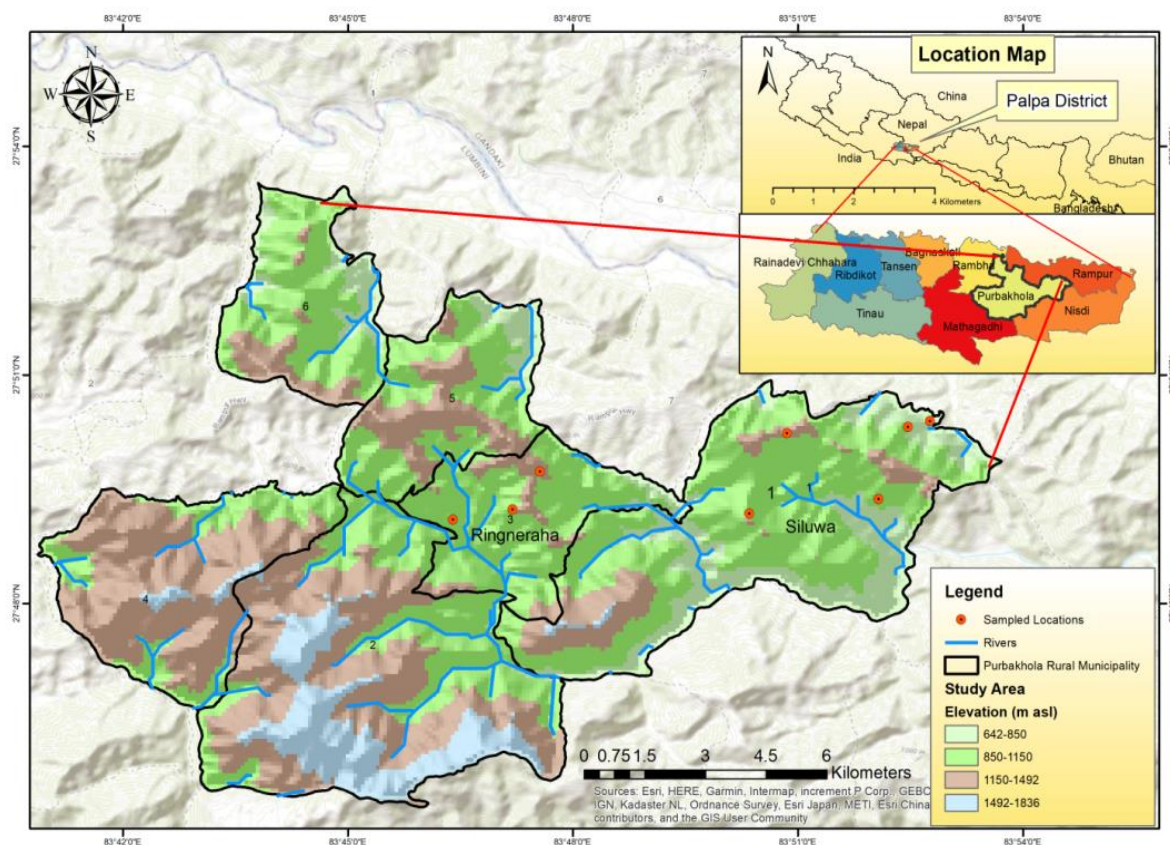


Figure 1. Map of the study area showing Purbakhola Rural Municipality (Source: Pangeni et al., 2020)

### 2.2 Preparation of plant extracts

Plant samples for laboratory investigation were air-dried in the shade at room temperature and stored in cotton bags for diffusion tests. They were stored in a dark and cool place to minimize chemical degradation. The plant extracts were

prepared following published papers (Taylor & Towers 1998; Parajuli et al., 2001; Taylor et al., 2002) with some minor modifications. The plant parts were ground and then 2 g sample powder of each plant material was immersed in 25 mL methanol (MeOH) for 24 hours. The sample was then extracted using suction-filtered through Whatman number 1 filter paper and the

residue was again immersed with another 25 mL MeOH for the next 24 hours. This process was continued until the extract was turning into colorless. The filtrates were then dried with the help of an electric table fan. After being complete dryness of samples, the extract was re-suspended in 2 mL of methanol. The final concentration of the extract was 1g dried plant material per mL methanol. A paper disk of 6 mm diameter was prepared from whatman filter paper no.1. Three types of test disks were made by using tetracycline (positive control-test disk dipped in 0.25 mgmL<sup>-1</sup> tetracycline), methanol (negative control-paper disk dipped in MeOH), and plant extracts (test disk dipped into plant extract). Thus, formed all the disks were allowed to dry at room temperature for antibacterial testing.

### 2.3 Bacterial Strains used

A total of four bacterial species including two Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and two of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) were used for this study. These bacterial strains were kindly received from the Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Inoculums of each bacterial strain was suspended in 5 mL of nutrient broth and incubated overnight at 37°C. These cultures were diluted (1/10) with nutrient broth before use.

### 2.4 Antibacterial Activity

The disc diffusion method was adopted to screen the antibacterial activity (Taylor, Manandhar & Towers, 1995; Bhattarai et al., 2008). The *in-vitro* antibacterial property was carried out by using standard sterile filter paper disks of 6 mm saturated with plant extracts. Overnight cultures were prepared by suspending 3-4 isolated colonies in 5mL of nutrient broth and incubating for 24 hours at 37°C. The overnight culture was used to inoculate the nutrient agar test plates. The test plates were inoculated with a suitable bacterial overnight culture medium on a sterile cotton swab. After inoculation, the test disks and the control disks were added. These plates were incubated upside down for about 24 hours at room temperature. Finally, the results were recorded as the presence or absence of a zone of inhibition, and testing was repeated for at least three times to ensure the reliability of the laboratory results.

## 3. Results

The information about the uses of ethnomedicinal plants by indigenous people (including Magars) and local communities from the study area by interviewing the faith healers, knowledgeable persons, and elder people are compiled in Table 1. The main outcomes of laboratory testing are summarized in Table 2. Twenty-five species of ethnomedicinally used plant extracts were examined; out of them, sixteen plant species showed antibacterial property i. e. produce a clear zone of inhibition against *Escherichia coli* (64%). The species were *Acorus calamus*, *Aesandra butyracea*, *Amaranthus spinosus*, *Anemone vitifolia*, *Bergenia ciliata*, *Cassia fistula*, *Centella asiatica*, *Cissampelos pareira*, *Clerodendrum viscosum*, *Curcuma amada*, *Eclipta prostrata*, *Fragaria nubicola*, *Oxalis corniculata*, *Rhododendron arboreum*, *Swertianervosa* and *Woodfordia fruticosa*, Nineteen extracts (*Acorus calamus*, *Amaranthus spinosus*, *Anemone vitifolia*, *Asparagus racemosus*, *Bergenia ciliata*, *Cassia fistula*, *Centella asiatica*, *Clematis b Buchananiana*, *Clerodendrum viscosum*, *Curcuma amada*, *Eclipta prostrata*, *Fragaria nubicola*, *Mallotus philippensis*, *Oxalis corniculata*, *Rhododendron arboreum*, *Solanum torvum*, *Swertia nervosa*, *Woodfordia fruticosa* and *Zingiber officinale*) showed positive effects against *Staphylococcus aureus* (76%).

Similarly, fifteen plant extracts (*Acorus calamus*, *Anemone vitifolia*, *Asparagus racemosus*, *Bergenia ciliata*, *Cassia fistula*, *Centella asiatica*, *Clerodendrum viscosum*, *Curcuma amada*, *Fragaria nubicola*, *Mallotus philippensis*, *Oxalis corniculata*, *Rhododendron arboreum*, *Swertia nervosa*, *Woodfordia fruticosa* and *Zingiber officinale*) exhibited antibacterial property against *Pseudomonas aeruginosa* (60%), and 19 plant extracts (*Acorus calamus*, *Centella asiatica*, *Rhododendron arboreum*, *Bergenia ciliata*, *Oxalis corniculata*, *Fragaria nubicola*, *Swertia nervosa*, *Curcuma amada*, *Cassia fistula*, *Anemone vitifolia*, *Clerodendrum viscosum*, *Woodfordia fruticosa*, *Aesandra butyracea*, *Cissampelos pareira*, *Clematis b Buchananiana*, *Mallotus philippensis*, *Zingiber officinale*, *Asparagus racemosus*, *Amaranthus spinosus*) showed positive results against *Bacillus subtilis* (76%) (Figure 2).

The examined plant extracts produced clear zone of inhibition recording a higher percentage in Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*, 76%) than in Gram-negative bacteria (*Escherichia coli*, 64% followed by *Pseudomonas aeruginosa*, 60%) (Figure 3). The results were found similar following the previous research work (Mccutcheon et al., 1992; Bhattarai et al., 2009; Bhattarai & Basukala 2016; Gupta et al., 2016). These outcomes may be due to the presence of a more complex cell wall in the structure of Gram-negative bacteria.

## 4. Discussion

Twenty-five extracts of different types of medicinal species were screened for their antibacterial property against four strains of pathogenic bacteria which can cause various diseases like cuts and wound, fever, diarrhea, dysentery, sinusitis, tonsillitis, pneumonia, urinary problems, respiratory ailments, boils, etc. The extracts were made from different parts of the medicinal plant like root, fruit, leaves, stem, bark, flower, inflorescence, seed, etc. The plant species that were used to treat various ailments/diseases in traditional herbal remedies potentially caused by human pathogenic bacteria, were examined.

Out of 25 medicinal plant extracts, tested, four plant species namely: *Artemisia dubia*, *Corchorus aestuans*, *Eryngium foetidum*, and *Lobelia pyramidalis* were unable to show the zone of inhibition towards any one of the tested bacteria. The results do not mean that the medicinal plants were valueless for traditional practice. There may be several possible reasons for this, as the polarity nature of methanol solvent made most of the plant species to show zone of inhibition in tested pathogenic bacteria and the methanol solvent was selected as it is easily available in the market. In the present investigation, we used an only little number of bacteria for the bioassay process; it may be also considered that the medicinal plants used here may contain antibacterial properties against pathogenic bacteria other than those tested, or the solvent used was unable to extract the active constituents.

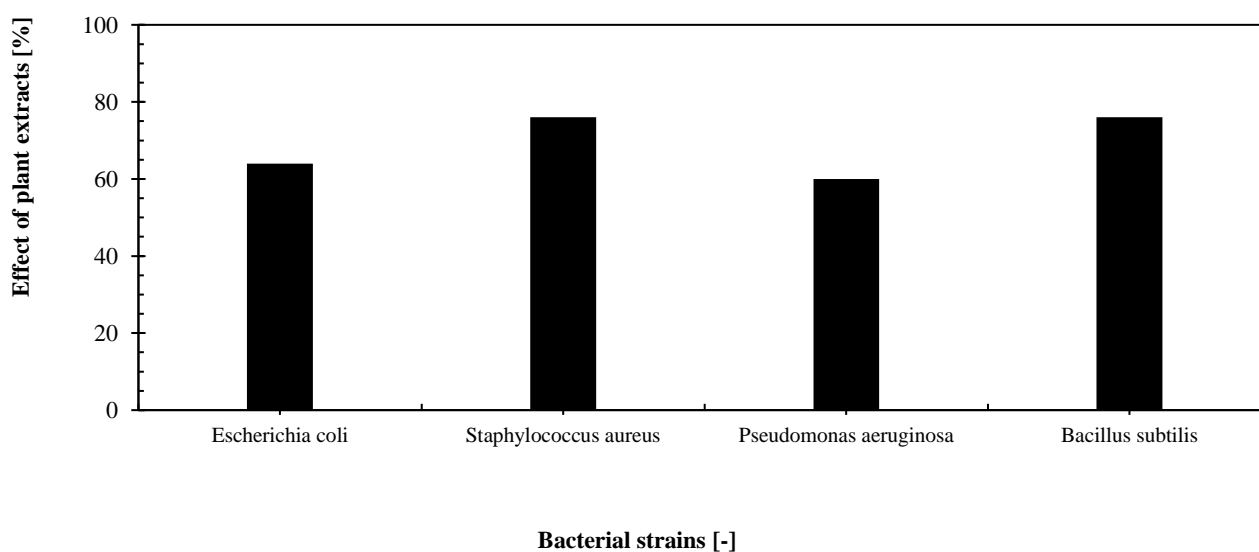
Gram-positive bacteria were found as more active to show more comparable results in tested extracts than that of Gram-negative bacteria. In the current research, Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were found to show the more positive and sensitive effect towards 38 plant extracts among 50 samples (76%), tested. Similarly, Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were found to show the positive effect for 31 plant extracts (62%) (Figure 3). As clear from the figure, the difference in showing positive effects for several extracts, between Gram-positive and Gram-negative bacteria can be well described to the morphological differences between these microorganisms and mostly to the differences in the permeability of the cell wall (Bereksi et al., 2018).

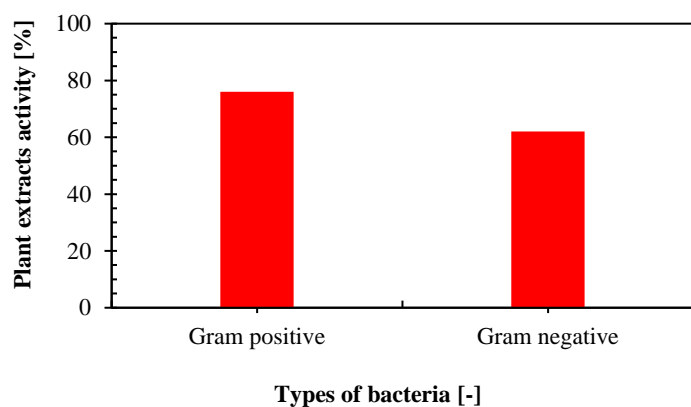
**Table 1.** Traditional use of ethnomedicinal plants in Palpa district

Plant scientific name	Family	Parts used	Origin/Voucher specimen	Traditional uses
<i>Amaranthus spinosus</i> L.	Amaranthaceae	Leaves, stem	Wild/cultivated [B.Pangeni 24, TUCH]	Boils, burns, cough, cold, dizziness
<i>Acorus calamus</i> L.	Araceae	Root	Cultivated [B.Pangeni 323, TUCH]	Cough, sore throat
<i>Aesandra butyracea</i> Roxb.	Sapotaceae	Bark, seeds	Wild [B.Pangeni 303, TUCH]	Sinusitis, stomachache
<i>Anemone vitifolia</i> Buch.-Ham. ex DC.	Ranunculaceae	Root, leaves	Wild/[B.Pangeni 12, TUCH]	Dysentery, dandruffs
<i>Artemisia dubia</i> Wall ex Besser	Asteraceae	Leaves, root	Wild/[B.Pangeni 317, TUCH]	Cuts and wound, asthma
<i>Asparagus racemosus</i> Willd.	Liliaceae	Tuber, shoot	Wild/Cultivated [B.Pangeni 360, TUCH]	Fever, urinary troubles
<i>Bergenia ciliata</i> (Haw.)Sternb.	Saxifragaceae	Rhizome	Wild [B.Pangeni 355, TUCH]	Rheumatism, diarrhea and dysentery
<i>Cassia fistula</i> L.	Fabaceae	Fruit, seeds, root	Wild [B.Pangeni 306, TUCH]	Fever, tonic and diabetes
<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Whole plant	Wild [B.Pangeni 06, TUCH]	Stomachache, indigestion, asthma and gastric
<i>Cissampelos pareira</i> L.	Menispermaceae	Rhizome, Leaves	Wild [B.Pangeni 328, TUCH]	Malarial fever, common cold and cough
<i>Clematis buchananiana</i> DC.	Ranunculaceae	Leaves	Wild [B.Pangeni 14a, TUCH]	Sinusitis
<i>Clerodendrum viscosum</i> Vent.	Verbenaceae	Seed, leaves	Wild/Cultivated [B.Pangeni 348, TUCH]	Gastric, stomachache
<i>Corchorus aestuans</i> L.	Tiliaceae	Whole plant	Wild/Cultivated [B.Pangeni 05, TUCH]	Fever, production of milk for women after post-delivery
<i>Curcuma amada</i> Roxb.	Zingiberaceae	Rhizome	Cultivated [only observed]	Skin allergy
<i>Eclipta prostrata</i> (L.)L.	Asteraceae	Aerial parts	Wild/Cultivated [B.Pangeni 09, TUCH]	Diarrhea and Dysentery
<i>Eryngium foetidum</i> L.	Apiaceae	Leaves	Cultivated [B.Pangeni 320, TUCH]	Headache
<i>Fragaria nubicola</i> Lindl.ex Lacaita	Rosaceae	Whole plant	Wild/Cultivated [B.Pangeni 308, TUCH]	Dysentery
<i>Lobelia pyramidalis</i> Wall	Lobeliaceae	Leaves, inflorescence	Wild [B.Pangeni 338, TUCH]	Asthma, bronchitis
<i>Mallotus philippensis</i> (Lam.) Mull-Arg.	Euphorbiaceae	Bark	Wild [B.Pangeni 301, TUCH]	Diarrhea and dysentery
<i>Oxalis corniculata</i> L.	Oxalidaceae	Whole plant	Wild [B.Pangeni 313, TUCH]	Eye infection
<i>Rhododendron arboreum</i> Sm.	Ericaceae	Flower	Wild/Cultivated [B.Pangeni 345, TUCH]	Dysentery
<i>Solanum torvum</i> Swartz.	Solanaceae	Fruit	Wild [B.Pangeni 305, TUCH]	Headache, dizziness
<i>Swertia nervosa</i> (G.Don) C.B. Clarke	Gentianaceae	Whole plant	Wild/cultivated [B.Pangeni 14b, TUCH]	Diarrhea, stomachache, tonic
<i>Woodfordia fruticosa</i> (L.) Kurz	Lythraceae	Flower	Wild [B.Pangeni 300, TUCH]	Dysentery, stomachache
<i>Zingiber officinale</i> L.	Zingiberaceae	Rhizome	Cultivated [only observed]	Diarrhea, common cold and cough

**Table 2.** Antibacterial activities of Medicinal Plants with different strains of bacteria

Plant scientific name	Results of bioassay test			
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
Tetracycline (Positive control)	+	+	+	+
Methanol (Negative control)	-	-	-	-
<i>Amaranthus spinosus</i> L.	+	+	-	+
<i>Acoru scalamus</i> L.	+	+	+	+
<i>Aesandra butyracea</i> Roxb.	+	-	-	+
<i>Anemone vitifolia</i> Buch.-Ham. ex DC.	+	+	+	+
<i>Artemisia dubia</i> Wall ex Besser	-	-	-	-
<i>Asparagus racemosus</i> Willd.	-	+	+	+
<i>Bergenia ciliata</i> (Haw.) Sternb.	+	+	+	+
<i>Cassia fistula</i> L.	+	+	+	+
<i>Centella asiatica</i> (L.) Urb.	+	+	+	+
<i>Cissampelos pareira</i> L.	+	-	-	+
<i>Clematis b Buchananiana</i> DC.	-	+	-	+
<i>Clerodendrum viscosum</i> Vent.	+	+	+	+
<i>Corchorus aestuans</i> L.	-	-	-	-
<i>Curcuma amada</i> Roxb.	+	+	+	+
<i>Eclipta prostrata</i> (L.) L.	+	+	-	-
<i>Eryngium foetidum</i> L.	-	-	-	-
<i>Fragaria nubicola</i> Lindl. ex Lacaita	+	+	+	+
<i>Lobelia pyramidalis</i> Wall	-	-	-	-
<i>Mallotus philippensis</i> (Lam.) Mull-Arg.	-	+	+	+
<i>Oxalis corniculata</i> L.	+	+	+	+
<i>Rhododendron arboreum</i> Sm.	+	+	+	+
<i>Solanum torvum</i> Swartz.	-	+	-	-
<i>Swertia nervosa</i> (G. Don) C.B. Clarke	+	+	+	+
<i>Woodfordia fruticosa</i> (L.) Kurz	+	+	+	+
<i>Zingiber officinale</i> L.	-	+	+	+

**Figure 2.** Antibacterial activity of medicinal plant extracts from Palpa District against the tested bacterial strains



**Figure 3.** Percentage of plant extracts showing inhibitory activity against tested bacterial strains.

Among the medicinal plants tested, 12 plant extracts (*Centella asiatica*, *Rhododendron arboreum*, *Bergenia ciliata*, *Acorus calamus*, *Oxalis corniculata*, *Fragaria nubicola*, *Swertia nervosa*, *Curcuma amada*, *Cassia fistula*, *Anemone vitifolia*, *Clerodendrum viscosum*, *Woodfordia fruticosa*) showed the most promising antibacterial properties with all the four tested strains of bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*), indicating the potential for discovery of antibacterial principles. Four plant extracts (*Aesandra butyracea*, *Cissampelos pareira*, *Clematis b Buchananiana*, and *Eclipta prostrata*) showed positive results for only any two of the tested bacteria (i. e. *Aesandra butyracea* and *Cissampelos pareira* with *Bacillus subtilis* and *Escherichia coli*; *Clematis b Buchananiana* with *Bacillus subtilis* and *Staphylococcus aureus*; *Eclipta prostrata* with *Escherichia coli* and *Staphylococcus aureus*). Similarly, plant extract (*Solanum torvum*) showed positive effect with only one of the tested bacteria (*Staphylococcus aureus*), four plant extracts (*Amaranthus spinosus*, *Asparagus racemosus*, *Mallotus philippensis* and *Zingiber officinale*) with any three of the tested bacteria (i.e. *Mallotus philippensis*, *Asparagus racemosus* and *Zingiber officinale* with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*; *Amaranthus spinosus* with *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*), and four plant species extracts (*Artemisia dubia*, *Corchorus aestuans*, *Eryngium foetidum* and *Lobelia pyramidalis*) with none of the tested bacteria. The results also indicate that scientific research conducted on medicinal plants having traditional claims of effectiveness might correlate with laboratory tests results.

Extracted plant medicines are safe, effective, cheaper, and have no or little side effects (Hassan & Ullah, 2019). The active compounds (phytochemicals) are responsible for biological activity such as antibacterial against infectious pathogens and provide a quite significant role in the discovery of new antibiotic herbal medicines. The present study investigated the antibacterial potential of a medicinal plant for the first time in the Purbakhola Rural Municipality of Palpa District in Province number five of Nepal.

## 5. Conclusion

Thus, it may be concluded that due to the presence of useful phytoconstituents in the tested plant extracts towards their antibacterial properties for four strains of bacteria, they show quite significant and clear zone of inhibition, therefore, these traditional medicinal plants could be used as potent sources of natural antibacterial agents as a substitute for the commercially available synthetic drugs which are quite expensive and may

have a large number of side effects. Further phytochemical studies are required to determine the type of compounds responsible for the antibacterial effects of these species. Further extensive research is also required for the separation and recognition of active biomolecules and principles present in these extracts so that they could be utilized for the pharmaceutical purpose at the industrial scale.

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## Declaration of interest

The authors report no conflict of interest.

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