International Journal of Herbal Medicine
Available online at www.florajournal.com

I J H M International Journal of Herbal Medicine

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2021; 9(3): 39-47
Received: 13-02-2021
Accepted: 15-03-2021

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Phytochemical screening, free radical scavenging and *In-vitro* anti-bacterial activity of ethanolic extracts of selected medicinal plants of Nepal and effort towards formulation of antibacterial cream from the extracts

Rajib Tiwari, Rishiram Baral, Nissa Parajuli, Riken Shrestha, Sapana Pun, Ashmita Pahari and Shila Gurung

Abstract
Bacteria are single-celled microorganism which lack nuclear membrane but are metabolically active causing many human diseases making itself an important therapeutic target in pharmacological sciences. Antibacterial agents are the group of materials that fight against such pathogenic bacteria, killing or reducing their metabolic activity. Meanwhile, various infectious diseases caused by virus, bacteria and other sources are being treated by locally available herbal remedies and are gaining interest among various indigenous and ethnic group.
This study was conceived to figure out the scientific background of these selected medicinal plants by taking their traditional and local use as a reference basis for selection of these plants. Quantitative phytochemical screening revealed that the ethanolic extract of *Cassia fistula* showed highest Total Phenolic content (TPC) with 94.63 ±5.22 µg GAE/mg of extract and *Aleuritopteris bicolor* showed highest Total Flavonoid Content (TFC) with 429.16±7.21µg QE/mg of extract. DPPH free radical scavenging study showed that *Aleuritopteris bicolor* showed the highest DPPH radical scavenging activity withIC50 value 46.76 µg/ml. Well diffusion method of antibacterial test showed that *Cassia fistula* showed best antibacterial activity against three bacteria followed by *Crinum amoneum*. This result coincides with the local and traditional use of these plant materials and provide scientific evidence for their anti-microbial property. This study even paved marvelous pathway for further study of these plant materials.

Keywords: Ethnomedicinal plants, phenols, flavonoids, Free radical scavenging activity, Antibacterial activity

1. Introduction
Bacteria are single-celled microorganisms which lack nuclear membrane, are metabolically active and divide by binary fission [1]. Pathologically, various bacteria of Gram-positive and Gram-negative stain cause different human diseases making itself as important therapeutic target in pharmacological sciences [2]. Therefore, various anti-bacterial agents which either kills i.e. bactericidal or stop the multiplication of bacteria i.e. bacteriostatic are being developed with different generation and potency [3]. Modern antibacterial drug discovery is associated with a variety of targets such as DNA polymerases and topoisomerases (Quinolones), or those that affect the cell-wall synthesis (Penicillins, Cephalosporins) or cause disruption in the cell membrane (polymyxin) as well as inhibiting protein synthesis (Tetracycline, Aminoglycosides) [4]. However, the diversity of the microbial world and the relatively specific activities of the synthesized antibacterial agents leads towards the widespread resistance among bacteria. Therefore, antimicrobial resistance is driven by inescapable evolutionary pressures to overcome the challenge [5]. On the other hand, existing synthetic antibacterial agent even consist of profound side effects such asdiarrhea, weakness, blood disorder, fungal infection of mouth and digestive tract, joint swelling, dehydration etc. [6]. By taking these into account, various infectious diseases caused by virus, bacteria and other sources are being treated by locally available herbal remedies and are gaining interest among various indigenous and ethnic group [7]. But, evidence based scientific study are still lacking to prove efficacy, safety and therapeutic potency of these medicinal plants and herbal species scientifically [8]. Therefore, this study was carried out to extrapolate the safety, efficacy, and therapeutic potency of the few traditionally used medicinal plants as a source of anti-bacterial and free radical scavenger.
Free radicals namely Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

Are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implication in the process of ageing [9]. Free radical scavenger or antioxidant molecules from phytochemicals of plants can either destroy the free radicals or inhibit or limit their production. Plants are considered as main sources of antioxidants which include phytochemicals such as flavonoids (anthocyanins, 3 flavanols, and flavones) and several classes of non-flavonoids (phenolic acids, lignins, stillbenes, terpenoids, etc) [10]. Meanwhile, various study revealed that different extracts of plants with free radical scavenging activity even showed anti-bacterial and anti-microbial effect revealing the relation between anti-oxidant and anti-bacterial properties of plant samples [11, 12].

Medicinal plants are possible source of new antibiotics and free radical scavengers which can be used as an alternative treatment for pathogenic strains [13]. Due to the increasing disease forms, resistance to existing drugs and demands for drugs with lesser side effect researcher group are more concerned to explore the best source of medicine from plants with modern scientific and technological ideas [14]. Traditional healers claim that some medicinal plants are more effective to treat infections in compared to synthetic compounds. In this study *Azadirachta indica* A. Juss, *Cassia fistula* L. and *Urtica parviflora* Roxb. are selected based on literature reviews while *Crinum amoenum* Roxb. ex Ker Gawl. and *Aleuritopteris bicolor* (Roxb.) Fraser-Jenk are selected based on their ethnomedicinal values and traditional use by different ethnic populations as antimicrobial agents [15].

Azadirachta indica is an evergreen tree of about 15 meter high, distributed throughout Nepal at the height of 900 m and belongs to the family Maeliaceae [16]. Leaf and bark of *Azadirachta indica* has been shown to reduce blood glucose level and lipid peroxidation as well as increase antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. In another study by Biswas *et al.* 2002, the study mentioned that *Azadirachta indica* have anti-inflammatory, spermicidal, anti-malarial, anti-bacterial, anti-fungal and diuretics activity [17]. Cassia fistula is a deciduous tree belonging to the family Meliaceae which is about 10 m high and even used as an ornamental plant due to its beautiful, bright yellow flowers [18]. Fruit pulp of Cassia fistula is widely used as mild laxative and purgative for children and pregnant women [19] while it even shows immunomodulators, wound healing, antifertility, antitumor, antifungal, antioxidant, antibacterial and antiparasitic effects [20].

Crinum amoenum is a herb found in Himalayas belonging to the family Amaryllidaceae, the rhizome of which is used to treat dysentery while certain ethnic group of Eastern Nepal use *Crinum amoenum* even to treat cholera [21]. Meanwhile, *Aleuritopteris bicolor* is a fern found in shady region belonging to the family Pteridaceae. The juice of the plant is taken to treat gastric troubles and Rai, an ethnic group of Eastern Nepal use *Aleuritopteris bicolor* to treat snake bite [21]. *Urtica parviflora* commonly called as Sishnu (Nepali), and Nettle plant (English) belongs to the family Urticaceae. The roots are used for the treatment of fractures of bone and dislocations of joints while leaves are used in dysentery, joint pain, and liver disorders. The inflorescence are used as cleansing agent after parturition and in the treatment of dermatitis [22]. This study was conceived to figure out the scientific background of these selected medicinal plants by taking their traditional and local use as a reference basis for selection of these plants. In this study, qualitative and quantitative phytochemical screening, free radical scavenging activity and In-vitro anti-bacterial activity of the crude extracts of those selected medicinal plants were evaluated. Finally, an effort was done to formulate the anti-bacterial cream from the plant extracts which showed potent activity against given stain of bacteria.

2. Material and Method

2.1 Chemicals, reagents, and test organisms

1,1 Diphenyl-2 picryl hydrazyl radical (DPPH) was purchased from Wako Pure Chemicals, Japan. The test organisms *Staphylococcus aureus, Pseudomonas aureginosa, Escherichia coli, Streptococcus pneumoniae* and *Enterococcus species* were provided as generous gift from Manipal Teaching Hospitals. The standard drug Amikacin which was used as positive control was provided as a generous gift from Asian pharmaceuticals Pvt. Ltd, Bhairahawa, Nepal. Ascorbic acid, a positive control for DPPH assay was purchased from Fisher Scientific, India. All chemicals and reagents used were of analytical reagent grade.

2.2 Collection and identification of plants sample

Selected medicinal plants (as shown in Table 1) were collected from different area of Kaski district, Nepal. The herbaria were prepared and identified with the help of taxonomist from National herbarium and Plant Laboratories, Godawari, Kathmandu, Nepal. The voucher specimen of each collected medicinal plants was deposited in the crude drug museum of School of Health and Allied Sciences, Pokhara University. The collected sample was chopped into small pieces and was shaded dried. It was incubated in hot air oven at 40° C for complete removal of moisture, which is detected by weight variation test at different time intervals. After complete drying of sample, it was powdered with the help of grinder.

Table 1: List of Selected Plants

S.N.	Name	Local Name	Family	Parts Used
1	*Azadirachta indica* A. Juss	Neem	Meliaceae	Leaves
2	*Urtica parviflora* Roxb.	Sishnu	Urticaceae	Leaves
3	*Cassia fistula* L.	Rajbrikshya	Leguminosae	Fruit pulp
4	*Crinum amoenum* Roxb.ex Ker Gawl.	Harde lasun	Amaryllidaceae	Bulb
5	*Aleuritopteris bicolor* (Roxb.) Fraser-Jenk	Ranisinke	Pteridaceae	Whole

2.3 Sample extraction

The crude samples were extracted by maceration process using ethanol (60% v/v) and distilled water as solvent in the ratio of 1:6. The maceration was carried out at room temperature for 24 hours and the extract was filtered using Whattman no.1 filter paper. The residue left was again subjected to second maceration under similar conditions as in first maceration. Then, the filtrate obtained from first and second maceration of all the plant extracts were concentrated in rotary evaporator at 175 mmHg pressure at 40 rpm and 5 °C chilling temperature. Further drying was performed in vacuum desiccator at pressure of 60 mbar until dry. Thus, obtained dried ethanolic extract was stored at 4 °C in refrigerator for further use.

2.4 Phytochemical Screening
2.4.1 Qualitative Phytochemical Screening
Qualitative phytochemical screening was performed for all five ethanolic extracts as per the method given by Bhatnagar *et al*. 2012 [23]. For each extract, stock solution of concentration 1 mg/ml was prepared in ethanol.

2.4.2 Quantitative Phytochemical Screening
2.4.2.1 Total Phenolic Content (TPC)
The TPC were determined by the Folin-Ciocalteu (FC) method as given by Pourmorad *et al*. 2006 [24]. 1 ml of sample was mixed with 5 ml of distilled water and 1ml of Folin reagent. After 5 minutes of incubation 1 ml of 10% Na_2CO_3 was added and incubated for one hour in the dark at room temperature. The absorbance was measured at 725 nm using UV spectrophotometer. Each assay was performed in triplicates and the values were expressed as mean ± SD. Total phenolic content was expressed as µg of Gallic acid equivalent per mg (GAE/ mg) of extract.

2.4.2.2 Total Flavonoid Content (TFC)
The TFC were determined by the Aluminium chloride method as given by Babu *et al*. 2017 [25]. 1 ml of 1 mg/ml plant extract was added with 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution which was allowed to remain for 5 minutes. Then 0.3 ml of 10% of Aluminium chloride was added followed by addition of 2 ml of 1 M sodium hydroxide. The absorbance was taken at 510 nm using UV-Vis spectrophotometer. Each assay was performed in triplicates and the values were expressed as mean ± SD. Total flavonoid was expressed as µg of quercetin equivalent per mg (QE/mg) of the plant extract.

2.5 Antioxidant Activity Analysis
2.5.1 DPPH Free Radical Scavenging Activity
DPPH free radical scavenging activity was performed as per the method given by Irshad *et al*, 2012, with slight modification [26]. 2 ml of different concentrations of extract solution was mixed with 2 ml of DPPH solution. Then it was left for 30 minutes at dark condition and the absorbance was measured at 517 nm using UV-Vis Spectrophotometer. Each assay was performed in triplicates. Radical scavenging activity was calculated by using following equation.

$$\%\,DPPH\ Scavenging\ activity = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\%$$

Where,
$Abs_{control}$ = Absorbance of control
Abs_{sample} = Absorbance of sample

The scavenging activity (%) was then plotted against concentration and graph was created. Half maximal inhibitory concentration (IC50) value was calculated by using linear regression analysis with Microsoft office excel 2010.

2.6 Antibacterial Assay
Antibacterial activity of all five ethanolic extracts was performed against *Staphylococcus aureus, Pseudomonas aureginosa, Escherichia coli, Streptococcus pneumoniae and Enterococcus species.*

2.6.1 Well Diffusion method
The antibacterial assay was carried out by using well diffusion method as given by Bhalodia *et al*. 2012 [27]. Wells of 6 mm diameter were prepared by using sterilized borer. Then the agar plates were swabbed with selected bacterial strains under sterile conditions. 100µl of 50 mg/ml, 100 mg/ml and 200 mg/ml solution were added in well with the help of micropipette. 10% DMSO (v/v) was used as negative control and reference antibiotic (Amikacin) disc was used as positive control. The solution in each well was allowed to diffuse for one hour into the plates and then incubated at 37 °C for 24 hours in inverted position. The results were obtained by measuring zone of inhibition. Each assay was performed in triplicates.

2.7 Formulation of Cream
2.7.1 Preparation of Cream Base
The formulation of cream base was performed as per the method given by Wibowo and Ng 2001 [28]. For the formulation of cream, cetyl alcohol and white petrolatum were used as oily phase, purified water as aqueous phase, glycerol as humectant, sodium lauryl sulphate as surfactant and methyl paraben as preservative. Cetyl alcohol also acted as emulsifier stabilizer.
To prepare oil phase cetyl alcohol was melted in the beaker by keeping it in the hot water bath at 70 °C. Then, white petrolatum was added, and the temperature was maintained at 70°C. Subsequently sodium lauryl sulphate and methyl paraben were added and stirred. To prepare aqueous phase, purified water was heated at 70 °C and glycerol was added and stirred. Then, oil phase and aqueous phase were mixed in the mortar which was maintained at 70°C and continuously stirred and cooled to obtain cream base.

2.7.1 Incorporation of Plant Extract in Cream Base
The cream base was melted in mortar and pestle by heating it in water bath, and the calculated amount of extract (5% and 10% w/w) was added in the molten base. The mixture was triturated until the homogeneous mixture was formed. The cream was stored for further evaluation.

2.7.2 Evaluation of Cream
2.7.2.1 Organoleptic Characterisation
Various organoleptic characters as colour, grittiness, odour, homogeneity and physical stability were determined as per the method given by Fatima *et al*. 2017 [29].

2.7.2.2 pH of Cream
The pH was measured using a calibrated pH meter at 4 and 7 pH. 5 gm of cream was weighed and dispersed in 45 ml of water to determine the pH of suspension at room temperature.

2.7.2.3 Percentage Residue
Percentage residue indicates the water content present in formulation. It was determined as per the method given by Fatima *et al*. 2017 [29]. 5 grams of cream was weighted in a cleaned and tarred Petri dish. Then, it was kept in oven at 105°C until constant weight was obtained. Then, it was cooled in desiccator and weighted. Percentage residue was calculated by using following equation:

$$\%residue = \frac{initial\ weight\ of\ cream}{final\ weight\ of\ cream} * 100\%$$

2.7.2.4 Determination of Type of Emulsion
The dilution test as given by Dhase *et al*, 2014 was used to

determine the type of emulsion [30]. In this test, the cream was diluted with either oil or water. Dilution with the oil refers to oily dispersion medium and dilution with water refers to aqueous dispersion medium.

2.7.2.5 Antibacterial Test of Cream

Antibacterial activity of cream (5% and 10%) was performed against Staphylococcus aureus, Streptococcus pneumoniae and Enterococcus Muller-Hinton agar plates were prepared by pouring the agar into sterilized Petri dish and allowed to cool. Then, bacteria were swapped in the plate and in one half of the Petri dish, prepared cream was swapped. Similarly, Gentamycin cream available in the market was used as positive control and the cream base without extract was used as negative control. It was then incubated in incubator at 37 °C for 24 hours and results were studied. All the experiments were carried out in triplet.

2.7.2.6 Stability Test

Phase separation was performed for stability analysis. The cream was kept in closed container and stored in room temperature. Phase separation was observed visually in 5 days interval up to 30 days.

2.8 Data Analysis

The relevant data were collected and presented in suitable tables and bar diagrams. The experimental data were expressed as Mean ± SD and were analysed by linear regression analysis with Microsoft office excel 2010.

3. Results

3.1 Phytochemical Screening

3.1.1 Qualitative Phytochemical Screening

The presence or absence of phytochemicals and their content was determined as per their colour intensity. The phytochemical screening of ethanolic extracts of most of the plants showed the presence of saponins, flavonoids, polyphenols, and terpenoids whereas alkaloids, glycosides and tannins were absent in all plant extracts. The detail of the phyto chemical screening is shown in the Table2.

Table 2: Phytochemical screening of Ethanolic Extracts of Plant Sample

Test	*Azadirachta indica*	*Urtica parviflora*	*Cassia fistula*	*Crinum amoenum*	*Aleuritopteris bicolor*
Saponin	+	-	+	+	+++
Phenols	++	+	+++	-	+++
Flavonoids	++	+	++	+	+++
Alkaloids	-	-	-	-	-
Tannins	-	-	-	-	-
Glycosides	-	-	-	-	-
Terpenoids	+	+	+++	-	++

Where, +++ Represents high presence of phytochemical, ++ Represents moderate presence of phytochemical, + Represents low presence of phytochemical, and - Represents absence of phytochemical.

From Table 2, it is seen that *Aleuritopteris bicolor* showed high presence of saponins, phenols, flavonoids, and moderate presence of terpenoids. Meanwhile, *Azadirachta indica* and *Cassia fistula* also showed significant presence of phenols, flavonoids and terpenoids.

3.1.2 Quantitative Phytochemical Screening

3.1.2.1 Total Phenolic Content (TPC)

Total phenolic content was quantified for all plant extracts by FC method using gallic acid as standard with Gallic acid calibration curve as shown in Figure 1. Results were expressed as µg gallic acid equivalent per mg of extracts as shown in Figure **2**. Among the selected plants *Cassia fistula* (94.63 ±5.22 µg GAE/mg of extract) showed highest and *Crinum amoenum* (25.22±2.41µg GAE/mg of extract) showed the lowest phenolic content.

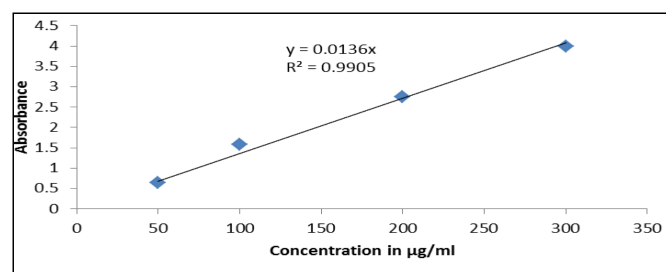

Fig 1: Calibration Curve of Gallic Acid

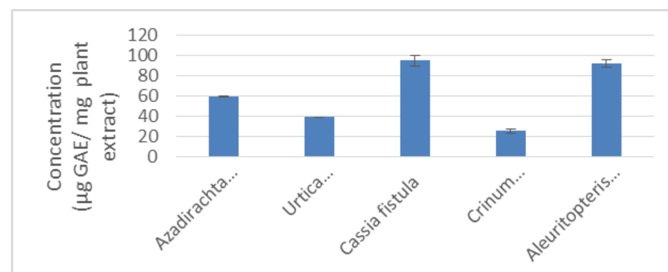

Fig 2: Total Phenol Content of Plant Extract

3.1.2.2 Total Flavonoid Content (TFC)

Total flavonoid content was quantified for all plants extract by AlCl3 method using quercetin as standard. Results were expressed as µg quercetin equivalent per mg of extract with the aid of quercetin equivalent calibration curve as shown in Figure 3.Among the selected plants *Aleuritopteris bicolor*, (429.16±7.21µg QE/mg of extract) showed highest and *Crinum amoenum* (104.16±5.05µg QE/mg of extract) showed least flavonoid content as shown in Figure 4.

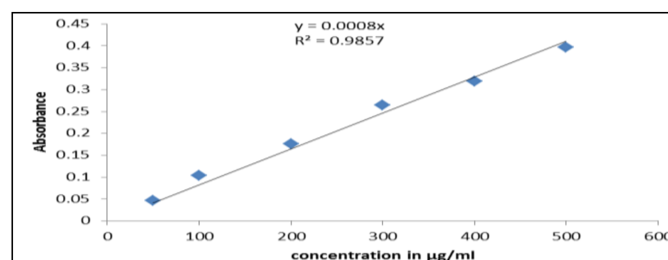

Fig 3: Calibration Curve of Quercetin

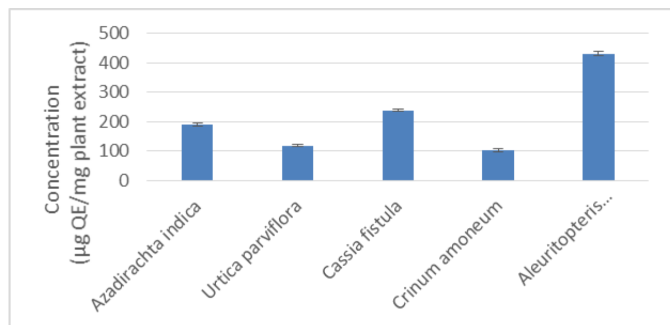

Fig 4: Total Flavonoid Content in Plant Extracts

3.2 Antioxidant Activity

DPPH free radical scavenging activity of five medicinal plants *viz, Azadirachta indica Urtica parviflora, Cassia fistula, Crinum amoenum* and *Aleuritopteris bicolor* were evaluated toassess their antioxidant activity at three different concentration as shown on Table 3.

Table 3: DPPH scavenging activity of plant extracts with IC50 values

Test	1 µg/ml	10 µg/ml	100 µg/ml	IC50 µg/ml
Ascorbic acid	5.01±0.005	59.64±0.008	87.95±0.036	35.6

Plants	100µg/ml	200µg/ml	300µg/ml	IC50 µg/ml
Azadirachtaindica	49.16±0.014	51.05±0.012	51.83±0.008	148.4
Urtica parviflora	1.11±0.002	2.23±0.002	4.57±0.002	2937.43
Cassia fistula	48.16±0.003	52.28±0.002	51.61±0.002	160
Crinum amoenum	1.67±0.07	10.70±0.006	18.84±0.009	661.76
Aleuritopteris bicolor	50.84±0.002	51.95±0.003	53.62±0.002	46.76

Among the selected plants, *Aleuritopteris bicolor* (100µg/ml, 200µg/ml and 300µg/ml) showed the highest DPPH radical scavenging activity with lowest IC50 value (46.76 µg/ml) which was found to be comparable with IC50 value of Ascorbic acid (35.6 µg/ml). Followingly, *Azadirachtaindica* also showed potent DPPH free radical scavenging effect with IC50 value of 148.4µg/ml.

3.3 Antibacterial Activity

Bacterial sensitivity test of five plant extracts was determined against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pneumoniae* and *Enterococcus.* Their potential activities were assessed by measuring zone of inhibition which is shown in Figure 5, 6 and 7.

Urtica parviflora of concentration of 200mg/ml showed antibacterial activity against *Streptococcus pneumonie* (ZOI= 14.5 mm) only. *Cassia fistula* in different concentration of 50 mg/ml, 100 mg/ml and 200 mg/ml showed antibacterial activity against *Sterptococcus aureus* (ZOI= 14.75 mm, 14.25 mm and 16 mm respectively), *Enterococcus* (ZOI= 12.33 mm, 13.2 mm and 13 mm respectively) and *Streptococcus pneumonie* (ZOI= 18 mm, 21 mm and 24 mm respectively). The data indicates that the extracts displayed a variable degree of antibacterial activity on different tested strains.

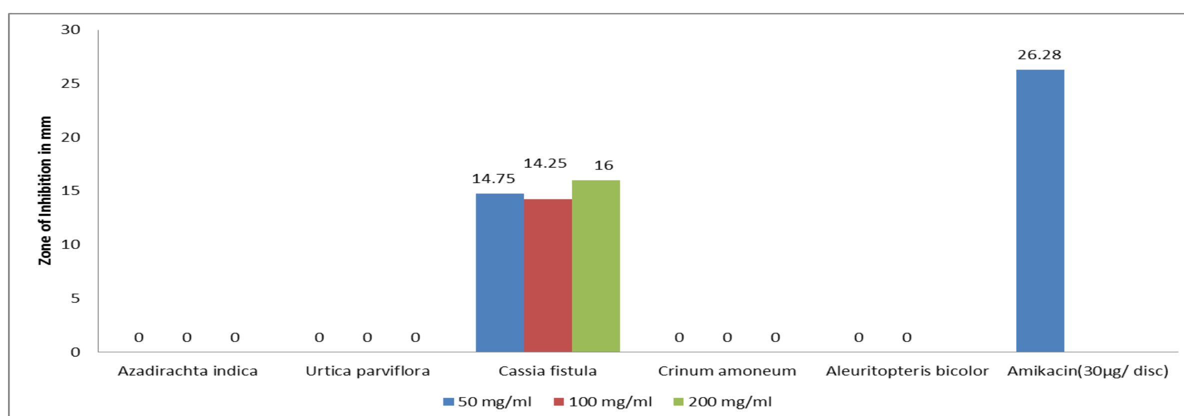

Fig 5: Antibacterial Activity of Plant Extracts against *Staphylococcus aureus*

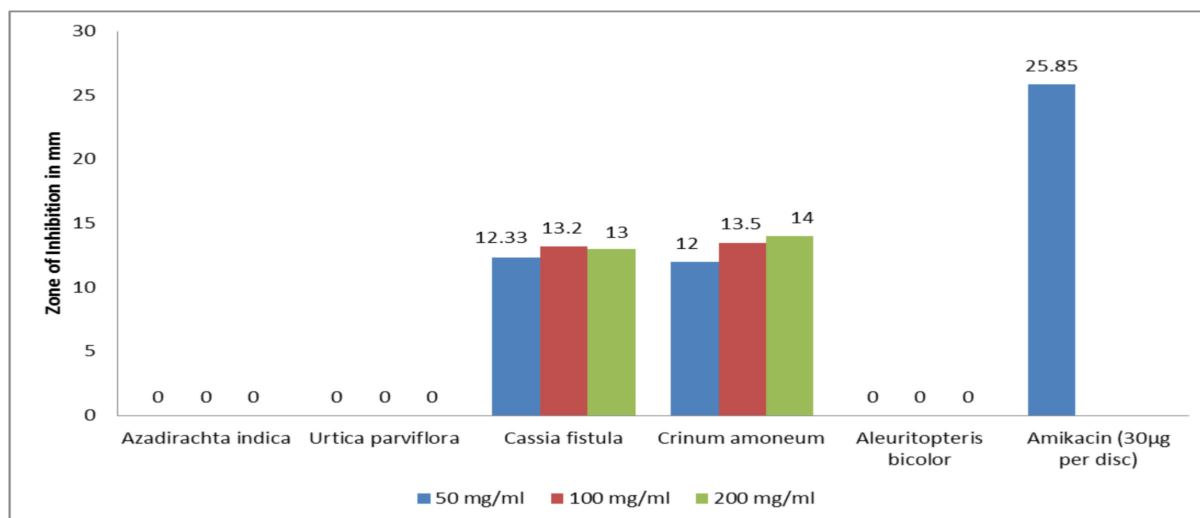

Fig 6: Antibacterial Activity of Plant Extracts against *Enterococcus*

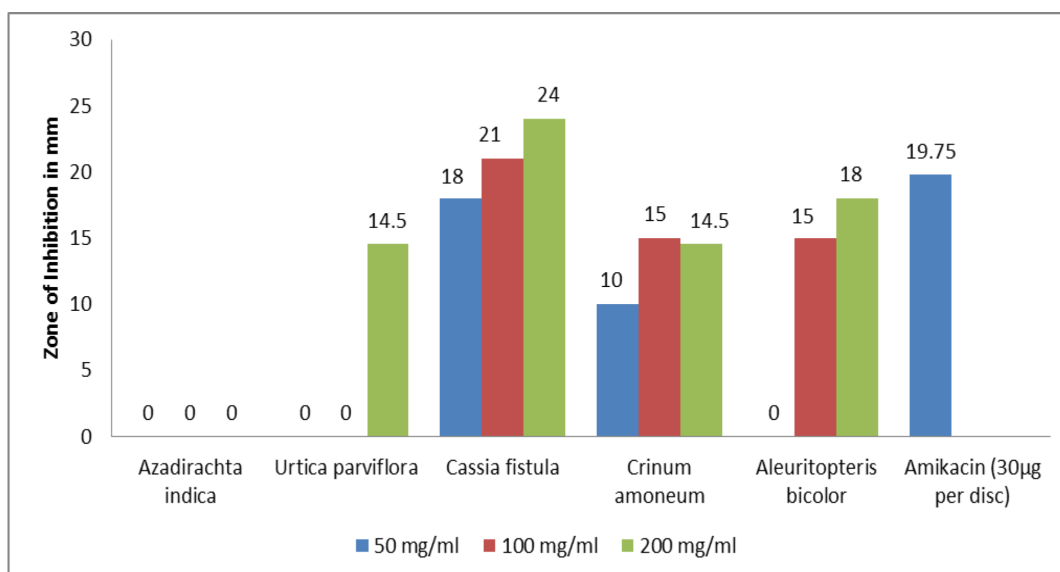

Fig 7: Antibacterial Activity of Plant Extracts against *Streptococcus pneumonia*

Aleuritopteris bicolor showed the zone of inhibition of 15 mm and 18 mm in 100 mg/ml and 200 mg/ml respectively against *Streptococcus pneumonia* only. Antibacterial activity was not observed in any plant extract against gram negative bacteria (*Pseudomonas aureginosa* and *Escherichia coli*). Amikacin disc showed zone of inhibition of 20 mm and 15.5 mm against *Pseudomonas aureginosa* and *Escherichia coli,* respectively. Overall, *Cassia fistula* showed best antibacterial activity against three bacteria followed by *Crinum amoneum*.

3.4 Evaluation of Cream

3.4.1 Organoleptic Characters and pH

Various observations of organoleptic characters and pH of cream are given below in the Table 4.

Table 4: Evaluation of Antibacterial Cream

Organoleptic characters	5%cream	10%cream
Color	Light Brown	Brown
Grittiness	Smooth	Smooth
Odor	Pleasant	Pleasant
Homogeneity	Smooth and homogenous	Smooth and homogenous
pH	6.44	6.50

Organoleptic evaluation of 5% cream showed the cream to be of light brown in color, pleasant in odour, smooth and homogenous while that of 10% cream showed the cream to be of dark brown in color, pleasant in odour, smooth and homogenous.

3.4.2 Percentage Residue

The percentage residue of 5% antibacterial cream was found out to be 77.8% while that of 10% residue was found to be 71%. According to BIS, percentage of total residue by mass should not be less than 10% and the percentage residue of the formulated cream was in the acceptable range [29].This also indicates that the formulated cream had less amount of water, thereby making the cream water in oil type of emulsion.

3.4.3 Determination of Type of Emulsion

The cream was diluted in oil but not in water, so the dispersion medium was oil phase. Hence, the emulsion was found to be water in oil.

3.4.4 Stability Test

The stability test was carried out for 25 days at room temperature. Color change and phase separation of cream was not observed.

Table 5: Stability Test of Cream

No of days	Color	Phase separation
5	Not changed	Negative
10	Not changed	Negative
15	Not changed	Negative
20	Not changed	Negative
25	Not changed	Negative

3.4.5 Antibacterial Test of Cream

10% cream inhibited the growth of *Staphylococcus aureus* and *Streptococcus pneumoniae,* but 5% cream did not show any inhibition. Both 10% and 5% cream did not inhibit the growth of Enterococcus.

4. DISCUSSION

From ancient time to modern period plant-based system have been used for the treatment of various sort of ailment. According to World Health Organization (WHO), approximately 80% of world population in developing countries relies on traditional medicines which are primarily derived from plants [31]. Epidemiological study have shown that many of the phytochemicals from plants possess various anti-inflammatory, antimicrobial, antimutagenic, antibacterial, anti-viral or several other activities [32, 33]. Based on these facts, the plant species used in this study were selected based on their ethno medicinal use for the treatment of respective ailments by different ethnic group of Kaski district Nepal or based on literature review which highlight the use of such medicinal plants as anti-septic, disinfectant, anti-microbial and other disease related to Reactive Oxygen Species (ROS) and free radicals.

Followingly, research have shown that till the recent time almost 4000 phyto constituents have been found on the medicinal plants and almost 150 of them have been studied in detail till today [34]. The mechanism behind such disease curing property of plant species is no other than secondary metabolites present in plant which are alkaloid, flavonoid, glycoside, saponins, tannin, carbohydrates, terpenoids and many others [35]. Phenolic compounds are widely known for their antioxidant or free radical scavenging activity. The antioxidant activity of phenolic compounds are mainly due to their redox properties which can play important role in absorbing and neutralizing free radical, quenching singlet oxygen or triplet oxygen or decomposing peroxides [36]. In a study by Wei *et al*. 2001, the relation between the antioxidant activity and phenolic content of plant was studied and the study revealed that there is positive linear correlation between the free radical scavenging activity and phenolic content of plant samples [37]. Typical phenolic compounds with radical scavenging effect are phenolic acid (caffeic acid, ferulic acid and vanillic acid). The flavonoid aglycon which show antioxidant properties are quercetin, myricetin, kaempferol, and their glycosides [38]. Three major criteria defined by the Bors to be best free radical scavengers are i) the presence of two hydroxy groups in the 3’, 4’ position on the B ring resulting in stability to the radical formed mainly in the 3’ position; ii) a double bond in the 2,3-position providing higher conjugation with other double bonds; iii) 3- and 5-hydroxyl groups with a 4-oxo function. These criteria are fulfilled by Quercetin. Followingly, flavan-3-ols include monomeric units such as epicatechin and catechin; gallate derivatives of the monomeric flavon-3-ols such as epigallocatechin, epicatechin gallate, epigallocatechin gallate and oligomers of the monomeric flavan-3-ols [39]. These are the compounds which act as best free radical scavengers.

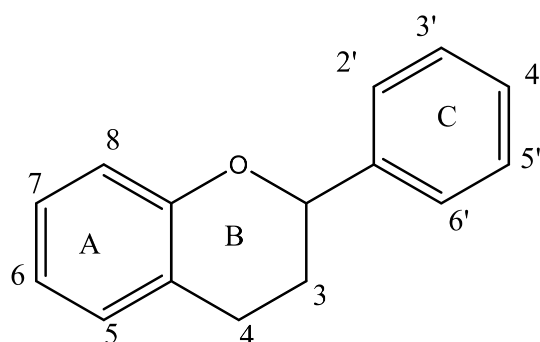

Fig 8: Chemical structure of flavonoids indicating rings (A, C, and B) and substitution numbers.

In our study, *Aleuritopteris bicolor* showed highest DPPH free radical scavenging activity with lowest IC50 value (46.76 µg/ml) which could be directly correlated with the high presence of Total Phenolic Content (TPC) and Total flavonoid content (TFC) as shown by quantitative phytochemical screening. Subsequently, the potent antioxidant activity after *Aleuritopteris bicolor* is shown by *Azadirachtaindica* and *Cassia fistula* which are 148.4µg/ml and 160 µg/ml and result showed that the set plants extract also contain high amount of phenol and flavonoids as compared to the leftover plant samples.

On the other hand, various plant phytochemicals are also known for their antimicrobial, antiseptic, disinfectant and for their antibacterial properties. In a study by Maria *et al*. 2010, phenolic compounds and flavonoid compounds were tested for their synergistic antibacterial activity against *Escherichia coli* and the study revealed that the combinations of gallic and protocatechuic acids, gallic and caffeic acids, rutin and quercetin were the best antibacterial agents, with synergistic effects [40]. On the other hand, several research groups have sought to elucidate the antibacterial mechanism of action of selected flavonoids. Quercetin is found to have DNA gyrase inhibition effect while (-)-epigallocatechin gallate inhibits cytoplasmic membrane function and licochalcones A and C inhibits energy metabolism [41]. In another study by Arthur *et al*. 2019, 13 common flavonoids i.e.chrysin, flavone, apigenin, luteolin, vitexin, orientin, vitexin 2’’-O-rhamnoside, isovitexin, isoorientin, kaempferol, quercetin, naringin and rutin were tested for their antibacterial activity and from the study it was revealed that these compounds inhibited both the gram positive and gram negative bacterial strain of which gram negative were strongly inhibited [42]. Other plant phytochemicals aside from phenol and flavonoids are alkaloids, saponins, tanninsand steroids which have known to be biologically active and thus partially responsible for the antimicrobial activities of plants [43]. In our study, *Cassia fistula* showed highest zone of inhibition at concentration of 200mg/ml plant extract against three-gram positive bacterial strain which are *Staphylococcus aureus* (16mm), *Enterococcus* (13mm) and *Streptococcus pneumonia* (24mm). This result could be correlated with the maximum presence of phenolic compounds as well as flavonoids. But in contrast, the *Aleuritopteris bicolor* plant extracts though it showed maximum positive result in most of the phyto constituents showed bacterial growth inhibition against Streptococcus pneumonia only which may be due to the presence of active constituents in phenolic and flavonoid group which are specific for this strain of bacteria only.

In theoretical science, antioxidant activity is all about the

reduction or removal of free radicals and nascent oxygen from an environment. On the other hand, many bacteria could also depend on these nascent oxygens for survival in any given environment. Thus, by implication, reduction or removal of free radicals or oxidants could relate to antimicrobial activity. This could explain in parts why antioxidant properties could correlate positively with antimicrobial activity.
Therefore, in our study, the antibacterial properties of *Cassia fistula* and *Aleuritopteris bicolor* could also be correlated with the best DPPH free radical scavenging effect of these two plant extracts.
Meanwhile, the cream which was formulated with the plant extracts showed adequate organoleptic character, appropriate stability as well as 10% cream showed inhibition to the growth of *Staphylococcus aureus* and *Streptococcus pnemoniae*. Attempt to formulate cream from these plant extracts is novelty of this study and no literature review had claim till now about the cream formulation of these plant extracts. Therefore, further study and scientific validation in molecular and synthetic level of active constituents from these plant extracts may ease in the formulation of topical or dermal antibacterial agent as well.

5. CONCLUSION
From the study it was revealed that *Cassia fistula* and *Aleuptopteris bicolor* showed promising antibacterial and antioxidant activity. This result coincides with the local and traditional use of these plant materials and provide scientific evidence for their anti-microbial property. This study even paved marvelous pathway for further study of these plant materials. Further isolation of the active phytoconstituent from the plant material could be done for the preparation of lead compound which could serve as active antibacterial agents.

6. Acknowledgments
This work was supported by School of Health and Allied Sciences, Faculty of Health Science, Pokhara University, Pokhara-30, Nepal. We would like to express our gratitude to National Herbarium and plant laboratory, Godawari, Kathmandu Nepal for their co-operation in plant identification used in research.

7. Author Contribution
Rajib Tiwari, Nissa Parajuli and Rishiram Baral conceived and designed the experiments. Rajib Tiwari, Nissa Parajuli, Riken Shrestha, Sapana Pun, Ashmita Pahari and Rishiram Baral performed the experiments. Shila Gurung supervised the research activity and setup methodology of experiment. Nissa Parajuli, Riken Shrestha, Sapana Pun and Ashmita Pahari analyzed the data. Rishiram Baral wrote the paper.

8. Conflict Of Interest
The author declares no conflict of interest.

9. References
1. Doetsch RN, Cook TM. Introduction to bacteria and their ecobiology. Springer Science & Business Media 2012.
2. Van BJD, Dąbrowska K, Vaneechoutte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria and the mammalian immune system, Journal of Viruses 2019;11(1):10.
3. Pucci MJ, Bush K. Investigational antimicrobial agents of Clinical microbiology reviews 2013;26(4):792-821.
4. Tang KW, Yang SC, Tseng C. Design, synthesis and anti-bacterial evaluation of triazolyl-pterostilbene derivatives, International journal of molecular sciences 2019;20(18):4564.
5. Patel JB, Richter SS. "Mechanisms of resistance to antibacterial agents," in Manual of Clinical Microbiology, Eleventh Edition: American Society of Microbiology 2015, 1212-1245.
6. Labu ZK, Jahan K, Afrin A. Glimpse of Usage of Antimicrobial Agents and its Unwanted Effects, International Journal 2013;4(4):768-774.
7. Shale TL, Stirk WA, Van SJ. Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity, Journal of Ethnopharmacology 1999;67(3):347-354.
8. Taylor PW. Alternative natural sources for a new generation of antibacterial agents, International journal of antimicrobial agents 2013;42(3):195-201.
9. Mosquera OM, Correa YM, Buitrago DC, Niño J. Antioxidant activity of twenty five plants from Colombian biodiversity, Memorias do Instituto Oswaldo Cruz 2007;102(5):631-634.
10. Moukette BM, Pieme CA, Njimou JR, Biapa CPN, Marco B, Ngogang JY. In vitro antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as spice: Monodora myristica, Journal of Biological Research 2015;48(1):1-17.
11. Safari M, Ahmady-Asbchin S. Evaluation of antioxidant and antibacterial activities of methanolic extract of medlar (Mespilus germanica L.) leaves, Journal of Biotechnology and Biotechnological Equipment 2019;33(1):372-378.
12. Chanda S, Dudhatra S, Kaneria M. Antioxidative and antibacterial effects of seeds and fruit rind of nutraceutical plants belonging to the Fabaceae family, Journal of Food function 2010;1(3):308-315.
13. Rios JL, Recio MC. Medicinal plants and antimicrobial activity, Journal of ethno pharmacology 2005;100(1-2): 80-84.
14. Chandran R, Abrahamse H. Identifying plant-based natural medicine against oxidative stress and neurodegenerative disorders, Journal of Oxidative Medicine and Cellular Longevity. 2020
15. Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections, BMC complementary and alternative medicine 2006;6(1):1-6.
16. Manandhar NP, Plants and people of Nepal. Timber press 2002.
17. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica), Current science 2002, 1336-1345.
18. Chewchinda S, Wuthi-udomlert M and Gritsanapan W. HPLC quantitative analysis of rhein and anti dermatophytic activity of Cassia fistula pod pulp extracts of various storage conditions, BioMed research international 2013.
19. Mozaffarpur SA, Naseri M, Esmaeilidooki MR, Kamalinejad M, Bijani A. The effect of cassia fistula emulsion on pediatric functional constipation in comparison with mineral oil: a randomized, clinical trial, DARU Journal of Pharmaceutical Sciences 2012;20(1):1-9.

20. Seyyednejad SM, Motamedi H, Vafei M, Bakhtiari A. The antibacterial activity of Cassia fistula organic extracts, Jundishapur Journal of Microbiology 2014, 7(1).
21. Rai R, Singh NB. Medico-ethnobiology in Rai community: a case study from Baikunthe Village development committee, Bhojpur, eastern Nepal, Journal of Institute of Science and Technology 2015;20(1):127-132.
22. Pandey S, Sah SP, Sah ML, Mishra D. An antioxidant potential of hydromethanolic extract of Urtica parviflora Roxb, Journal of basic and clinical pharmacy 2010;1(3): 191.
23. Bhatnagar S, Sahoo S, Mohapatra AK, Behera DR. Phytochemical analysis, Antioxidant and Cytotoxic activity of medicinal plant Combretum roxburghii (Family: Combretaceae), International journal of drug development and research 2012;4(1):193-202.
24. Pourmorad F, Hosseinimehr SJ and Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants, African journal of biotechnology 2006, 5(11).
25. Soyer Y, Microbial Control and Food Preservation, 2017.
26. Irshad M, Zafaryab M, Singh M, Rizvi M. Comparative analysis of the antioxidant activity of Cassia fistula extracts, International journal of medicinal chemistry 2012.
27. Bhalodia NR, Nariya PB, Acharya RN and Shukla VJ. In vitro antibacterial and antifungal activities of Cassia fistula Linn. fruit pulp extracts, Journal of Ayurveda and integrative medicine 2012;33(1):123.
28. Wibowo C, Ng KM. Product-oriented process synthesis and development: creams and pastes, AIChE Journal 2001;47.(12):2746-2767.
29. Fatima S, Zaman R, Haider N, Shamsi S, Alam A. Design and development of Unani anti-inflammatory cream, Journal of Ayurveda and integrative medicine 2017;8(3):140-144.
30. Dhase AS, Khadbadi SS, Saboo SS. Formulation and evaluation of vanishing herbal cream of crude drugs, AJEthno 2014;1:313-318.
31. Chivian E. Biodiversity: its importance to human health, Center for Health the Global Environment, Harvard Medical School, Cambridge, MA 2002, 23.
32. Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil, European Journal of Cancer 2000;36(10):1235-1247.
33. Sala A *et al.*, Anti-inflammatory and antioxidant properties of Helichrysum italicum, ournal of Pharmacy and Pharmacology 2002;54(3):365-371.
34. Saxena Mamta, Saxena Jyoti, Nema Rajeev, Singh Dharmendra, and Gupta Abhishek Phytochemistry of medicinal plants, Journal of pharmacognosy and Phytochemistry 2013, 1(6).
35. Vaishnav P, Demain AL. Unexpected applications of secondary metabolites, Biotechnology Advances 2011; 29(2):223-229.
36. Osawa T. Novel natural antioxidants for utilization in food and biological systems, Postharvest biochemistry of plant food-materials in the tropics 1994;1:241-251.
37. Zheng W. Wang SY. Antioxidant activity and phenolic compounds in selected herbs, Journal of Agricultural and Food chemistry 2001;49(11):5165-5170.
38. Kähkönen MP *et al.* Antioxidant activity of plant extracts containing phenolic compounds, Journal of agricultura food chemistry 1999;47(10):3954-3962.
39. Fraga CG. Plant polyphenols: how to translate their in vitro antioxidant actions to in vivo conditions, IUBMB life 2007;59(4-5):308-315.
40. Rodriguez VMJ, Aredes FPA, Manca de NMC, Strasser de SAM. Phenolic compound combinations on Escherichia coli viability in a meat system, Journal of agricultural and food chemistry 2010;58(10):6048-6052.
41. Cushnie TPT Lamb AJ. Antimicrobial activity of flavonoids, International journal of antimicrobial agents 2005;26(5):343-356.
42. Adamczak A, Ożarowski M, Karpiński TM. Antibacterial activity of some flavonoids and organic acids widely distributed in plants, Journal of clinical medicine 2020; 9(1):109.
43. Wintola OA, Afolayan A. The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africana Thunb. used as antidysenteric in Eastern Cape Province, South Africa, BMC complementary and alternative medicine 2015;15(1):1-12.