ORIGINAL RESEARCH ARTICLE

# Analysis of Phytoconstituents and Biological Activities of Different Parts of Mahonia *nepalensis* and *Berberis aristata*

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## Abstract

The phytochemicals and biological activities of extracts from leaves and stem of *Mahonia nepalensis* and *Berberis aristata* were carried out. Phytochemical screening showed the presence of alkaloids, steroids, polyphenols, quinones, glycoside, flavonoid, terpenoid and cardiac glycoside in the hexane, ethyl acetate and methanol extracts of leaf and stem of these two plants. The column chromatography of methanol extract of stem of *Mahonia nepalensis* resulted in isolation of four pure compounds $MN_1$, $MN_2$, $MN_3$ and $MN_4$. Out of four isolated compounds, two were identified as $MN_1$:β- sitosterol and $MN_2$: Berberine by comparison of melting point, Co-TLC, IR and UV spectra of authentic sample. Potent pharmacological activity of *Mahonia nepalensis* and *Berberis aristata* were revealed from antimicrobial activity and brine shrimp bioassay. Methanol extracts of stem of *Mahonia nepalensis* and *Berberis aristata* showed significant zone of inhibition of 18 mm and 21 mm respectively against the *Staphylococcus aureus*. Methanol extract of *Berberis aristata* were comparatively little stronger against *Staphylococcus aureus* than methanol extract of *Mahonia nepalensis*. $LC_{50}$ values (µg/ml) of methanol extracts of stem of *Berberis aristata* and *Mahonia nepalensis* were found to be $8.058x10^{-4}$ and 8.3 whereas methanol extracts of leaf of *Mahonia nepalensis* and *Berberis aristata* were 389.04 and 1303.166 respectively.

**Key words:** *Mahonia nepalensis*, Phytochemical, *Berberis aristata*, $LC_{50}$ values,

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## Introduction

Nepal is well known for superb collection of medicinal and aromatic plant resources that grow luxuriously in tropical forests to alpine meadow [1]. Since past, parts of these medicinal plants or their extracts have been used as traditional medicine. Traditional medicines are safe, easily accessible and affordable form of health care for much of the rural population in Nepal. Plants used in traditional medicine are important sources of novel biomolecules with application for the manufacture of pharmaceuticals and cosmeceuticals [2].

Berberidaceace is a family of shrubs and herbs, mainly of the Northern temperate zone, with simple, pinnate, or peltate leaves with spines. There are 14 genera in this family, and *Mahonia* and *Berberis* are among them. *Mahonia* is readily distinguished from *Berberis* by its compound leaves, spineless stems and inflorescence of several dense spikes. *Berberis* is distinguished by its undivided spiny toothed leaves and its spiny stems with yellow wood [3].

*Mahonia nepalensis* belongs to the family Berberidaceae and is known vernacularly as "*Jamanemandro*" in Nepali and "*Michiki swan*" in Newari. It is medium sized fully hardy perennial evergreen shrub with yellow flowers in winter. This shrub has an ultimate height of 6m /19.7ft. Its origin is in Nepal. It is widely distributed in the high mountainous areas at altitude of about 1000m - 2000m in Nepal, Sikkim, Bhutan, China, Vietnam, etc. It is useful in architectural and security barrier in garden, traditional essential flower for conducting Bel Bibaha and Bratabanda in Newar community. The stem and wood of this plant have anti-inflammatory, anti-bacterial, anti-fungal activity. It is particularly used for the treatment of skin diseases like eczema, psoriasis, etc. This plant contains alkaloids as the major compounds which belong to class protoberberines and bisbenzylisoquinolines [4] Berberine [5], Jattrrorzhine, O-methyl puljabine [6], Isotetradine, Homoaromaline etc. were isolated from the stem of this plant [7].

*Berberis aristata* also belongs to Berberidaceace family and is commonly known as "*Chutro*" in Nepali, "*Daruhaldi*" in Hindi and "*Indian Berberry*" in English. It is spinous herb native to northern Himalaya region, widely distributed from Himalayas to Sri Lanka, Bhutan and hilly areas of Nepal. It is used in ayurvedic medicine from very long time. The plant is used traditionally in inflammation, diarrhea, wound healing, skin disease, menohrrhagia, jaundice, and affection of eyes. A very valuable ayurvedic

preparation" Rashut" is prepared from this plant [5]. It is useful in treatment of jaundice, diabetes, cancer, malaria etc. and has good anti-oxidant property, anti-pyretic activity, analgesic activity, anti-fungal activity and anti-microbial property, anti-inflammatory, anti-platelet activating factor [8].

Berberine, Berbamine [9], Oxycanthine, Epiberberine, Palmatine, Dehydrocaroline, Jattrorhizine, Columbamine, Dihydrokarachine, Karachine [10], Taximaline [11], Oxyberberine, Aromaline [12], Pakistanine, 1-O-methyl Pakistanine [13], Pseudo Palmatine chloride, Pseudo berberine chloride, Lanost-5-en-β-ol [14] were isolated from its stem while citric acid, malic acid from fruit and E-caffeic acid, quercetin, chlorogenic acid, meratin and rutin from flower have also been isolated from this plant [15].

Due to their important pharmaceutical importance, we have tried to isolate the plant metabolites and determine the photo-constituents of different parts of these two valuable plants and studied their biological properties, isolated some of the important compounds and tried to characterize and identify the isolated compound as well.

# Materials and Method

## Sample collection and extraction

The stem and leaves of *Mahonia nepalensis* and *Berberis aristata* were collected from Bhaktapur and Palpa district respectively in April, 2013 and thoroughly dried in shade. About 50 g of stem and leaves of the two plants were ground to fine powder. The grinded parts were then successively extracted with hexane, ethyl acetate and methanol on the basis of their increasing polarity by using Soxhlet apparatus. The extracts were concentrated using rotatory evaporator and left for removal of solvent. After the solvents were completely removed, they were used for different purposes like phytochemical screening, biological activities study and isolation of chemical constituents.

## Phytochemical screening

A small amount of dry extracts of plant materials were subjected to phytochemical screening. The method employed was based on the standardized procedure with slight modification [16-18].

## Test for Tannin/Polyphenols

To a portion of extract diluted with water, 3-4 drops of 10% $FeCl_3$ is added, a blue color was observed for gallic, tannins and green color for catecholic tannin.

## Test for Reducing Sugar

To 0.5ml of plant extract, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick Red precipitate indicated the presence of reducing sugar.

## Test for Quinines

To the extract, Freshly Prepared $FeSO_4$ Solution (1ml) and Ammonium Thiocyanate (few crystals) were added and treated with conc. $H_2SO_4$ drop by drop. The deep red color was persistent indicating the presence of quinines.

## Test for Glycosides

For testing the glycosides, the protocol was slightly modified. To the extract, 5ml Molisch's reagent was added and conc.$H_2SO_4$ was added drop wise without disturbing the solution. A violet ring at the junction of two liquids was observed and on shaking the solution turned violet completely indicating the presence of glycosides.

## Test for Flavonoid

i. Shinoda test: 4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of conc. HCl was added and red color was observed for flavonoid and orange color for flavones.

ii. 5ml of dilute $NH_3$ solution was added to the aqueous filtered solution of each fraction followed by the addition of conc.$H_2SO_4$. The appearance of yellow color indicated the presence of flavonoid. The yellow color disappeared after some time.

## Test for Terpenoid

About 0.2g of each sample was mixed with 2ml of chloroform first and 3ml of conc.$H_2SO_4$ was

added to each mixture. The formation of a reddish brown coloration at the interface indicates the presence of terpenoid.

## Test for Alkaloids

I. Meyer's Test: To 2ml of extract, 1ml of Meyer's reagent (picric acid solution) was added. White precipitate and pale yellow precipitate indicates presence of alkaloids.

ii. Dragendroff's Reagent Test: 2ml of extract and each fraction were warmed with 2%. $H_2SO_4$ for 2min. After filtration of the reaction mixture a few drops of Dragendroff's reagent were added to each filtrate. Orange red precipitate indicates presence of alkaloids.

## Test for Saponins

About 2g of powdered sample was boiled in 20ml of distilled water in a water bath and filtered.10ml of filtrate was mixed with 5ml of distilled water and shaken vigorously. The appearance of frothing indicated the presence of saponins.

## Test for Volatile oils

2ml extract was shaken with0.1ml of NaOH and a small quantity of dil. HCl. A white precipitate was formed which indicated presence of volatile oils.

## Test for Cardiac Glycosides

5ml of plant extract was treated with 2ml of glacial acetic acid containing one drop of $FeCl_3$ solution. A brown ring at the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring was appeared below the brown ring, while in acetic acid; a greenish ring was formed just gradually throughout thin layer which showed the presence of cardiac glycosides.

## Test for Steroids

1gm of plant extract was dissolved in few drops of acetic acid. It was gently warmed and cooled under tap and a drop of conc.$H_2SO_4$ was added alongside of tube. Appearance of green color indicated presence of steroids.

## Separation of compound by using column chromatography

23.2g methanol extract of stem of *Mahonia nepalensis* was adsorbed in 20.07g silica gel (60-120) mesh and loaded in the column having diameter 3cm and length 60cm packed with150 mg activated silica gel (60-120)mesh. The 40.5cm long column was eluted with gradient of hexane, ethyl acetate, and methanol to obtain no. of fractions which resulted in isolation of compound $MN_1$, compound $MN_2$, compound $MN_3$ and compound $MN_4$. Compound $MN_1$was obtained on concentration of fraction (25-27) in rotator evaporator while Compound $MN_2$ from fraction (190-191), compound $MN_3$ and $MN_4$ from fraction (200-206),

Compound $MN_1$: Needle Shaped white crystals, Melting Point (MP) 135° C, showed single spot on TLC with R.F value 0.43 (20% Ethyl acetate in hexane).

Compound $MN_2$: Needle shaped yellow crystals, MP - 200$^0$C-205$^o$C. (decomposed), showed single spot on TLC with R.F value 0.45 (20% methanol in chloroform).

Compound $MN_3$: Needle shaped brown colored crystals, MP - 140$^{\circ}$C, showed single spot on TLC with R.F value 0.81 (30% methanol in chloroform).

Compound $MN_4$: Needle shaped dark orange brown colored crystalline, MP - 140$^{\circ}$C and decomposed at 160$^{\circ}$C, showed single spot on TLC with R.F value 0.86 (30% methanol in chloroform).

## Antimicrobial tests

The extracts of *Mahonia nepalensis* and *Berberis aristata* were screened for their antimicrobial activity i.e. determination of zone of inhibition against tested organism by Agar well diffusion method [19].

Four strains of bacteria namely *Staphylococcus aureus, Kleibsella pneumonia* (ATCC 700603), *Salmonella typhimurium* (ATCC 242), and *Salmonella typhimurium* (ATCC 14028) were used for antibacterial assay. Staphylococcus aureus and *Kleibsella pneumonia* were obtained from National Public Health Laboratory, Teku, Nepal and *Salomonella* typhimurium and *Salmonella typhimurium* were obtained from Institute of Medicine,

Maharajgunj, Nepal. These organisms were placed in Muller-Hinton Agar (MHA) in the refrigerator at 4°C prior to subculture.

**Table 1**: Percentage yield of different plant extracts and their physical characteristics.

| Plant | part | Extract | % Yield | color | consistency |
|---|---|---|---|---|---|
| *M. nepalensis* | stem | hexane | 0.53 | yellow | sticky |
| *M. nepalensis* | stem | ethyl acetate | 4.2 | yellow | sticky |
| *M. nepalensis* | stem | methanol | 12.6 | dark orange | powdered |
| *M. nepalensis* | leaf | hexane | 4.6 | green | sticky |
| *M. nepalensis* | leaf | ethyl acetate | 6.8 | dark green | sticky |
| *M. nepalensis* | leaf | methanol | 22.4 | dark green | powdered |
| *B. aristata* | stem | hexane | 0.88 | yellow | sticky |
| *B. aristata* | stem | ethyl acetate | 5.8 | yellow | sticky |
| *B. aristata* | stem | methanol | 22.6 | dark orange | powdered |
| *B. aristata* | leaf | hexane | 1.2 | green | sticky |
| *B. aristata* | leaf | ethyl acetate | 1.8 | dark green | Sticky |
| *B. aristata* | leaf | methanol | 3.8 | Dark green | powdered |

Agar well diffusion method was used to test the anti-bacterial properties of the crude extract and

Table 2: Phytochemical screening of different plant extracts

| Test | Tannin | Reducing sugar | Quinone | Glycoside | Flavonoid | Terpenoid | Alkaloid | Saponir | Volatile compd | Cardiac glycoside | Steroid |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HSM | - | - | ++ | + | - | +++ | + | - | - | + | +++ |
| ESM | - | - | + | + | - | +++ | + | + | - | + | +++ |
| MSM | - | - | +++ | + | +++ | +++ | +++ | + | - | + | +++ |
| HLM | - | - | ++ | + | - | +++ | - | - | - | + | +++ |
| ELM | - | - | + | + | - | +++ | - | + | - | + | +++ |
| MLM | - | - | +++ | + | +++ | +++ | +++ | + | - | + | +++ |
| HSB | - | - | ++ | + | - | +++ | + | - | - | + | +++ |
| ESB | - | - | + | + | - | +++ | + | - | - | + | +++ |
| MSB | - | - | +++ | + | +++ | +++ | +++ | - | - | + | +++ |
| HLB | - | - | ++ | + | - | +++ | - | - | - | + | +++ |
| ELB | - | - | + | + | - | +++ | - | - | - | + | +++ |
| MLB | + | + | +++ | + | +++ | +++ | +++ | - | - | + | +++ |

(+ sign indicate presence, - sign indicate absence)

MHA was used as medium. The bacterial inoculums were sub-cultured in Nutrient Broth for 12-18 hours at 37°C. Sterile petri plates were taken and MHA was poured, allowed to set and maintained the thickness of media at 4mm in each plate. Seven wells were bored in the medium and extracts of five plant material, a standard antibiotic disc, Nalidixic acid 30µg, as positive control and DMSO as solvent control was added into them. The inoculated plates were incubated at 37°C for 18-24hrs and the diameters of the zone of inhibition of microbial growth were measured in millimeters.

### Brine Shrimp Bioassay

Brine shrimp Bioassay was performed using standard procedure. Brine shrimp eggs were hatched in artificial sea water. The Brine Shrimp larvae were cultured under prescribed laboratory condition [20] and used against methanol extracts. The number of survived shrimps was counted and $LC_{50}$ value was calculated.

## Result and Discussion

### Isolation of Plant Metabolites

The different parts of plants specially leaf and stem selected on the basis of their medicinal use in Ethno medicine were successively extracted on the basis of increasing polarity. The yield of these extract and their physical characteristic were shown in (**Table 1**).

The highest yield % was observed for the methanolic extract of stem of *B.aristata* and was found to be 22.6 while the yield % for methanolic extract of stem of *Mahonia nepalensis*

was only 12.6%.Similarly the highest yield % of methanolic leaf extract of *Mahonia nepalensis* was 22.4 whereas the yield % of methanolic leaf extract of *B.aristata* was only 3.8%. Hexane extract were generally sticky and has comparatively low yield while ethylacetate extract yield was medium.

## Phytochemical Screening

The phytochemical screening of all plant materials were done on the basis of the procedure given by Alamzeb K (2013), Talukdar and Chaudhary (2010), and Prof. I. Culie (1990) [16-18]. The results obtained are given below in **Table 2**.

## Isolation and Identification from Stem of *M. nepalensis*

**Compound $MN_1$**: The compound $MN_1$ was white crystalline having MP 135°C and showed single spot on TLC in 20% Ethylacetate in Hexane solvent system with R.F value 0.43. It gave greenish red test in Liberman Burchard Test which indicated that the compound was sterol. It was identified as β-Sitosterol with the help of Co-TLC and melting point. The structure of β-Sitosterol (**Figure 1**).

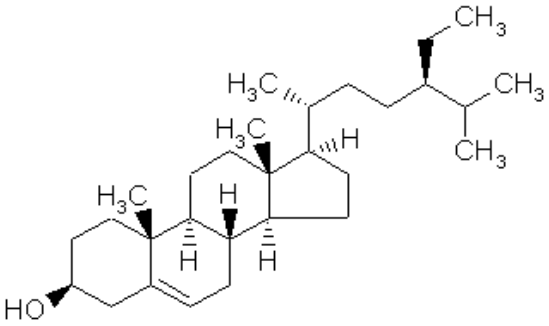


**Figure 1**: Chemical Structure of β-Sitosterol

**Compound $MN_2$**: The compound $MN_2$ is needle shaped yellow colored crystalline compound having MP. 200°C-205°C (decomposed). It showed a clear single spot on TLC in 20% methanol in $CHCl_3$ solvent system with R.F value 0.4. It gave pale yellow precipitate in Meyer's test and orange red precipitate in Dragendroff's test which indicated that the compound was alkaloid. The IR spectra showed peaks of 3549$cm^{-1}$, 3402 $cm^{-1}$,3317 $cm^{-1}$, 3224 $cm^{-1}$ (N-H stretch ), 3055 $cm^{-1}$ (C-H stretch in aromatic functional group) ,2908 $cm^{-1}$ , 2846 $cm^{-1}$(C-H stretch of alkenes), 2121 $cm^{-1}$(C triple bond N stretch), 1605 $cm^{-1}$ (C-C stretch in ring, aromatic), 1396 $cm^{-1}$, 1365.60 $cm^{-1}$(C-H bending)), 1293.02 $cm^{-1}$, 1204.44 $cm^{-1}$ (C-O stretch C-N stretch in aromatic amines), 1111 $cm^{-1}$, 1041 $cm^{-1}$(C-O-C bending), 970 $cm^{-1}$(O-H bending), 887 $cm^{-1}$, 825 $cm^{-1}$, 640 $cm^{-1}$,524 $cm^{-1}$, 424 $cm^{-1}$, 393 $cm^{-1}$(C-H out of plane bending) which were identical with that of the authentic sample of Berberine (**Figure 3** and **4**).The UV spectrum of the isolated compound showed that max wavelength of 353.8 nm at 0.861A, min wavelength of 307nm at 0.310A and max wavelength 271.6nm at 2.267A and min wavelength 251.8nm at 0.335A. These UV spectral data of isolated compound were also found to be identical with that of authentic sample of Berberine (**Figure 5 and 6**) respectively. Comparing the spectral data of IR Spectra and UV spectra and melting point with reference to the authentic sample, it was identified that the compound $MN_2$ was Berberine. The structure of Berberine (**Figure 2**).

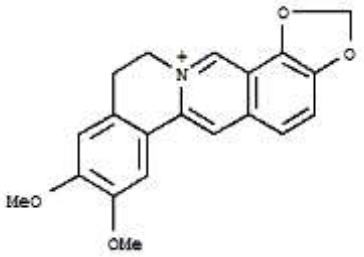


**Figure 2**: Chemical Structure of Berberine

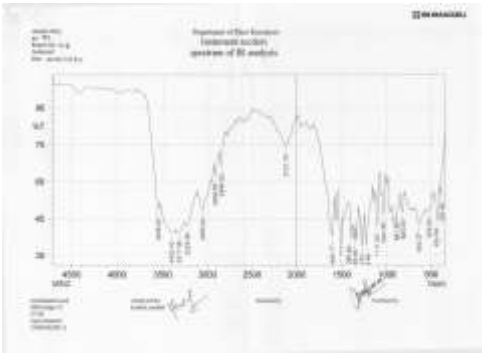

**Figure 3**: IR Spectrum of $MN_2$

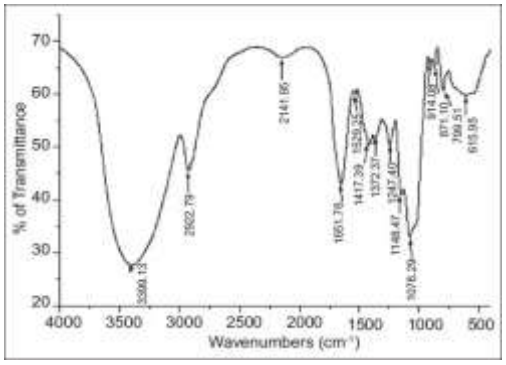


**Figure 4:** IR Spectrum authenticated berberine

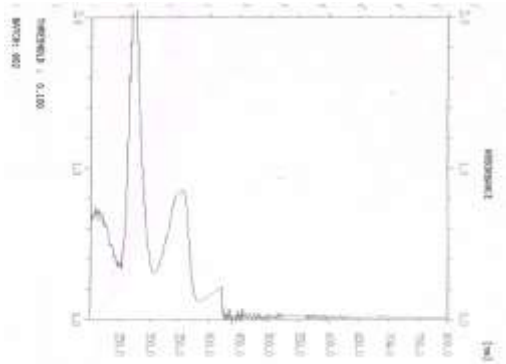

**Figure 5:** UV Spectrum of $MN_2$

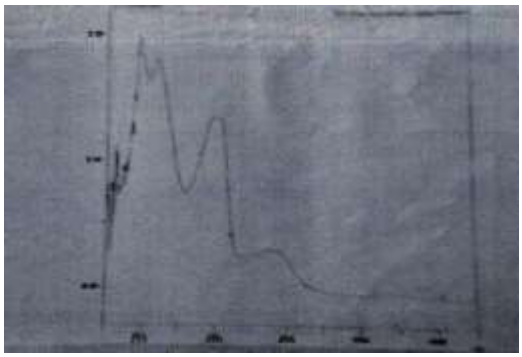

**Figure 6**: UV Spectrum of authenticated berberine

**Compound $MN_3$**: The compound $MN_3$ is needle shaped orange red colored crystalline compound having MP-140$^0$C. It showed a clear Spot was observed in 30% methanol in $CHCl_3$ with R.F value 0.81.It gave pale yellow precipitate in Meyer's test and orange red precipitate in Dragendroff's test which indicated that the compound was alkaloid**.** The IR peaks were 4420cm$^{-1}$, 3996.51 cm$^{-1}$, 3340.71 cm$^{-1}$,3240.41 cm$^{-1}$(N-H stretch), 3062.96 cm$^{-1}$ , 3016.67 cm$^{-1}$, 2947.23 cm$^{-1}$, 2885.52 cm$^{-1}$, 2831.50 cm$^{-1}$ (C-H stretch in aromatic functional group), 2762.06 cm$^{-1}$, 2654.05 cm$^{-1}$, 2623.19 cm$^{-1}$, 2376.30 cm$^{-1}$, 2345.44 cm$^{-1}$ (N-H stretch), 2121.70 cm$^{-1}$, 2052.26 cm$^{-1}$ (C triple bond N stretch) ,1928.82 cm$^{-1}$,1604.77cm$^{-1}$,1527.62 cm$^{-1}$ (C-C stretch in aromatic),1442.75 cm$^{-1}$ (C-H bending), 1018.41 cm$^{-1}$ (C-O-C stretch) ,972.12 cm$^{-1}$(O-H bending),900.40 cm$^{-1}$, 879.54 cm$^{-1}$, 807.82 cm$^{-1}$, 600.8 cm$^{-1}$, 509.21 cm$^{-1}$,362.62 cm$^{-1}$ (C-H out of plane bending). The IR peaks of authentic sample of 7,8 dihydro methoxy berberine were 1605cm$^{-1}$, 1510 cm$^{-1}$, 1050 cm$^{-1}$, 975 cm$^{-1}$, and 850 cm$^{-1}$ [21]. The fingerprint region peaks of isolated compound were very much identical with that of the authentic sample 7,8-Dihydro-8-methoxy berberine for IR spectrum of $MN_3$ (**Figure 7**)

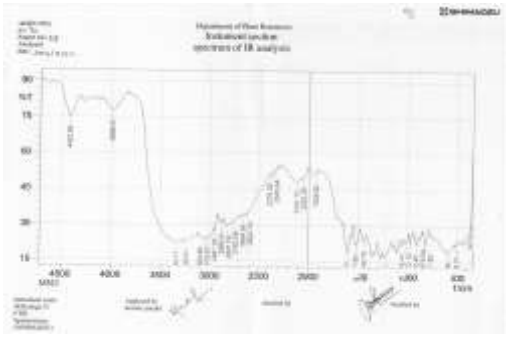

**Figure 7**: IR Spectrum of $MN_3$

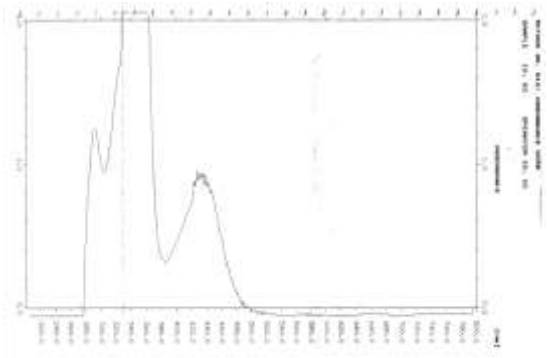

**Figure 8**: UV Spectrum of $MN_3$

The UV spectral data λmax/nm (EtOH) of the authentic sample were 285 and 365. The UV spectral data of 7,8-Dihydro-8-methoxy berberine were very much identical with that of the authentic sample 7,8-Dihydro-8-methoxy berberine (**Figure 9**). Comparing these spectral data of IR Spectra and UV spectra and melting point with reference to the authentic sample, compound $MN_3$ may be 7,8-Dihydro-8-methoxy berberine.The confirmation of compound is in progress. The structure of 7, 8-Dihydro-8-methoxy Berberine (Error! Reference source not found.**9)**.

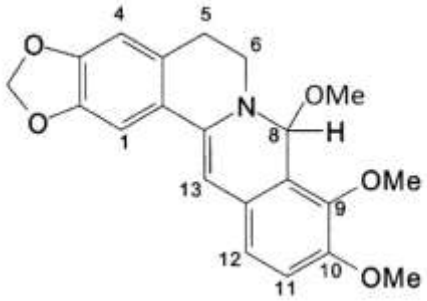


**Figure** 9: Chemical structure of 7, 8-Dihydro-8-methoxy Berberine

**Compound $MN_4$**: A needle shaped dark Orange brown colored crystalline compound $MN_4$ was obtained in pure and dry state. A sharp melting point was found to be 140˚C and decomposed at160˚C. A clear Spot was observed in 30% methanol in $CHCl_3$ with R.F value 0.86. Furthermore, it gave pale yellow precipitate in Meyer's test and orange red precipitate in Dragendroff's test which indicated that the compound was alkaloid. The exact structure and name of the compound will be confirmed after analysis of NMR, Mass and UV spectra.

**Table 3**: Antimicrobial Susceptibility test

| Crude extract | Vol. of extract | *Staphylococcus aureus* | | *Kleibsella pneumonia* | |
|---|---|---|---|---|---|
| | | | control | | control |
| Hexane stem extract of *M.nepalensis* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Ethyl acetate stem extract of *M.nepalensis* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Methanol stem extract of *M.nepalensis* | 30µl | 18mm | 21mm | Resistant | 30mm |
| Hexane stem extract of *B. aristata* | 30µl | 7mm | 21mm | Resistant | 30mm |
| Ethyl acetate stem extract of *B. aristata* | 30µl | 14mm | 21mm | Resistant | 30mm |
| Methanol stem extract of *B. aristata* | 30µl | 21mm | 21mm | Resistant | 30mm |
| Hexane leaf extract of *B. aristata* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Ethyl acetate leaf extract of *B. aristata* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Methanol leaf extract of *B. aristata* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Hexane leaf extract of *M.nepalensis* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Ethyl acetate leaf extract of *M.nepalensis* | 30µl | Resistant | 21mm | Resistant | 30mm |
| Methanol leaf extract of *M.nepalensis* | 30µl | Resistant | 21mm | Resistant | 30mm |

## Biological activities of plant extract

### The antimicrobial susceptibility test

The study involved the antimicrobial activity tests of the different extracts. Different bacteria were used for the test of the activity of the extracts. The results of the qualitative antimicrobial screening of different extracts were as shown in (**Table 3**).

### Comparative Study of anti- microbial activity

The results of the antimicrobial susceptibility test of the different extracts showed that the crude hexane extracts and ethyl acetate extract of stem and leaf of *M. nepalensis* were resistant to all of the bacteria species tested while ethyl acetate extract of *B. aristata* was found to be moderately active against *Staphylococcus aureus* while hexane extract of stem of *B. aristata* was slightly active against *S. aureus*. But the methanol extracts of stem of both plant species were found to be strongly active towards the gram positive bacteria *Staphylococcus aureus*. The methanol extract of *B. aristata* showed same pharmacological effect of Nalidixic acid control while methanol extract of *M. nepalensis* showed little lower pharmacological effect than that of control. Hence both of these methanol extract of *M. nepalensis* and *B. aristata* were pharmacologically active against *Staphylococcus aureus* while they were resistant to bacteria *Kleibsella pneumonia*. The methanol extract of leaf of both plant species were also found to be resistant to above bacteria species. DMSO was used as solvent control and it showed no effect. It was also found that *Salmonella typhimurim* and *Salmonella typhimurium* strains of bacteria were resistant to Nalidixic acid (Data not shown)

### Brine Shrimp Bioassay

For the study of biological activity of plant material, the newly hatched brine shrimp nauplii were exposed to the plant extracts. The biological activities were evaluated on the basis of their toxicities towards these nauplii. In this

method, $LC_{50}$ values (µg/ml) for different fractions were determined and those having less than 1000 are considered as pharmacologically active. The results obtained during this study are given in (**Table 4**).

**Table 4**: cytotoxicity value of different plant extracts

| Methanol Extract | $LC_{50}$ (µg/ml) |
|---|---|
| Stem of M.nepalensis | 8.3 |
| Leaf of M.nepalensis | 389.04 |
| Stem of B. aristata | $8.058*10^{-4}$ |
| Leaf of B. aristata | 1303.166 |

### Comparative Study of Cytotoxicity against Brine shrimps

The results of Brine shrimp bioassay showed that stem and leaf of *Mahonia nepalensis* have 8.3 (µg/ml) and 389.04 (µg/ml) $LC_{50}$ values while stem of *Berberis aristata* have $8.058*10^{-4}$ (µg/ml) $LC_{50}$ values which showed their bio-activity. Stem of *Berberis aristata* and *Mahonia nepalensis* were comparatively highly cytotoxicity while leaf of *Mahonia nepalensis* was moderately cytotoxic and leaf of *Berberis aristata* was found to pharmacologically inactive against brine shrimp.

## Conclusion

Phytochemical screening of hexane extract, ethyl acetate extracts and methanol extracts of the two plants revealed the presence of quinones, glycoside, terpenoid, cardiac glycoside, and steroid. However, Alkaloids was found in all type of stem extracts. Hence, distribution of different group of compounds was somewhat phytochemical equivalent in both species. The column chromatography of methanol extract of stem of *Mahonia nepalensis* resulted in isolation of four different compounds $MN_1$, $MN_2$, $MN_3$ and $MN_4$ in which compound $MN_1$ was identified as β-Sitosterol for the first time and $MN_2$ as Berberine. Metabolic stem extract of *Mahonia nepalensis* and *Berberis aristata* both were strongly pharmacologically active as that of Nalidixic acid against *Staphylococcus aureus.* The hexane and ethyl acetate extract of stem and leaf of *Mahonia nepalensis* were resistant to all of the bacterial species whereas hexane extract ethyl acetate extract of *Berberis aristata* were pharmacologically active against *Stappylococcus aureus.* In addition, all the other extracts were found to be inactive against Gram negative Bacteria like *Kleibsella Pneumoniae, Salmonella typhimurim, Salmonella typhimurim.* Methanol stem extract of *Mahonia nepalensis* and methanol stem extract of *Berberis aristata* were highly cytotoxic against Brine shrimp while leaf of *Mahonia nepalensis* was moderately cytotoxic and leaf of *Berberis aristata* was pharmacologically inactive against brine shrimp nauplii.

## Conflict of interest

The authors declare no conflict of interest.

## Author contribution

R.T was responsible for performing research under the supervision of S.M. R.T performed bibliographic researches and participated in discussion. The manuscript was designed, organized and written and edited by R.T. All authors have read and approved the final manuscript.

## Ethical Clearance

No ethical rules had been violated during sample collection and experimentation. Sample of *Mahonia nepalensis* and *Berberis aristata* had been collected from Bhaktapur and Palpa.

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