

Assessment of Antimicrobial and Antioxidant Activities of Four Ethnomedicinal Plants Used by Magars in Nawalpur District, Nepal

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Abstract

Ethnobotanical survey in the Magar villages in Nawalpur district, Gandaki province, Nepal revealed that root of *Phanera vahlii* (Wight & Arn.) Benth, bark of *Rhododendron arboreum* Sm. and flower of *Woodfordia fruticosa* (L.) Kurz, have been in use for diarrhea and dysentery. Root of *Thalictrum foliolosum* DC. has been used for the treatment of rheumatic pain. The study aimed to evaluate antimicrobial and antioxidant effects as well as total phenol content in *Phanera vahlii*, *Rhododendron arboreum*, *Woodfordia fruticosa* and *Thalictrum foliolosum*. Total phenol content (TPC) was estimated using Folin-Ciocalteu method. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical and hydrogen peroxide scavenging assays were used to evaluate the antioxidant capacity. Antibacterial effect was established by the Agar well diffusion assay. The minimum TPC value of 65.78±3.44 mg GAE/g dry extract was estimated in 70% methanolic extract of *Thalictrum foliolosum* and maximum TPC value of 258.40±6.26 mg GAE/g dry extract was estimated in 70% methanolic extract of *Woodfordia fruticosa*. IC₅₀ value range 21.59±0.26 µg/ml in *Rhododendron arboreum* to 1124.79±3.69 µg/ml in *Thalictrum foliolosum* was calculated in DPPH free radical scavenging assay. Scavenging of DPPH free radical range from 12.40% to 94% at 100 µg/mL concentration of 70% methanolic extracts were estimated and scavenging of hydrogen peroxide range from 36% to 73%. The maximum zone of inhibition (ZOI) against *Escherichia coli* at loading dose of 5 mg of the extract observed was 18±0.73 mm by 70% methanolic extract of *Woodfordia fruticosa* and its minimum inhibition concentration (MIC) was <1.56 µg /ml. The extracts efficiently inhibited the growth of *Escherichia coli* verifying the rural knowledge. At the same time, the extracts displayed efficient antioxidant activity.

Keywords: Antibacterial susceptibility assay, DPPH radical scavenging assay, Ethnobotany, Total phenol content

Introduction

Ethnobotanical study is an important tool for recording of plants along with associated traditional botanical knowledge of the ethnic people. These ethnic groups have cumulative knowledge of ethnomedicinal plant therapy which has been transmitted from generation to next generation. Ethnomedicine based treatments are gaining a high interest to the public due to several side effects of the synthetic drugs, lacking of curative treatment for several chronic diseases, elevated cost of newly discovered drugs, microbial resistance to the drugs in use and emergence of many new diseases.

Phanera vahlii (Wight & Arn.) Benth. is known as Bhorla in Nepali and Magar language; fruit is called as “pakshya” in Magar language. Root is used for

treatment of diarrhea and dysentery in Dang district (Manandhar, 1985) and bloody dysentery in Sindhuli district (Manandhar, 1990), gastritis in Morang district (Siwakoti & Siwakoti, 1998). *Phanera vahlii* is mostly used by tribal sovereignty for fever, diarrhea, bone fracture, skin irritancy, tonic and as vermifuge medicine (Pattanaik et al., 2007).

Rhododendron arboreum Sm. is known as Laligurans in Nepali and Pataksar in Magar language. The bark juice is used in the treatment of coughs, diarrhea and dysentery (Manandhar, 2002). In Ayurveda it is used in jaundice, diabetes, piles, splenomegaly (enlargement of spleen), liver disorder, worms and skin diseases. Ayurvedic preparation, such as Rohitakyadi churna, is prepared from *Rhododendron arboreum*. An extract of the flowers and barks

is used as an ingredient in commercial cosmetic preparations as a skin conditioner.

Thalictrum foliolosum DC is known as Bajuri in the study area. Root is used for treatment of indigestion (Manandhar, 1990), peptic ulcer (Manandhar, 1991), on skin itching (Manadhar, 1987). Root and leaf paste is given as anthelmintic by Sherpas of Helambu (Bhattarai, 1989). Root is used traditionally as tonic, diuretic, febrifuge, purgative and stomachic (Gangwar et al., 2010; Pandey et al., 2017). Chen et al. (2003) reported that its roots were used to treat virus hepatitis, dysentery, congestion of eyes, heat-type malnutrition of children, chickenpox and inadequate measles eruption in China. The root juice is taken for jaundice by Tamang people in Langtang valley (Shrestha & Shrestha, 2000).

Woodfordia fruticosa (L.) Kurz is known as Chhebok in Magar language. Flower is used for diarrhea, dysentery and headache (Manandhar, 1990). Decoction of bark is applied on sprain and swelling (Manandhar, 2002). Fruit juice is used to treat urinary trouble at Dhading district (Manandhar, 1992). It has been used traditionally for treatment of diarrhea, dysentery, fever, hemorrhoids, herpes, leprosy, burning sensation, skin diseases, internal hemorrhage, impaired hepatic function and leucorrhea (Das et al., 2007; Kumar et al., 2016).

On the ethnobotanical survey of the study areas, it is found that Magar community in the study area used these plant species for the treatment of different diseases in different ways. They used these plants for treatment of bacterial diarrhea. To validate the ethnomedicinal knowledge, the present research was focused on evaluation of antibacterial along with antioxidant activities.

Materials and Methods

Methanol and Hexane were purchased from Fisher Scientific. Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were purchased from HiMedia Laboratories Pvt. Ltd. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich. Folin–Ciocalteu 2 s reagent was purchased from Loba Chemie Pvt. Ltd., Gallic acid from Sisco

Research Laboratories Pvt., Ltd., Spectrophotometry was carried out using an Elisa microplate reader (EPOCH2, BioTek Instruments).

Ethnomedicinal survey

The study was carried out in Dhauwadi, Jugepani, Jhalbase, Girubari and Upallo Arkhala villages of Nawalpur district, Gandaki province, Nepal. The study area extends from 150 to 1900m altitude. The tentative coordinates of the study area ranges from 27°36'N to 27°45'32"N latitude and 84°05'E to 84°09'E longitude. Study sites were visited in March 2015, December 2015 and December 2016.

Ethnomedicinal data of medicinal plants from Magar community were collected through questionnaires, structural and un-structural interview among healers and knowledgeable people (12 Key informants). Herbaria were prepared following Bridson and Forman (1998) and herbarium specimens were identified by using relevant references (Lawrence, 1967; Malla et al., 1976; Polunin & Stainton, 1997). The specimens were confirmed through comparison with authenticated specimens at National Herbarium and Plant Laboratories, Godawari. The specimens were deposited to TUCH for future reference. The plant materials for laboratory tests were dried in shade at room temperature.

Preparation of hexane and methanol extract

Air dried plant materials were ground. The ground plant materials (100g) were successively extracted with hexane (800 ml, 7hrs.) and 70% methanol (800 ml, 22hrs.) using a Soxhlet extractor. These plant extracts were concentrated under vacuum by using rotary evaporator. The concentrated extracts were stored in refrigerator at 4°C until further use.

Antimicrobial screening

The hexane and the 70% methanolic extracts of four plants species (eight extracts) were screened against total of seven bacterial strains. The test bacteria were *Pseudomonas aeruginosa* (ATCC 27263), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Enterococcus faecalis* (ATCC 29212),

Bacillus subtilis (ATCC 6051) and *Salmonella enteric* subsp. *enteric* serovar *Typhi*.

Preparation of inoculums: Three to four isolated colonies from freshly prepared subculture were suspended into 5ml of sterile MHB (Muller Hinton Broth) in test tube and mixed by using vortex mixer. The turbidity of suspension was adjusted to that of McFarland standard 0.5. The inoculums were used within 30 min. The antibacterial screening of these medicinal plant extracts were evaluated by using the agar well diffusion technique. The sterilized MHA plates were swabbed by the bacterial suspension. The wells were punched on the agar gel using sterile borer of 6 mm diameter. The wells were filled with 50 μ l of plant extracts of 0.1g/ml concentration. Ampicillin and Gentamicin (Mast diagnostics) of 10 μ g per disc were used as standard reference. Dimethyl sulfoxide (DMSO) was used as control. The plates were incubated at 37°C for 18-24 hours. Tests were performed in triplicate. Zone of inhibition was measured in millimeter (mm) and absence as negative.

Determination of MIC: In a microplate, 50 μ L of the extract solutions of 0.1 g/ml concentration were mixed with Mueller-Hinton broth (50 μ l) and then the content was serially double diluted. The bacterial suspension adjusted to 1×10^8 cfu ml⁻¹ (equivalent to McFarland 0.5 standard) was further diluted to 1:100 using MHB and then 50 μ l of the suspension was inoculated. After incubation for 24 hrs. at 37°C, the MIC value was taken as the lowest concentration that inhibited the visible growth of the tested bacteria.

Estimation of TPC

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method of Singleton et al. (1999) with some modifications. Gallic acid solutions of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 μ g/ml were prepared. The wells of 96 well microplate were filled with 50 μ l of gallic acid solution of each concentration, 25 μ l of Folin-Ciocalteu's reagent (FCR) and 100 μ l of aqueous Na₂CO₃ solution (75g/l). Distilled water (175 μ l) was taken as blank. Solutions of the aq. methanolic extract of the plants at concentration of 4mg/10ml was prepared and

mixed with Folin-ciocalteu's reagent. The absorption at 760 nm was measured on spectrophotometer after 1h keeping in the dark. A linear curve of the standard gallic acid concentrations versus absorbance was constructed. The total phenolic content was calculated using the formula: $C = c V/m$ where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in ml, m = mass of extract in gram. Total phenolics content of the extract was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

Antioxidant assay

DPPH Free Radical Scavenging Assay: Only aqueous methanol extract were used for the DPPH free radical scavenging assay. The antioxidant capacities were determined following Nemkul, et al. (2018). Different concentrations of extracts such as 1500, 1000, 750, 500, 250, 100, 50 and 25 μ g/ml (total volume of 50 μ l) were filled in wells of microplates. Next, as a positive control, 50 μ l of gallic acid solutions of concentrations 20, 10 and 5 μ g/ml were used to obtain a linear curve. To each well was added 250 μ l of DPPH solution (0.1mM). For blank, 300 μ l of distilled methanol was used. For control, 250 μ L of DPPH solution and 50 μ l of distilled methanol was used. The microplate was shaken at room temperature for 30 min in the dark and the absorbance was determined at 517 nm wavelength. The DPPH radical scavenging ability was calculated according to the following equation.

DPPH scavenging rate (%) =

$$1 - \frac{\text{Absorbance (sample)} - \text{Absorbance (blank)}}{\text{Absorbance (control)} - \text{Absorbance (blank)}} \times 100$$

Where, A_{sample}, A_{blank} and A_{control} are the absorbances measured for the sample, blank and control, respectively. Concentration of the extract for 50% inhibition of DPPH radical was calculated using formula $IC_{50} = (50 - c) / m$, where c is the intercept and m is the slope of the linear curve.

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging activity was measured

following the instructions of commercial kit (Radical catch; Hitachi Ltd., Tokyo, Japan). Briefly, 25µl of reagent A (that is 5 mM cobalt chloride solution) was mixed with 25µl of reagent B (luminol solution). Then 10µl of the experimental sample solution was added (The experimental sample solution was made by dissolving 0.1 mg of aqueous methanol extract in 1ml of distilled methanol). Subsequently, the mixture solution was incubated at 37°C for 5 min in an incubator (Varioskan LUX Multimode Microplate Reader, Thermo Fisher Scientific, Waltham, MA, USA). Then 25µl of reagent C (hydrogen peroxide) was added. After the mixture reacted with hydrogen peroxide solution the luminescence of light for 120 s in the incubator was measured. The luminescence was observed to subtract an amount of 120 s to 80 s. 70% methanol was used as control. Hydrogen scavenging activity was calculated following the equation below.

Hydrogen scavenging activity (%) =

$$\frac{\text{Luminescence (control)} - \text{Luminescence (sample)}}{\text{Luminescence (control)}} \times 100$$

Statistical analysis

Statistical analysis was done using Microsoft excel program. Antimicrobial susceptibility assay, MIC determination, % of scavenging, IC₅₀ determination, determination of total phenolic contents and hydrogen peroxide scavenging were carried out in triplicates (n = 3) and presented as average±SEM (standard error mean) using Microsoft excel program.

Results and Discussion

Ethnomedicinal data and Medicinal plants used in different ailments have been shown in Table 1.

Antibacterial susceptibility assay

S. aureus (ZOI = 32.5±0.5 mm) was found most susceptible to the standard drug ampicillin, on the other hand, *B. subtilis* (ZOI = 8.5±0.5 mm), *K. pneumoniae* (ZOI = 8.5±0.5mm) and *Pseudomonas aeruginosa* (no ZOI) were found resistant (Table 2).

Methanolic extract of *Rhododendron arboreum* and *Woodfordia fruticosa* showed larger zone of inhibition (ZOI ≥ 15mm) against Gram –ve as well as Gram +ve bacteria. Only extracts of *W. fruticosa* had shown antimicrobial activity against *S. typhi* (Gram –ve). All the extracts have shown antimicrobial activity against ampicillin resistant *P. aeruginosa* (Table 2).

Phanera vahlii showed antimicrobial activity against all the tested bacteria except *S. typhi*. Dugasani et al. (2010) have also reported antimicrobial activity of *Phanera vahlii* against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis* and *B. subtilis*. Taylor et al. (1996) had reported that methanol extract of root of *P. vahlii* showed antimicrobial activities against *B. subtilis*, *S. aureus* and *P. aeruginosa*.

R. arboreum showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. Nisar et al. (2013) investigated antibacterial activity of the methanolic extract of the barks of *R. arboreum* and reported significant to low antibacterial activity

Table 1: Ethnomedicinal data

Scientific name	Family	Nepali name	Magar name	Part use	Ailments	Form
<i>Phanera vahlii</i> (Wight & Arn.) Benth.	Leguminosae	Bhorlla	Bhorlla, fruit is called "pakshya"	Root, bark	Menstrual problem, diarrhea	decoction
<i>Rhododendron arboreum</i> Sm.	Ericaceae	Laliguras	Pataksar	Flower, bark	diarrhea, dysentery	Juice, decoction
<i>Thalictrum foliolosum</i> DC.	Ranunculaceae	Bajara	Bajuri	Root	Rheumatic pain	powder
<i>Woodfordia fruticosa</i> (L.) Kurz	Lythraceae	Dhanyar	Dhainra, chhebok	Flower	Diarrhea, gastritis	juice

in the order of *S. typhi*, *E. coli*, *B. subtilis* and *S. aureus*. Shakya et al. (2008) reported antibacterial activity of 50% ethanol extract of whole plant of *R. arboreum* against *B. subtilis* and *E. coli* whereas no antibacterial activity against *S. typhi* and *S. aureus*.

T. foliolosum showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. Joshi and Sati (2017) investigated antibacterial activity of methanol, chloroform, hexane and aqueous extracts of the leaves of *T. foliolosum* and reported that methanol and hexane extracts showed highest activity against *B. subtilis* while the aqueous extract had almost no inhibitory effect. Shakya et al. (2008) reported that 50% ethanol extract of whole plant of *Thalictrum foliolosum* showed antibacterial activity against *B. subtilis*, *E. coli* and *S. typhi* but not against *S. aureus*.

The result of antibacterial susceptibility assay of *W. fruticosa* showed larger ZOI against *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* than standard antibiotics. Methanolic extract of *W. fruticosa* showed antibacterial activity against *E. coli*, *K.*

pneumoniae, *P. aeruginosa* and *Staphylococcus aureus* (Kumar et al., 2013; Parekh & Chanda, 2007).

W. fruticosa showed larger zone of inhibition (ZOI ≥ 15 mm) to *P. aeruginosa*. *P. aeruginosa* frequently displays resistance to multiple antimicrobial agents (Carmeli et al., 1999). Savas et al. (2005) reported increasing resistant of *P. aeruginosa* to multiple antimicrobial agents including gentamicin. The result of this research showed susceptibility of *P. aeruginosa* to gentamicin. The susceptibility to gentamicin has been reported as low as 49.8% in Greece to as high as 96.6% in United Kingdom (Van Landuyt et al., 1986). But the resistant rate is increased and reported to be 70.7% (Savas et al., 2005).

The hexane extracts showed comparatively low efficacy than methanolic extracts (Table 3). The efficacy of different plant extracts depends on the presence of the antibacterial compounds in it. But the efficacy also depends on the different bacterial species.

Table 2: Antimicrobial activity of the 70% methanolic extract

Plant extract	Zone of inhibition \pm Standard error mean (mm)						
	Gram positive bacteria			Gram negative bacteria			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<i>Phanera vahlii</i>	16 \pm 0.57	14.5 \pm 0.88	12 \pm 0	9.3 \pm 0.61	-	9.66 \pm 1	12 \pm 0.59
<i>Rhododendron arboreum</i>	16.33 \pm 0.50	16 \pm 0.44	-	15.33 \pm 0.55	-	-	11.6 \pm 0.46
<i>Thalictrum foliolosum</i>	14.33 \pm 0.33	8 \pm 0	-	13.33 \pm 0.33	-	-	10.8 \pm 0.28
<i>Woodfordia fruticosa</i>	19.16 \pm 0.40	14.66 \pm 0.33	18.66 \pm 0.66	18 \pm 0.73	10 \pm 1	13.5 \pm 0.67	16.4 \pm 0.24
Ampicillin	32.5 \pm 0.5	8.5 \pm 0.5	17.75 \pm 0.25	25 \pm 1	15.5 \pm 0.5	8.5 \pm 0.5	-
Gentamicin	16.75 \pm 0.25	15.5 \pm 0.5	18.5 \pm 0.5	17.5 \pm 0.5	12.66 \pm 0.33	11.33 \pm 0.88	14.66 \pm 0.33
DMSO	-	-	-	-	-	-	-

Table 3: Antimicrobial activity of Hexane extracts

Plant extract	Zone of inhibition \pm Standard error mean (mm)						
	Gram positive bacteria			Gram negative bacteria			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<i>Phanera vahlii</i>	NA	11 \pm 0	-	10 \pm 0	-	-	NA
<i>Rhododendron arboreum</i>	-	-	9.5 \pm 0.5	-	-	14 \pm 0.57	10 \pm 0
<i>Thalictrum foliolosum</i>	-	13 \pm 0	-	-	-	-	10.33 \pm 0.33
<i>Woodfordia fruticosa</i>	10.66 \pm 0.76	12 \pm 0	11 \pm 0	12.8 \pm 0.48	11 \pm 0	11 \pm 0	13 \pm 0
DMSO	-	-	-	-	-	-	-

The values of MIC varied from lowest <1.56 mg/ml to highest 25 mg/ml. The lowest MIC value was shown by *W. fruticosa* against *E. coli* and *P. aeruginosa*. The highest MIC value was shown by *T. foliolosum* against *B. subtilis*. Likewise, the MIC value of *W. fruticosa* was 3.12 mg/ml against *E. faecalis* and 6.5 mg/ml against *S. aureus* and *B. subtilis* (Table 4). It showed that *W. fruticosa* was potential antibacterial against Gram +ve as well as Gram -ve bacteria. The magar use the plant species *P. vahlii*, *R. arboreum* and *W. fruticosa* for the treatment of diarrhea. The efficacy of 70% methanolic extract of these plant species against *E. coli* were in the order of *W. fruticosa* (MIC <1.56 mg/mL), *P. vahlii* (12.5mg/ml) and *R. arboretum* (12.5mg/ml). The result showed that the most potential antibacterial against *E. faecalis* were *W. fruticosa* followed *P. vahlii*. The MICs of the extracts might have bacteriostatic or bactericidal effect.

The efficacy of hexane extract of the plant used against *E. coli* were in the *P. vahlii* (12.5 mg/ml) and *W. fruticosa* (12.5mg/ml) (Table 5). Comparatively more potential hexane extracts against *K. pneumoniae* was *R. arboreum*. The antibacterial efficacy of the plant extract also depends on polarity of the solvent

used for extraction. Antibacterial activity depends upon extraction procedure, type of plant parts used, solvents used for extraction and bacterial strain (Devi et al., 2014).

Total Phenol Contents (TPC)

To estimate TPC, a linear curve of standard gallic acid ($Y = 0.023x + 0.088$, $R^2 = 0.996$) was obtained from the measured absorbance values using different gallic acid concentrations (Figure 1) at 760 nm wave length. The TPC content in the 70% methanolic extracts of *P. vahlii*, *R. arboreum*, *W. fruticosa*, *T. foliolosum* was calculated using the regression equation.

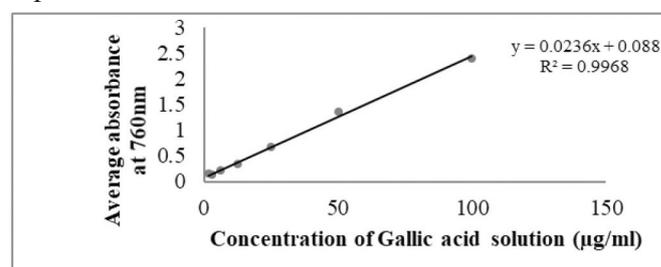


Figure 1: Standard gallic acid curve

The result showed that total phenol contain in the plant extracts had variation ranging from 65.78 ± 3.44 to 258.40 ± 6.26 mg of GAE/g of dry extract (Figure 2).

Table 4: MIC of 70% methanolic extracts

Plant extract	Minimum inhibitory concentration (MIC) of the 70% methanolic extract (mg/mL)						
	Gram positive bacteria			Gram negative bacteria			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<i>Phanera vahlii</i>	12.5	12.5	3.12	6.25	-	12.5	6.25
<i>Rhododendron arboreum</i>	6.25	6.25	-	6.25	-	-	3.12
<i>Thalictrum foliolosum</i>	6.25	25	-	3.12	-	-	12.5
<i>Woodfordia fruticosa</i>	3.12	3.12	1.56	<1.56	12.5	25	<1.56

Table 5: MIC of hexane extracts

Plant extract	Minimum inhibitory concentration (MIC) of the hexane extract (mg/mL)						
	Gram positive bacteria			Gram negative bacteria			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<i>Phanera vahlii</i>	NA	12.5	-	12.5	-	-	NA
<i>Rhododendron arboreum</i>	-	-	25	-	-	6.25	12.5
<i>Thalictrum foliolosum</i>	-	6.25	-	-	-	-	12.5
<i>Woodfordia fruticosa</i>	6.25	6.25	25	12.5	-	12.5	12.5

The total phenol contain of bark of *R. arboreum* was found to be 257.55±4.48 mg GAE/g of dry extract. Bhandari and Rajbhandari (2014) reported total phenol contain of methanolic extract of the barks of *R. arboreum* was 240±00 mg GAE/g of dry extract and 330 mg GAE/g of dry extract in 50% methanolic extract. Our result of the total phenol contain data of *R. arboreum* is similar to that of Bhandari and Rajbhandari (2014). Painuli et al. (2018) estimated total phenol contain of the methanolic extract of the leaves and it was reported to be 102.7±0.017 mg GAE/g of dry extract. It indicates that total phenol contain is comparatively less in the leaf extract.

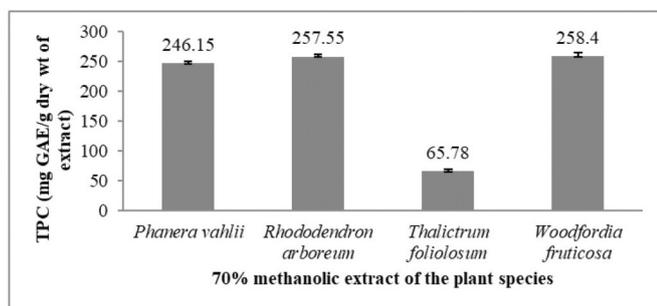


Figure 2: TPC of the extracts

DPPH free radical scavenging assay

Linear curves of standard gallic acid ($Y = 3.043 + 12.03x$, $R^2 = 0.998$) (Figure 3) was obtained from values of DPPH free radical inhibition and concentrations of gallic acid. IC_{50} of the gallic acid solution was calculated 12.47 µg/ml.

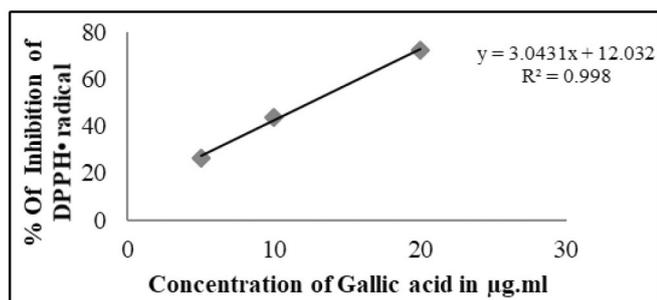


Figure 3: DPPH radical scavenging of gallic acid

We found DPPH free radical scavenged by 70% methanolic extract of the plant species at 100µg/ml concentration and IC_{50} are shown in Table 6. The values of DPPH free radical scavenged were high by the extracts in comparison to the Gallic acid (Figure 4). This study showed that *R. arboreum* and *W. fruticosa* possessed higher antioxidant capacity in term of DPPH free radical scavenging followed by *P. vahlii* at 100 µg/ml concentration.

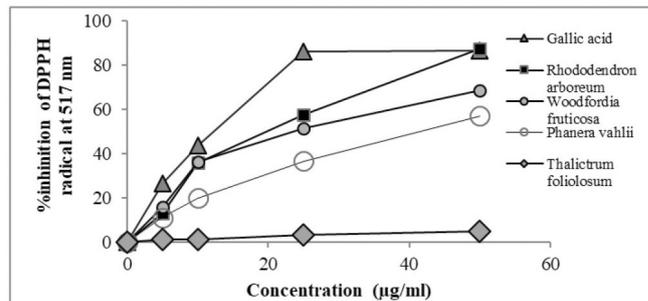


Figure 4: DPPH radical scavenging activity of the plant extracts compared to positive control gallic acid

Painuli et al. (2018) investigated antioxidant activity of the aqueous and methanolic extracts of leaves of *R. arboreum* and reported that methanol extract showed highest scavenging activity (91.67%). Our result was found 93%, comparable with it.

W. fruticosa showed 94% of DPPH radical scavenging activity at 100 µg/ml concentration. Grover et al. (2014) reported that flowers extracts scavenge 93.48±0.26% DPPH radical at 200 µg/ml concentration. It showed that our sample scavenge DPPH radical comparatively strongly.

Hydrogen peroxide scavenging assay

Percentage of hydrogen peroxide scavenging by the 70% methanolic extracts at 100 µg/ml was presented in Figure 5.

The phenolic compounds scavenge different percent of H_2O_2 and DPPH depending on many factors,

Table 6: DPPH radical scavenging

Plant species	$IC_{50}(\mu\text{g/ml})$	% of DPPH• radical scavenging at 100µg/mL
<i>Phanera vahlii</i>	43.52±1.13	78.22
<i>Rhododendron arboreum</i>	21.59±0.26	93.00
<i>Thalictrum foliolosum</i>	1124.79±3.69	12.40
<i>Woodfordia fruticosa</i>	33.68±0.52	94

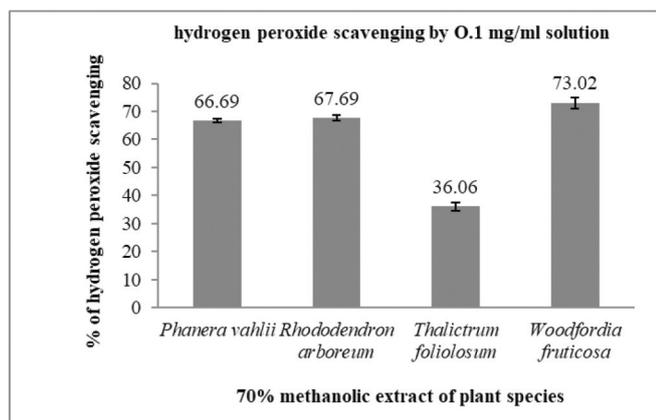


Figure 5: Hydrogen peroxide scavenging

such as the number of hydroxyl groups bonded to the aromatic ring, the site of bonding and mutual position of hydroxyls in the aromatic ring (Sroka & Cisowski, 2003). This could be a reason of the difference between the correlation of TPC with DPPH scavenging and H_2O_2 scavenging activities.

Hydrogen peroxide has positive role in energy production in *in vivo* system, phytochemicals, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds (Packer et al., 2008). But H_2O_2 itself can be toxic to the cells (Halliwell & Aruoma, 1991). Incubation of cells with H_2O_2 causes damage of deoxyribonucleic acid (DNA). Therefore, removal of H_2O_2 is obviously biologically advantageous (Chance et al., 1979). Plant base H_2O_2 scavenger is important for human health.

Conclusion

The community uses decoction of *W. fruticosa*, *R. arboreum*, *P. vahlii* to treat diarrhea and dysentery. This work showed that 70% methanolic extracts of *W. fruticosa*, *R. arboreum*, *P. vahlii* and exhibit antibacterial activity against *E. coli*, causal bacteria of bloody diarrhea, in the support of traditional knowledge. The result showed that the 70% methanolic extract of *W. fruticosa* ($MIC \leq 1.56$ mg/ml) was the most potential of the all the tested extracts against *E. coli*. The 70% methanol extract of *W. fruticosa* showed stronger antimicrobial activity than standard antibiotics to *Enterococcus faecalis*. Therefore, the plant could be used for urine infection along with diarrhea and dysentery.

The *W. fruticosa*, *R. arboreum* and *P. vahlii* were good antioxidant with high total phenol content. These plants would be used as antioxidants. The traditional knowledge of the local Magars has scientific value.

Author Contributions

C. M. Nemkul visited the study site, collected plant materials and performed phytochemical screening, GC-MS analysis and antimicrobial assays in the laboratory. G. B. Bajracharya helped in chemical analysis by GC-MS and reviewed the manuscript. I. Shrestha helped on the ethnobotanical part of the manuscript.

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References

- Bhandari, L. & Rajbhandari, M. (2014). Isolation of quercetin from flower petals, estimation of total phenolic, total flavonoid and antioxidant activity of the different parts of *Rhododendron arboretum* Smith. *Scientific World*, 12(12), 34-40.
- Bhattarai, N. K. (1989). Traditional phytotherapy among the Sherpas of Helambu, central Nepal. *Journal of Ethno-pharmacology*, 27(172), 45-54.
- Bridson, D., & Forman, L. (1998). *The herbarium handbook* (3rd ed). Royal Botanic Gardens Kew.
- Carmeli, Y., Troillet, N., Eliopoulos, G. M., & Samore, M. H. (1999). Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: Comparison of risks associated with different anti pseudomonas agents. *Antimicrob Agents Chemother*, 43, 1379-1382.

- Chance, B., Sies, H., & Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiological reviews*, 59(3), 527-605.
- Chen, S. B., Chen, S. L., & Xiao, P. G. (2003). Ethnopharmacological investigations on *Thalictrum* plants in China. *Journal of Asian Natural Products research*, 5(4), 263-271. <https://doi.org/10.1080/1028602031000111941>
- Das, P. K., Goswami, S., Chinniah, A., Panda, N., Banerjee, S., Sahu, N. P., & Achari, B. (2007). *Woodfordia fruticosa*: Traditional uses and recent findings, Review. *Journal of Ethnopharmacology*, 110, 189-199.
- Devi, M. A., Prasad, B. D., & Rambabu, B. (2014). Study of antimicrobial properties of *Tinospora sinensis* by agar well diffusion method. *American Journal of Biological and Pharmaceutical Research*, 1(2), 55-59.
- Dugasani, S., Balijepalli, M. K., Tandra, S., & Pichika, M. R. (2010). Antimicrobial activity of *Bauhinia tomentosa* and *Bauhinia vahlii* roots. *Pharmacognosy Magazine*, 6(23), 104-207.
- Gangwar, K., Deepali, K., & Gangwar, R. S. (2010). Ethnomedicinal plant diversity in Kumaun Himalaya of Uttarakhand India. *Nature and Science*, 8, 66-78.
- Grover, N., Khandelwal, M., & Patni, V. (2014). Antimicrobial and antioxidant screening of methanolic extract from leaf and flower parts of *Woodfordia fruticosa*. *Journal of Phytol Research*, 27(1-2), 7-18.
- Halliwell, B., & Aruoma, O. I. (1991). DNA damage by oxygen-derived species, its mechanism and measurement in mammalian systems. *Federation of European Biochemical Societies*, 281(1,2), 9-19
- Joshi, S., & Sati, S. C. (2017). Screening of antibacterial potentiality of *Thalictrum foliolosum* leaves extracts. *Biomedicine and Nursing*, 3(1), 98-102, [doi:10.7537/marsbnj030117.14](https://doi.org/10.7537/marsbnj030117.14).
- Kumar, D., Sharma, M., Sorout, A., Saroha, K., & Verma, S. (2016). *Woodfordia fruticosa* Kurz.: a review on its botany, chemistry and biological activities, *Journal of Pharmacognosy and Phytochemistry*, 5(3), 293-298.
- Kumar, S., Tarannum, A., Saleem, A., & Ali, M., Y. (2013). Antibacterial activity of the flowers of *Woodfordia fruticosa* on different micro-organism. *International Journal of Pharmaceutival sciences and Research*, 4(8), 3225-3228.
- Lawrence, G. H. M. (1967). *Taxonomy of vascular plants*. Oxford and IBH Publishing Co. Pvt. Ltd.
- Malla, S. B., Shrestha, A. B., Rajbhandari, S. B., Shrestha, T. B., Adhikari, P. M., & Adhikari, S. R. (1976). *Flora of Langtang and cross section vegetation survey (central zone)*. Bull.No.6. Department of Medicinal Plants.
- Manandhar, N. P. (1987) Traditional medicinal plants used by tribebals of Lamjung district, Nepal. *International Journal of Crude Drug Research*, 25(4), 236-240.
- Manandhar, N. P. (1985). Ethnobotanical notes on certain medicinal plants used by Tharus of Dang-Deukhuri district, Nepal. *International Journal of crude drug Research*, 23(4), 153-159.
- Manandhar, N. P. (1990). Traditional phytotherapy of Danuwar tribes of Kamlakhonj in Sindhuli district, Nepal. *Fitoterapia*. 61(4), 324-332
- Manandhar, N. P. (1991). Medicinal plant-lore of Tamang tribe of Kabhrepalanchok district, Nepal. *Economic. Botany*, 45(1), 58-71.
- Manandhar, N. P. (1992). Folklore medicine of Dhading district, Nepal. *Fitoterapia*, 63(2), 163-177.
- Manandhar, N. P. (2002). *Plants and people of Nepal*. Timber press, Inc.
- Nemkul, C. M., Bajracharya, G. B., & Shrestha, I. (2018). Phytochemical, antibacterial and DPPH free radical scavenging evaluations of the barks of *Aegle marmelos* (L.) Correa. *Journal of Pharmacognosy and Phytochemistry*, 7(4), 1637-1641.
- Nisar, M., Ali, S., & Qaisar, M. (2013). Antibacterial and cytotoxic activities of the methanolic

- extracts of *Rhododendron arboretum*. *Journal of Medicinal Plants Research*, 7(8), 398-403. <https://doi.org/10.5897/JMPR012.628>
- Packer, L., Cadenas, E., & Davies, K. J. A. (2008). Free radicals and exercise: An introduction. *Free Radical Biology and Medicine*, 44, 123-125.
- Painuli, S., Joshi, S., Bhardwaj, A., Meena, R. C., Misra, K., Rai, N., & Kumar, N. (2018). *In vitro* antioxidant and anticancer activities of leaf extracts of *Rhododendron arboretum* and *Rhododendron campanulatum* from Uttarakhand region of India. *Pharmacognosy Magazine*, 14(57), S294-S303.
- Pandey, G., Khatoon, S., Pandey, M. M., & Rawat, A. K. S. (2017). Altitudinal variation of berberine, total phenolics and flavonoid content in *Thalictrum foliolosum* and their correlation with antimicrobial and antioxidant activities. *Journal of Ayurveda and Integrative Medicine*, 30, 1-8. <http://dx.doi.org/10.1016/j.jaim.2017.02.010>
- Parekh, J., & Chanda, S. (2007). *In vitro* antibacterial activity of crude methanol extract of *Woodfordia fruticosa* Kurz. Flower (Lythraceae). *Brazilian Journal of Microbiology*, 38, 204-207.
- Pattanaik, C., Reddy, C. S., Das, R., & Reddy, M. (2007). Traditional medicinal practices among the tribal people of Malkangiri district, Orissa, India. *Natural Product Radiance*, 6, 430-435.
- Polunin, O., & Stainton, A. (1997). *Flowers of the Himalaya*. Oxford University Press.
- Savas, L., Duran, N., Savas, N. Onlen, Y., & Ocak, S. (2005). The prevalence and resistance patterns of *Pseudomonas aeruginosa* in intensive care units in a university hospital. *Turkish Journal of Medical Science*, 35, 317-322.
- Shakya, D. M., Pradhan, R., & Ranjitkar, R. (2008). A preliminary screening of some Nepalese medicinal plants for antimicrobial activity. *Plant Resources*, 30, 87-94.
- Shrestha, I., & Shrestha, K. (2000). Ethno-Medico-Botanical Studies of Langtang National Park, Nepal. *Proceeding of Nepal-Japan Join Symposium on Conservation and Utilization of Himalayan Medicinal Resources*, 178-181.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.
- Siwakoti, M., & Siwakoti, S. (1998). Ethnobotanical uses of plants among the Limbu of Morang district, Nepal. *Ecoprint: International Journal of Ecology, Nepal*, 5(1), 79-84.
- Sroka, Z., & Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food and Chemical Toxicology*, 41, 753-758.
- Taylor, R. S. L., Edel, F., Manandhar, N. P., & Towers, G. H. N. (1996). Antimicrobial activities of southern Nepalese medicinal plants. *Journal of Ethnopharmacology*, 50(2), 97-102.
- Van Landuyt, H. W., Boelaert, J., Glibert, B., Gordts, B., & Verbruggen, A. M. (1986). Surveillance of aminoglycoside resistance. European data. *The American Journal of Medicine*, 80(6B), 76-81.