BIOLOGICAL ACTIVITIES OF SOME SELECTED NEPALESE MEDICINAL PLANTS AND ISOLATION OF CHEMICAL CONSTITUENTS FROM CALLICARPA MACROPHYLLA

KHAGA RAJ SHARMA*, KHEMINDRA RANA
*Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Email: khagaraj.sharma33@yahoo.com

Received: 25 Jan 2020, Revised and Accepted: 23 Mar 2020

ABSTRACT

Objective: The main objectives of this study was to analyze the phytochemicals, determine the total flavonoid content, brine shrimp toxicity, antibacterial activity, evaluate the antioxidant, antimicrobial, anti-diabetic activities of nine medicinal plants Callicarpamacrophylla, Bauhinia purpurea, Plumeriarubra, Girardinia diversifolia, Acacia nilotica, Woodfordiafratrica (Bark) Woodfordiafruticosa (flower), Terminalialatia, and Premnabarbata.

Methods: The cold percolation method was adopted for the extraction of secondary metabolites in methanol. The preliminary phytochemical analysis was performed by colour differentiation methods. The radical scavenging activity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The antidiabetic activity was performed by α-amylase enzyme inhibition activity. The chemical constituent was isolated by column chromatography from the biologically active plant fraction.

Results: The phytochemical investigation has shown plants are the rich source of secondary metabolites as quinones, saponins, terpenoids and glycosides. Among the nine tested plants, Terminalialatia showed the highest radical scavenging activity 96.41±0.47 with IC50 value 6.353 µg/ml followed by Girardinia diversifolia 97.26±0.67 with IC50 value 11.52 µg/ml whereas ascorbic acid has 39.85 µg/ml as standard. Bauhinia purpurea showed significant inhibition to the α-amylase enzyme having inhibitory concentration IC50 17.05±13.00 SD in a dose-dependent manner. Woodfordiafratrica demonstrated significant toxicity to A. salina with LC50 value of 457.08 µg/ml. Callicarpamacrophylla bark showed a potential inhibitory activity against the growth of Straphylococcusaureus as compared to standard chloramphenicol. Active plant extract of Callicarpamacrophylla was subjected for column chromatography. Conclusion: Out of nine plant samples Terminalialatia showed the highest radical scavenging activity. The plant extract of Bauhinia purpurea showed significant inhibition to the α-amylase enzyme inhibition. Woodfordiafruticosa demonstrated significant toxicity to A. salina whereas Callicarpamacrophylla showed the potent antibacterial activity. The active plant extract was subjected for column chromatography and different fractions were collected in solvent polarity basis.

Conclusion: The phytochemical investigations showed that plant extracts are the rich sources of secondary metabolites such as alkaloids, flavonoids, saponins, glycosides, polyphenols, coumarins and reducing sugars which showed they are supposed to be responsible for different biological activities. IC50 values showed the varied degree of antioxidant property of which Plumeriarubra and Acacia nilotica exhibit good antioxidant property with IC50 value close to the standard ascorbic acid.

Keywords: Phytochemical, Medicinal plants, Antioxidant, Cytotoxic, Antimicrobial, Anti-diabetic, α-amylase

INTRODUCTION

Nepal is rich in all three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In Nepal, large number of medicinal plants are known to have medical values and peoples have been using since many years to cure specific diseases [1]. Nepal has been regarded as the natural showroom of biodiversity because of its geotopography which is reflected in its dramatic contrast of climatic condition, which in turn is reflected in floral and faunal variations. Such biodiversity has supported the livelihood of people who live in remote areas of Nepal. These local people of a different ethnic group traditionally acquired a diversity of knowledge regarding the utilization of plant and animal resources for various purposes like food, medicine, clothing construction, dyes, ritual performances, energy, etc. About 80-90% people living in rural areas of Nepal depend directly or indirectly on the formal and informal system of traditional medicine involves the use of plant extracts [2]. Antioxidants are natural or synthetic substances that may prevent or delay oxidative cell damage in human beings. In humans, free radicals have been blamed, at least partially, for the development of several chronic ailments, for example, Alzheimer’s disease, atherosclerosis, cancers and many others [3].

Fig. 1: Location map of study site (medicinal plants collection): Palpa district of Nepal
Oxidative stress is a pathological state in which reactive oxygen/nitrogen species (ROS/RNS) overwhelm antioxidative defense of the organism, leading to oxidative modification of lipids, proteins, DNA, tissue injury and accelerated cellular death [3]. Commercially available antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylated hydroquinone (TBHQ). But, these antioxidants have side effects and toxicity when taken in vivo. Hence, their use is being restricted and have an urgent need to find out safer and bioactive natural antioxidant [4, 5]. Diabetes mellitus is a metabolic disorder characterized by loss of glucose homeostasis occurring due to defects in insulin secretion or insulin action resulting from impaired metabolism of glucose, lipids and proteins. Hyperglycemia, the primary clinical diagnosis of diabetes, is thought to be contribute to diabetic complications by altering vascular cellular metabolism in human body. Diabetes is a multifactorial disease leading to several complications require a multiple therapeutic approach [6, 25]. α-amylase is an enzyme that breaks bonds of large polysaccharides, such as starch and glycogen yielding glucose and maltose [8, 24]. It is the major form of amylase found in humans and other mammals [7]. α-amylase inhibitory agent is a protein family which inhibits mammalian α-amylases mainly by forming a stoichiometric complex with α-amylase [9].

An antimicrobial agent either kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the effect caused by microorganisms in human body [10]. Today, numerous antimicrobial agents exist to treat a wide range of infections. The development of new anticancer and antimicrobial therapeutic agents is one of the fundamental goals in medicinal chemistry. Owing to the growth of resistance, new techniques for the research on new anticancer and antimicrobial therapeutic agents are being introduced. The antimicrobials should be selectively toxic to the pathogenic microbes but not toxic to the host tissues [10]. More ethnomediapharmacological studies have been performed in Nepal but these results are not well documented and explored. The peoples of different communities of Nepal have been using such medicinal plants for cure of simple to life threatening diseases but the modes of preparation and administration of traditional herbal medicines are not well known. The evidences that show the relationship between pharmacological and phytochemical uses of plants are not well explored [13].

The most important part of this research is documenting the traditional knowledge and perform scientific validation of traditionally used different natural products, especially medicinal plants. Validation can be performed by different in vitro and in vivo experiments or by isolating the target secondary metabolites, which is useful for treating particular diseases or any health disorders [11, 12]. Present study focused on the collection of nine medicinal plants, Callicarpamacrophylla, Bauhinia purpurea, Plumeriarubra, Woodfordiafruticosa, Girardiniadiversifolia, Acacia nilotica, Woodfordiafruticosa (bark), Woodfordiafruticosa (flower), Terminaliaalata and Premnabarbarata from Palpa district of Nepal based on ethnomedicinal and traditional uses of plants and to perform their scientific validation as the primary source of medicine curing different diseases. Based on their biological activities, one of the plant fraction was selected to isolate the chemical compound by column chromatography.

**MATERIALS AND METHODS**

**Plant materials**

The plant materials were collected from the BoughaGumba VDC of Palpa district Nepal. The plants were identified by Dr. Munesh Gubhaju, Tribhuvan Multiple Campus, Tribhuvan University, Tansen, Palpa. The list of medicinal plants with voucher specimen number and their uses are shown in table 1.

**Table 1: List of selected medicinal plants and their therapeutic uses**

| Voucher specimen number | Code  | Scientific name               | Nepali name               | Used part                              | Altitude (m) | Therapeutic uses          |
|-------------------------|-------|-----------------------------|---------------------------|----------------------------------------|--------------|---------------------------|
| 2713                    | AB₁  | Callicarpamacrophylla       | Dahigola                  | Bark fruit and root (Plant juice)       | 1300-2700    | Fever, stomachitis         |
| 9423                    | AB₂  | Bauhinia purpurea           | Tanki                     | Bark (powder, paste)                   | 1300-2500    | Diarrhea, dysentery        |
| 1222                    | AB₃  | Plumeriarubra               | Golaichi                  | Whole part (fruit)                     | 1300-2000    | Anorexia, marasmus         |
| 4649                    | AB₄  | Girardiniadiversifolia      | Chalinsisnu               | Root (decoction, paste)                | Below 2000   | Cooling agent, constipation |
| 10076                   | AB₅  | Acacia nilotica             | Jukkharat                 | Bark (juice)                           | 900-3300     | Sprain, cut, ulcer         |
| 6038                    | AB₆  | Woodfordiafruticosa         | Dhyaro                    | Flower (juice)                          | Below 1500   | Dysentery, jaundice        |
| 6038                    | AB₆  | Woodfordiafruticosa         | Dhyaro                    | Bark (paste, juice)                    | 300-1800     | Gastritis                  |
| 5192                    | AB₇  | Terminaliaalata             | Saaj                      | Stem bark (juice)                       | 1100-2700    | Cooling agents, fever      |

**Sample preparation**

The bark of Callicarpamacrophylla, Bauhinia purpurea, Plumeriarubra, Acacia nilotica, Woodfordiafruticosa, Terminaliaalata, Premnabarbarata, and flower of Woodfordiafruticosa and the root of Girardiniadiversifolia were collected and washed with tap water to remove the contaminants. Then the collected plant parts were shade dried. The dried plant parts were grinded into powder form in electric grinder and stored in clean plastic bag at 4 °C until to perform different biological activities.

**Extract preparation**

The phytochemicals were extracted by cold percolation method using methanol as a solvent. Powdered plant parts (150 g) of mentioned plants were kept separately in the clean and dry conical flasks. Methanol (400 ml) was added to each nine different flask and kept for 72 h with frequent shaking. The mixtures were decanted and filtered with the help of cotton plug and thus obtained filtrates were concentrated with the help of rotary evaporator by distillation at temperatures below 60 °C. The concentrated filtrates were kept in a beaker wrapping with aluminum foil containing small pores to facilitate the evaporation of the solvent. After complete evaporation of the solvent extracts were obtained. These plant extracts were stored at 4 °C until doing biological activities. Biological activities were performed after sudden extraction. Percentage yield for each plant extracts was calculated.

**Phytochemical analysis**

This method involves the selective and successive extraction of phytochemicals where the method adopted was primarily based on the standard procedure. The analysis of the presence of main groups of natural compounds in the different plant extracts was done by the color reaction using different specific reagents [16].

**Antioxidant activity**

This method is rapid, simple and inexpensive to measure antioxidant capacity involves the use of the free radical, 2,2'-diphenyl-1-pirclyhydrazyl (DPPH). The ability of different plant extracts to scavenge DPPH free radicals was performed by adopting the standard protocol described by Jamuna et al. 2012 [17].

Different concentrations of test samples of 20, 40, 60, 80 and 100 μg/ml were made from stock solutions. Then 2 ml of each plant extracts were mixed with 2 ml of DPPH solution. The test tubes were
shaken vigorously for the uniform mixing then the solutions was kept for 30 min in the dark at room temperature. After 30 min, absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid of same concentrations was used as a standard.

The percentage of the DPPH free radical scavenging activity was calculated by using the equation:

\[
\text{Radical scavenging (\%)} = \left( \frac{A_0 - A_s}{A_0} \right) \times 100
\]

Where, \(A_s\) = Absorbance of the control (DPPH solution+methanol), \(A_0\) = Absorbance of test sample

The IC_{50} indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical. IC_{50} values were calculated using the inhibition curve by plotting extract concentration versus the corresponding scavenging.

Antidiabetic activity (α-amylase inhibition assay)

The antidiabetic activity of plant extracts was determined by using the α-amylase inhibition assay proposed by Kusano et al. 2011 with few modification. The undigested starch due to enzyme inhibition was detected through the blue starch iodine complex at 630 nm [18].

1000 µg/ml of stock solution of different dry extracts were prepared by dissolving 17 mg dry mass of extract in 17 ml dimethyl sulfoxide (DMSO). This stock solution was further used to prepare 5 different concentrations of each extracts viz. 640 µg/ml, 320 µg/ml, 160 µg/ml, 80 µg/ml and 40 µg/ml. Substrate was prepared by dissolving 200 mg of starch in 25 ml of NaOH (0.4M) by heating at 100 °C for 5 min. After cooling, pH was adjusted to 7.0 and the final volume was made up to 100 ml using distilled water.

400 µl of substrate was pre-incubated with 200 µl of varying concentrations (640 µg/ml, 320 µg/ml, 160 µg/ml, 80 µg/ml and 40 µg/ml) of plant extracts and acarbose separately at 37 °C for 5 min. After this 200 µl of α-amylase solution was added to each of them and then again incubated for 15 min at 37 °C. After incubation the enzymatic reaction was quenched with 800 µl of HCI (0.1M). Then, 1000 µl of iodine reagent was added, and the absorbance was measured at 630 nm. Then experiment was carried out in triplicate. Percentage of enzyme inhibition was calculated by using formula,

\[
\text{% inhibition} = 1 - \left( \frac{\text{Abs2-Abs1}}{\text{Abs4-Abs3}} \right) \times 100
\]

Where,

\(\text{Abs1}\) = absorbance of incubated mixture containing plant extract, starch and amylase, \(\text{Abs2}\) = absorbance of incubated mixture containing plant extract and starch, \(\text{Abs3}\) = absorbance of incubated mixture containing starch and α-amylase, \(\text{Abs4}\) = absorbance of incubated solution containing starch only. Graph was plotted by taking the concentration on the x-axis and percentage inhibition on the y-axis. With the help of this graph, IC_{50} values of each samples were calculated. The species having the lowest IC_{50} was considered to have the best α-amylase inhibition property.

Qualitative screening and evaluation of an antibacterial activity

Sterile Muller-Hinton Agar (MHA) plates were dried to remove excess of moisture from the surface of the media. The agar plates for the essay were prepared by labeling them with the name of the bacteria and the name code of the disc. The inoculums of bacteria were transferred into petri disc containing solid nutrient media of agar and the name code of the disc. The inoculums of bacteria were prepared by labeling them with the name of the bacteria and loaded into the respective wells with the help of micropipette. The plates were then kept for half an hour with the lid closed so that the extract diffused into the media. The plates were incubated overnight at 37 °C. After 24 h of incubation, the plates were observed for the presence of inhibition of bacterial growth indicated by a clear zone around the wells. The size of the zone of inhibition was measured and the antibacterial activity expressed in terms of the average diameter of zone of inhibition in millimeters. The absence of zone of inhibition was interpreted as the absence of activity. The ZOI were measured with the help of a millimeter ruler and the mean was recorded [19].

Brine shrimp bioassay (Toxicity test)

The eggs of brine shrimp are readily available at low cost and they remain viable for years in the dry state. Upon being placed in a brine solution, the eggs hatch within 48 h providing large number of larvae (nauplii). It determines the LC_{50} value (S) (µg/ml) for the crude extract (s). Extracts having LC_{50} values less than 1000 ppm (µg/ml) are considered as pharmacological active. Compounds/extracts having LC_{50} values less than 1000 ppm (µg/ml) are considered as pharmacological active. The assay was carried out by adopting the standard protocol of Meyer et al. 1982 [20].

\[
\text{LC}_{50} = \text{Antilog} x
\]

Where, Y is constant

\[
\text{LC}_{50} = \alpha + \beta x
\]

In the present work, brine shrimp bioassay of different plant extracts was carried out and the lethal concentration value was calculated.

Extraction and isolation of pure compounds

On the basis of biological activities, the plant extract of Callicarpum macrophylla was selected as an active sample for the isolation of compounds by chromatographic technique. Bark of C. macrophylla was dried and powdered. 150 g of powdered plant material was extracted with methanol by cold percolation. The solvent was filtered and evaporated in a rotatory evaporator to get methanolic extract. The yield of the methanolic extract obtained was 15.56 g. The methanolic extract was then fractionated with different solvents such as hexane, dichlomethane, ethyl acetate and methanol based on polarity.

Chromatographic separation

The hexane fraction weighing 8.01 g was adsorbed on 20 g silica gel and loaded on to a silica gel (120 g. Qualigens, and 60-120 mesh) packed column having an internal diameter of 3 cm with the adsorbent height 32 cm. The column was initially eluted with hexane and then the gradient of hexane in ethylacetate of increasing polarity and finally reported upto 100% ethyl acetate. Different fractions were collected and analyzed by thin-layer chromatography (TLC). Based on TLC report hexane fraction was selected for isolation of chemical constituents by column chromatography.
Table 2: The yield percentage of extracts of plant samples

| Name of sample plants       | Sample taken (g) | Extract (g) | Percentage yield |
|-----------------------------|------------------|-------------|------------------|
| Callicarpamacrophylla        | 150              | 15.56       | 10.37            |
| Bauhinia purpurea            | 150              | 11.10       | 7.4              |
| Plumeriarubra                | 150              | 8.25        | 5.5              |
| Girardiniadiversifolia       | 77               | 6.45        | 8.37             |
| Acacia nilotica             | 150              | 5.12        | 3.41             |
| Woodfordiafruticosa (bark)  | 150              | 13.12       | 8.74             |
| Woodfordiafruticosa (flower)| 150              | 10.78       | 7.18             |
| Terminalialatula            | 150              | 9.65        | 6.43             |
| Premnabarbata                | 150              | 12.75       | 8.5              |

The plant sample *Callicarpamacrophylla* showed the highest yield percentage (10.37%), indicating the plant extract is the rich source of secondary metabolites. The plant extract of *Acacia nilotica* showed the lowest yield percentage indicating the extract is the poor source of secondary metabolites as phytocomstituents.

**Phytochemical screening**

The results of the phytochemical analysis is shown in table 3.

Table 3: Phytochemical screening of plant extracts

| S. No. | Groups of compounds | AB₁ | AB₂ | AB₃ | AB₄ | AB₅ | AB₆ | AB₇ | AB₈ |
|--------|---------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| 1      | Basic Alkaloids     | -   | +   | -   | -   | +   | -   | -   | -   |
| 2      | Coumarins           | +   | +   | +   | +   | -   | -   | -   | +   |
| 3      | Flavonoids          | +   | +   | -   | -   | +   | +   | -   | -   |
| 4      | Glycosides          | +   | -   | +   | -   | -   | +   | -   | +   |
| 5      | Polyphenols         | +   | -   | -   | +   | -   | +   | -   | -   |
| 6      | Quinones            | +   | -   | +   | -   | +   | +   | +   | -   |
| 7      | Reducing sugars     | -   | -   | -   | -   | -   | -   | -   | -   |
| 8      | Saponins            | +   | +   | -   | +   | -   | +   | +   | +   |
| 9      | Terpenoids          | +   | +   | +   | +   | +   | +   | +   | +   |

Where, ‘+’ represents presence and ‘-’ represents absence, AB₁ = *Callicarpamacrophylla*, AB₂ = *Bauhinia purpurea*, AB₃ = *Plumeriarubra*, AB₄ = *Girardiniadiversifolia*, AB₅ = *Acacia nilotica*, AB₆ = *Woodfordiafruticosa* (bark), AB₇ = *Woodfordiafruticosa* (flower), AB₈ = *Terminalialatula*, AB₉ = *Premnabarbata*.

Results of the phytochemical analysis showed that quinones present in all the extracts except *Terminalialatula*, saponins and terpenoids are present on most of the plant extracts. Glycosides are present in *Callicarpamacrophylla*, *Plumeriarubra*, *Woodfordiafruticosa* (flower) and *Premnabarbata*, whereas there is an absence of reducing sugars in all plant extracts. Alkaloids are present only in *Plumeriarubra* and *Woodfordiafruticosa* (flower). The result of the phytochemical analysis slightly differs due to variation in altitude, different environmental conditions, method and time of sample collection, extraction procedure and also due to lab setup and chemical grades.

**Antioxidant activity**

Antioxidant activity of each plant extract were measured by using DPPH free radical scavenging. DPPH radical is scavenged by the plant antioxidants in which the donation of proton forming the reduced DPPH takes place. DPPH solutions show a strong absorbance band at 517 nm, appearing as deep violet color. The color changes from purple to yellow indicates reduction, which can be measured by its decrease of absorbance at wavelength 517 nm.
The degree of decolorization indicates the free radical scavenging potentials i.e. antioxidant potentials of the sample.

Percentage scavenging of the DPPH radical was gradually increased with the increase in the concentration of the methanolic plant extract from 20-100 μg/ml. The percentage inhibition of DPPH free radical of methanolic extract of bark of *Callicarpamacrophylla* and *Terminaliaalata* was found almost equal to the standard ascorbic acid taken whereas nearly equal to the bark of *Acacia nilotica* and flowers and bark of *Woodfordiafruticosa*. Graphical representations of DPPH assay of all the extracts is shown in fig. 1.

The linear regression of the percentage of radical scavenging versus concentration was used for the calculation of the concentration of each plant extract required for 50% inhibition of DPPH activity (IC$_{50}$). The antioxidant potential has an inverse relation with IC$_{50}$ value; lower the IC$_{50}$ indicates high antioxidant potential. The IC$_{50}$ values of the plant extracts along with the standard ascorbic acid is shown in table 4.

The diameter of zone of inhibition (ZOI) produced by plant extracts on particular bacteria was measured for the estimation of their antimicrobial activity. The methanolic extract of *Plumeriarubra, Bauhinia purpurea, Premnabarbarata, Woodfordiafruticosa* and *Terminaliaalata* did not show any zone of inhibition at 10 mg/ml.

Further, extracts of *Callicarpamacrophylla, Girardinia diversifolia* and *Acacia nilotica* were found not to be resistant against *E. coli*, whereas the same extract were found to be resistant against *S. aureus*. The extract of *Callicarpamacrophylla, Girardinia diversifolia* and *Acacia nilotica* found active for the inhibition of the growth of *S. aureus* only whereas negative response towards *E. coli*. The *Callicarpamacrophylla* showed the highest ZOI (12 mm) against *S. aureus*. *Terminaliaalata* against *E. coli* showed 20 mm of ZOI.

### α-amylase inhibition activity

The absorbance of different test samples was recorded by spectrophotometer. The graph was plotted concentration of plant extract against the percentage α-amylase inhibition, where acarbose was used as the positive control. The IC$_{50}$ values of each extracts were calculated with the help of plot.

The comparisons of percentage α-amylase inhibition between different plant extracts and acarbose as standard are shown in the fig. 2.

### Table 4: Comparison of IC$_{50}$ values of different plant extracts with standard ascorbic acid

| Plant sample/ascorbic acid       | IC$_{50}$ (μg/ml) |
|----------------------------------|-------------------|
| Standard Ascorbic acid           | 39.85             |
| *Callicarpamacrophylla*          | 17.77±1.568       |
| *Bauhinia purpurea*              | 23.57±1.491       |
| *Plumeriarubra*                  | 11.52±0.762       |
| *Girardinia diversifolia*        | 34.19±5.079       |
| *Acacia nilotica*                | 15.85±1.886       |
| *Woodfordiafruticosa* (bark)     | 24.45±2.982       |
| *Woodfordiafruticosa* (flower)   | 6.35±0.485        |
| *Terminaliaalata*                | 17.16±0.673       |

Values are expressed as mean±SD with $n=3$

The inhibitory concentration of *Terminaliaalata, Girardinia diversifolia* and *Callicarpamacrophylla* showed low IC$_{50}$ value with high antioxidant potential. These plant samples are the good sources of natural antioxidants. The rest of the plant extracts are moderate towards antioxidant activity with respect to the standard ascorbic acid. The antioxidant potential of plant sample was found comparable to the previously reported results [21, 22]. The results perform the scientific validation to the plant extracts that have been using by the peoples since many years to cure simple and life threatening diseases.

### Antibacterial activity

The diameter of zone of inhibition (ZOI) produced by plant extracts on particular bacteria was measured for the estimation of their antimicrobial activity. The methanolic extract of *Plumeriarubra, Bauhinia purpurea, Premnabarbarata, Woodfordiafruticosa* and *Terminaliaalata* did not show any zone of inhibition at 10 mg/ml.

### Table 5: The results of antimicrobial screening of different plant extracts

| S. No. | Plant extracts          | Bacteria    | ZOI (mm) of extracts at concentration 10 mg/ml | ZOI (mm) of chloramphenicol as control at 100 mg/ml |
|--------|-------------------------|-------------|-----------------------------------------------|---------------------------------------------------|
| 1      | *Callicarpamacrophylla*  | *E. coli*   | -                                             | 14                                                |
| 2      | *Bauhinia purpurea*      | *S. aureus* | 12                                            | 18                                                |
| 3      | *Plumeriarubra*          | *E. coli*   | -                                             | 14                                                |
| 4      | *Girardinia diversifolia*| *E. coli*   | -                                             | 14                                                |
| 5      | *Acacia nilotica*        | *E. coli*   | -                                             | 14                                                |
| 6      | *Woodfordiafruticosa*    | *S. aureus* | 10                                            | 18                                                |
| 7      | *Woodfordiafruticosa*    | *S. aureus* | -                                             | 14                                                |
| 8      | *Terminaliaalata*        | *E. coli*   | -                                             | 14                                                |
| 9      | *Premnabarbarata*        | *S. aureus* | -                                             | 18                                                |

(·) = No effective antibacterial activity, ZOI = Zone of Inhibition, *E. coli*: Gram-negative organism, *S. aureus*: Gram-positive organism
Table 6: Comparison of IC50 values of different plant extracts with standard acarbose

| S. No. | Plant samples/extracts                              | IC50 value (µg/ml) |
|--------|----------------------------------------------------|--------------------|
| 1      | Acarbose                                          | 361.01             |
| 2      | Callicarpa macrophylla                            | 475.00             |
| 3      | Bauhinia purpurea                                 | 17.05              |
| 4      | Plumeria rubra                                    | 133.50             |
| 5      | Girardinia diversifolia                          | 4308.25            |
| 6      | Acacia nilotica                                   | 329.57             |
| 7      | Woodfordia fruticosa (bark)                       | 76.78              |
| 8      | Woodfordia fruticosa (flower)                     | 366.52             |
| 9      | Terminalia alata                                  | 664.13             |
| 10     | Premna barbata                                    | 777.36             |

Fig 3: Percentage α-amylase inhibition against the concentration of plant extract (values are expressed as mean±SD with n=3)

The IC50 values of different plants extract along with standard acarbose were evaluated and found that the value ranges from 17.05 µg/ml to 4308.25 µg/ml. From the data the extract of Woodfordia fruticosa (flower) having IC50 value 366.52 µg/ml which is close to the standard acarbose with 361.01 µg/ml IC50 value. The plant extracts of Bauhinia purpurea, Woodfordia fruticosa (bark), Plumeria rubra, and Acacia nilotica are found potent than the acarbose. The rest of the plant extracts showed poor inhibitory activity against the α-amylase inhibition activity. Previous research reported that the aqueous leaves extracts of P. Americana possess hypoglycemic activity. Similarly, different fractions of R. Ellipticus fruits were reported for its antidiabetic activity on alloxan-induced diabetes and glucose tolerance test in rats. The results showed the similarity in α-amylase inhibition activity as reported by the previous researchers [23].

Brine shrimp bioassay (Toxicity test)

The toxicity of different plant extracts were evaluated for their toxicity towards newly hatched Brine Shrimp Larvae (A. salina leach) adopting the protocol Mayer et al. 1982. In this study, the lethal concentration that kills 50% of the exposed population of A. salina (LC50) values in µg/ml for different concentrations of plant extracts was determined and results obtained during these studies were recorded.

The degree of lethality was found to be directly proportional to the concentration of the extracts that is maximum mortalities of the brine shrimp larvae took place at the concentration of 1000 µg/ml and least mortalities were at 10 µg/ml. Those having LC50 values less than 1000 µg/ml are supposed to be pharmacologically active. It is cleared that the plant extract of sample AB1 was found toxic towards the brine shrimp larvae whereas rest of the plant extracts were found nontoxic. Although this method does not provide any adequate information regarding the mechanism of toxic action, it is a very useful method for the assessment of the toxic potential of various plant extracts. This method provides preliminary screening data that can be backed up by more specific bioassays once the active compounds have been isolated.

Isolation of compounds

On the basis of antioxidant activity and anti-diabetic nature of Callicarpa macrophylla extract was selected to separate the chemical constituents by column chromatography. The hexane fraction of methanolic extract weighing 3.5 g was adsorbed on 20 g of silica gel to make a slurry and loaded on silica gel packed column. The column was eluted in increasing order of solvent polarity and different fractions were collected/examined by thin-layer chromatography. The results of TLC examination for different fractions collected after elution is shown in table 7.
which showed they are supposed to be responsible for different saponins, glycosides, polyphenols, coumarins and reducing sugars rich sources of secondary metabolites such as alkaloids, flavonoids, The phytochemical investigations showed that plant extracts are the CONCLUSION scientific validation of this plant to the people who have been using included. By elucidating the structures of these isolated compounds, In this study, the characterization of these isolated compounds is not this research work. We would like to thank Dr. MuneshGubhaju, Tribhuvan Multiple Campuses, Tribhuvan University, Tansen, Palpa, for the identification of plants.

CONFLICTS OF INTERESTS The authors declare that they have no conflicts of interest for publishing this research article.

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Table 7: Isolation by column chromatography and TLC report of different fractions collected after elution

| S. No. | Elution solvent system | Fraction number | Volume of eluent (ml) | Solvent system for TLC | Remarks of TLC spots |
|--------|------------------------|-----------------|----------------------|------------------------|----------------------|
| 1      | 100% hexane            | 1 to 8          | 150                  | 1% EtOAC in hexane     | No spots             |
| 2      | 100% hexane            | 6 to 9          | 150                  | 1% EtOAC in hexane     | No spots             |
| 3      | 1% EtOAC in hexane     | 10 to 13        | 400                  | 3% EtOAC in hexane     | No spots             |
| 4      | 1% EtOAC in hexane     | 14-16           | 300                  | 3% EtOAC in hexane     | No spots             |
| 5      | 3% EtOAC in hexane     | 17-19           | 300                  | 5% EtOAC in hexane     | Tailing              |
| 6      | 2% EtOAC in hexane     | 20-21           | 200                  | 5% EtOAC in hexane     | No distinct spot     |
| 7      | 3% EtOAC in hexane     | 22-26           | 200                  | 5% EtOAC in hexane     | No spots             |
| 8      | 3% EtOAC in hexane     | 27-28           | 200                  | 5% EtOAC in hexane     | No spots             |
| 9      | 5% EtOAC in hexane     | 29-31           | 300                  | 5% EtOAC in hexane     | Single spot          |
| 10     | 5% EtOAC in hexane     | 32-33           | 200                  | 5% EtOAC in hexane     | Single spot          |
| 11     | 5% EtOAC in hexane     | 34-35           | 200                  | 5% EtOAC in hexane     | Single spot          |
| 12     | 5% EtOAC in hexane     | 36-38           | 300                  | 7% EtOAC in hexane     | Single spot          |
| 13     | 10% EtOAC in hexane    | 39-40           | 200                  | 15% EtOAC in hexane    | No clear spot        |
| 14     | 10% EtOAC in hexane    | 41-42           | 200                  | 15% EtOAC in hexane    | Tailing              |
| 15     | 10% EtOAC in hexane    | 43-44           | 200                  | 20% EtOAC in hexane    | No clear spot        |
| 16     | 10% EtOAC in hexane    | 45-46           | 200                  | 30% EtOAC in hexane    | No distinct spots    |
| 17     | 100% hexane            | 47-48           | 200                  | 30% EtOAC in hexane    | No distinct spots    |
| 18     | 25% EtOAC in hexane    | 49-51           | 300                  | 30% EtOAC in hexane    | No distinct spot     |
| 19     | 40% EtOAC in hexane    | 52-54           | 300                  | 50% EtOAC in hexane    | No spots             |
| 20     | 40% EtOAC in hexane    | 55-57           | 300                  | 50% EtOAC in hexane    | No distinct spots    |
| 21     | 40% EtOAC in hexane    | 58-59           | 200                  | 40% EtOAC in hexane    | Multiple spots       |
| 22     | 60% EtOAC in hexane    | 60-62           | 300                  | 70% EtOAC in hexane    | No distinct spots    |
| 23     | 60% EtOAC in hexane    | 63-64           | 300                  | 70% EtOAC in hexane    | Multiple spots       |
| 24     | 80% EtOAC in hexane    | 65-67           | 300                  | 90% EtOAC in hexane    | No distinct spots    |
| 25     | 80% EtOAC in hexane    | 68-69           | 200                  | 90% EtOAC in hexane    | No distinct spots    |
| 26     | 80% EtOAC in hexane    | 70-71           | 200                  | 90% EtOAC in hexane    | Tailing              |
| 27     | 100% EtOAC            | 72-74           | 300                  | 1% MeOH in EtOAC      | Multiple spots       |
| 28     | 100% EtOAC            | 75-77           | 300                  | 1% MeOH in EtOAC      | Tailing              |

EtOAC = Ethylacetate, MeOH = Methanol

Single spots were observed in thin layer chromatography in fraction no. 29-31, 32-33, 34-35 and 36-38, indicating the pure compounds. In this study, the characterization of these isolated compounds is not included. By elucidating the structures of these isolated compounds, their in vivo and in vitro study can be performed, which supports the scientific validation of this plant to the people who have been using as medicine since many years.

ACKNOWLEDGMENT
The authors are thankful to the Central Department of Chemistry, Tribhuvan University, for providing laboratory services to conduct this research work. We would like to thank Dr. MuneshGubhaju, Tribhuvan Multiple Campuses, Tribhuvan University, Tansen, Palpa, for the identification of plants.

AUTHORS'S CONTRIBUTIONS
Dr. Khaga Raj Sharma analyzed the data and wrote the manuscript, whereas KhimendraRana carried out the laboratory work. Both the authors read and approved the final manuscript.

FUNDING
The authors themselves bear the publication fees of this paper.
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