JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2017
VOL 1, NO. 1, PAGES 66–75
10.5455/jice.20171013045424

eJManager

ORIGINAL ARTICLE

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# Chemical profiling and biological activity analysis of cone, bark, and needle of *Pinus roxburghii* collected from Nepal

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## ABSTRACT

**Aim:** The present study aims to investigate chemical composition and biological activities of *Pinus roxburghii* collected from Kavre district of Nepal.

**Material and Methods:** Phytochemical screening, antibacterial activities, and antioxidant activities were measured. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the spectrophotometric analysis. Chemical composition was carried out using GC-MS **analysis.**

**Results:** Phytochemical analysis reveals the presence of interesting metabolites such as cardiac glycosides, saponin, protein, quinone, sterols, tannin and terpenoids. Highest TPC and TFC were observed in a bark crude methanol extract. The result further revealed that bark methanol extract showed the highest antioxidant activity. Furthermore, methanol and acetone extracts of cone, bark, and needle showed a range of *in-vitro* antibacterial activity against Gram positive and Gram negative pathogens. Gas chromatography mass spectroscopy analysis of crude acetone extract of bark revealed the presence of 14 different compounds.

**Conclusions:** This study showed that needle, cone, and bark of Pinus roxburghii are a source of biologically active metabolites. Furthermore, bark extract revealed the presence of diverse chemical constituent.

## ARTICLE HISTORY

Received October 13, 2017
Accepted November 30, 2017
Published January 09, 2018

## KEYWORDS

*Pinus roxburghii*; phytochemical screening; total phenol content; total flavonoid content; antioxidant activities and GC-MS

## Introduction

*Pinus roxburghii* is widely distributed in Himalayan region from Nepal, India and Pakistan and belong to the family Pinaceae [1]. In Nepal, it inhibits in the altitude range from 1,200 to 2,100 m in height. It was reported to have several medicinal importances, such as intestinal antiseptic, antidyslipidemic, and spasmolytic [2]. The wood, resin, gum, oil, seeds, needle, and bark from *P. roxburghii* have been used for the treatment of several diseases in many parts of the world [3]. Furthermore, it is the rich source of terpenoids, flavonoids, tannins, and xanthones [4]. The bark and needle were reported to have diverse chemical constituents. It includes taxifolin, quercetin, catechin, kaempferol, rhamnetin, sterols, and pinosylvin [5]. Furthermore, Pinus bark extract has been reported to act as an anti-proliferation effect on human breast cancer cells and shows strong 2, 2-diphenyl 1-picryl hydrazyl (DPPH) radical scavenging activity, analgesic and anti-Inflammatory activity [6]. Owing to the adverse effect of synthetic antioxidants and antimicrobial, much scientific effort is ongoing to find out the less toxic and cost-effective antioxidant and antimicrobial from natural sources.

Phenolic compounds are secondary metabolites produced by many plant species and played a vital role in defense response in the plant. Together with that many polyphenolic compounds derived from the plant has shown to be a potent antioxidant, antibacterial activities, and analgesic and anti-inflammatory activity [7]. Several scientific reports suggested that plant phenolic compounds such as phenolic acids and flavonoids reduce the risk of

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metabolic syndrome and its associated complication such as type 2 diabetes as well. However, different polyphenols have a different function. Aside from antioxidants activity, these molecules provide beneficial effects against virus, cancer, inflammation, and allergy [8].

The essential oil composition of *P. roxburghii* has been studied in detail in many parts of the world and revealed the presence of several sesquiterpenes as well as monoterpene alcohols. However, lacks the detailed investigation of total phenolic and flavonoid content as well as the comparisons of antioxidant and antibacterial activities of cone, needle, and bark of *P. roxburghii*. Especially, lacks enough scientific data of *P. roxburghii* from Kavre district of Nepal. Furthermore, plants grown in diverse climatic condition varies in the chemical constituents as well as antioxidant and antibacterial activities. Hence, these studies are carried out to compare the chemical constituents, antioxidant and antibacterial activities of cone, needle and bark of *P. roxburghii* collected from Kavre district of Nepal.

## Material and Methods

### *Collection of plant materials*

The plant materials were collected from Dhulikhel Latitude and Longitude of 27.6167 and 85.55, respectively. Dhulikhel is located in sub-locality, Dhulikhel locality, Bagmati District, Central Region State of Nepal Country 30.5 km away from the capital. The plant materials were identified by Mrs. Tirtha Maiya Shrestha, Assistant Professor, Department of Pharmacy, Kathmandu University.

### *Extraction*

The shade-dried needle, bark, and cone were grinded in coarse powder form and 20 gm of each were successively extracted at room temperature using 200 ml of solvent. All extracts were filtered separately with Whatman No 1 filter paper and evaporated by Vacuum Evaporator (Hanil P201502902-1) to get dry extracts. After drying, crude extracts were weighed and stored in stock vials and kept in the refrigerator (0–4°C) for further use.

### *Phytochemical analysis and determination of Total Phenolic Content(TPC)*

The phytochemical analysis of alkaloids, flavonoids, phenolic content, saponin, protein, quinone, sterols, Cardic glycoside, Tannin, Terpenoid, and reducing compound was performed following the standard protocol [9]. Total phenolic content (TPC) estimation was measured using Folin Ciocalteu's methods using gallic acid as a standard [10]. The 1 ml of test solution was placed into the separate test tubes followed by addition of 0.5 ml of Folin Ciocalteu's reagent, and 4.5 ml of distilled water was mixed and shaken well, after 5 minutes 4 ml of 7% sodium carbonate was added. Then the blue color mixture was shaken and incubated at 40°C in a water bath. UV-Vis Spectrophotometers was used to measure absorbance at 760 nm. The experiments were performed in triplicates. Results were expressed as mg of gallic acid equivalent per gram dry weight (mg GAE/g DW).

### *Determination of Total Flavonoid Content*

The aluminium chloride colorimetric assay was used for total flavonoid content (TFC) using quercetin as a standard [11]. The 1 ml aliquots of test solution was added into separate test tubes and followed by the addition of 0.3 ml of 5% sodium nitrite solution, 4 ml of distilled water, and shortly after 5 minutes, 0.3 ml of 10% aluminum chloride was added, and followed by the addition of 2 ml of 1 M sodium hydroxide was added. The final volume was adjusted to 10 ml with distilled water and mixed well until the yellowish color was developed. The absorbance was measured at 510 nm spectrophotometer using the UV-visible instrument. The experiments were carried out in triplicates. The standard quercetin was used to plot calibration curve. The total flavonoids were expressed as mg of quercetin equivalents per gram of dry weight (mg QE/g DW).

### *Free radical scavenging activity*

DPPH radical was used to determine the free radical scavenging capacity of the extracts using standard protocol [12]. The reaction mixture contained 3.7 ml of 0.004% freshly prepared DPPH methanol solution and 0.3 ml of test sample (final concentration was adjusted to 20–100 µg/ml, respectively). The mixture was vigorously shaken and left for 30 minutes in the dark (until stable absorption values were obtained). The range of reduction of the DPPH radical was dogged by determining the absorption at 517 nm. For reference standard, ascorbic acid was used and DPPH solution was used as the control.

### *Reducing power assay*

Total reducing power of selected medicinal plants was analyzed following the standard method with

some modifications [13]. The 1 ml of test sample (final concentration 200–1,000 µg/ml) was mixed with 2.5 ml of sodium phosphate buffer (pH 6.6, 0.2 M) which was then followed by the addition of 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 20 minutes. The mixture was then supplemented with trichloroacetic acid (10%, 2.5 ml) and centrifuged at 1,000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and ferric chloride solution (0.1%, 0.5 ml) and absorbance was measured at 700 nm, higher absorbance indicates higher reducing power. The above assays were carried out in triplicate and the results were expressed as mean values ± standard deviation. The results were expressed as effective concentration ($EC_{50}$) when the absorbance is 0.5 at 700 nm and ascorbic acid was used as a standard.

### *Antibacterial activity*

The extracts *in vitro* antibacterial screening were carried out against four pathogenic strains, viz., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Enterococcus spp.* by the disk-diffusion method [14,15]. The Mueller–Hinton agar plate dried surface was inoculated over the entire sterile agar surface by streaking the swab. The 20 µl of the plant extract was loaded in sterile filter paper disks of 6 mm diameter. Methanol was used as negative control and Ampicillin was used as positive control. The experiment was performed in triplicates under aseptic conditions. Plates were incubated for 18 hours at 37°C. The antibacterial activity was evaluated by measuring the zones of inhibition. The mean value of the diameter of the inhibition zone of the triplicates sets was taken as the final value.

### *GC-MS analysis*

The analysis of the essential oil was performed using Shimadzu GCMSQP2010 plus. For the analysis, Rtx5 MS (30 m length × 0.25 mm diameter × 0.25 micrometer thickness) was used. The carrier gas was helium at 1.3 ml/minutes in a constant flow mode. The injector temperature was 220°C, the injection volume 1 µl, and the split ratio 1:30. The initial oven temperature of 40°C was held for 3 minutes, then increased at a rate of 12°C/minutes up to 180°C, kept fat 180°C for 5 minutes, and finally ramped at a rate of 12°C $min^{-1}$ to 240°C kept at this temperature for 5 minutes.

## Results

### *Phytochemical screening, total phenolic content and total flavonoid content*

Phytochemical screening of *P. roxburghii* was carried out using the standard protocol described in material and methods. The needle, cone and bark metabolites were extracted using four solvent of different polarity index. Results revealed that methanol extract contains the higher amounts of alkoloids, saponin, xanthoprotein, quinone, sterol and reducing sugar, followed by acetone extract. The least phytonutrients were observed in aqueous and hexane extract. It is mainly because of the extraction efficiency of many phytochemicals by these solvents. The phytonutrients present in the needle, cone, and bark is summarized in Table 1.

Quantitative determination of total flavonoids and phenolic content was determined as described in material and methods. The TPC was

**Table 1.** Phytochemical screening of *Pinus roxburghii* crude extract. WhereThe signs +, ++ and +++ represents the relative higher activity towards the phytonutrients and (–) not detected.

| Plant parts | Solvent | Alkaloid | Saponin | Xantho protein | Quinone | Sterol | Cardiac glycoside | Tannin | Terpenoid | Reducing sugar |
|---|---|---|---|---|---|---|---|---|---|---|
| Needle | Water | + | + | + | + | + | – | + | – | + |
| | Methanol | ++ | + | ++ | ++ | + | ++ | ++ | ++ | + |
| | Hexane | – | – | – | + | + | – | + | – | – |
| | Acetone | + | + | + | + | + | + | ++ | + | + |
| Cone | Water | + | ++ | + | + | + | – | + | + | + |
| | Methanol | +++ | ++ | ++ | ++ | + | +++ | +++ | +++ | ++ |
| | Hexane | – | – | – | + | + | – | ++ | – | – |
| | Acetone | ++ | + | + | ++ | + | ++ | +++ | + | + |
| Bark | Water | – | – | + | + | + | – | + | – | – |
| | Methanol | ++ | + | + | ++ | + | + | ++ | ++ | + |
| | Hexane | – | – | – | + | + | – | ++ | – | – |
| | Acetone | + | – | + | + | + | + | ++ | + | + |

expressed as mg gallic acid equivalent per gram dry weight of the sample (mg GAE equivalent/g DW) and summarized in Table 2. In comparison of four different solvent extracts (water, methanol, acetone, and hexane), the methanol extract of bark showed the highest amount of phenolic content (69.23 ± 0.04 mg GAE equivalent/g DW), followed by needle and cone. Furthermore, the TFC of medicinal plants was expressed as mg quercetin equivalent per gram dry weight of the sample (mg QE equivalent/g DW) and is presented in Table 2. Among all solvent extracts compared, the methanol extract of bark showed highest flavonoids content (62.4 ± 0.03 mg QE equivalent/g DW), followed needle and cone. Results revealed the lowest amount of polyphenol and flavonoids in water and hexane extracts, this might be due to the poor extraction efficiency of the polyphenolic compounds. When compared with needle and cone extracts, bark extract revealed higher amount of TPC and TFC.

## Antioxidant Activity

The DPPH radical scavenging activity and $IC_{50}$ values of different medicinal extracts are summarized in Table 3. Generally, the higher % RSA and lower $IC_{50}$ values indicate a higher antioxidant activity. The DPPH radical scavenging properties were found to be concentration dependent. The *P. roxburghii* bark was found to have 36.57%, 38.94%, 52.30%, 57.60%, and 70.64% inhibition at 20, 40, 60, 80, and 100 mg/ml of crude methanol extract. The percentage inhibition of this radical was found to increase with the increase in the concentration of extract. At 20 µg/ml, the inhibition of methanol extract of *P. roxburghii* was 36.57%, whereas ascorbic acid was 38.94% (Fig. 1). In comparison of needle, cone, and bark crude extracts in four different solvents, methanolic extract of bark revealed the highest antioxidant activity (57.2 ± 0.23 µg/ml), whereas the methanolic extract of needle showed the least antioxidant activity with $IC_{50}$ value of 157.35 ± 1.60 µg/ml. The data were compared with ascorbic acid ($IC_{50}$ = 35.05 ± 0.11 mg/ml), as a

**Table 2.** Total flavonoid content (TFC) and total phenolic content (TPC) of *Pinus roxburghii* crude extract. All experiments were performed in triplicate. TPC was expressed as mg of gallic acid equivalent per gram dry weight (mg GAE/g DW) and TFC was expressed as mg of quercetin equivalents per gram of dry weight (mg QE/g DW).

| Solvent | Plant parts | TFC (mg QE equivalent/g DW) | TPC (mg GAE equivalent/g DW) |
|---|---|---|---|
| Water | Needle | 4.08 ± 0.05 | 5.39 ± 0.05 |
| | Cone | 2.9 ± 0.03 | 4.45 ± 0.07 |
| | Bark | 9.37 ± 0.04 | 10.75 ± 0.04 |
| Methanol | Needle | 50.98 ± 0.03 | 57.34 ± 0.05 |
| | Cone | 52.71 ± 0.04 | 54.19 ± 0.06 |
| | Bark | 62.4 ± 0.03 | 69.23 ± 0.04 |
| Hexane | Needle | 2.64 ± 0.05 | 3.18 ± 0.07 |
| | Cone | 4.59 ± 0.05 | 5.23 ± 0.06 |
| | Bark | 7.44 ± 0.04 | 7.68 ± 0.04 |
| Acetone | Needle | 41.23 ± 0.05 | 52.59 ± 0.07 |
| | Cone | 44.35 ± 0.03 | 54.1 ± 0.4 |
| | Bark | 48.17 ± 0.05 | 52.12 ± 0.04 |

**Table 3.** Antioxidant activity of *Pinus roxburghii* crude extract. All experiments were performed in triplicate and results were expressed as mean ± SD.

| Solvent | Plant parts | Reducing activity EC50 (mg/ml) | DPPH activity IC50 (mg/ml) |
|---|---|---|---|
| Water | Needle | 847.86 ± 1.62 | 100.92 ± 0.50 |
| | Cone | 661.2 ± 0.51 | 152.12 ± 2.05 |
| | Bark | 489.25 ± 0.78 | 134.61 ± 1.09 |
| Methanol | Needle | 673.5 ± 0.95 | 157.35 ± 1.60 |
| | Cone | 590.35 ± 1.26 | 80.19 ± 0.160 |
| | Bark | 410.1 ± 0.42 | 57.2 ± 0.23 |
| Acetone | Needle | 743.7 ± 2.19 | 75.18 ± 0.30 |
| | Cone | 624.05 ± 1.12 | 92.65 ± 0.57 |
| | Bark | 459.27 ± 1.36 | 63.32 ± 0.33 |
| Hexane | Needle | 970.9 ± 1.2 | 104.58 ± 0.54 |
| | Cone | 624.64 ± 0.29 | 145.3 ± 0.59 |
| | Bark | 552.7 ± 0.09 | 102.44 ± 0.18 |
| Reference | Ascorbic acid | 255.38 ± 1.04 | 35.05 ± 0.11 |

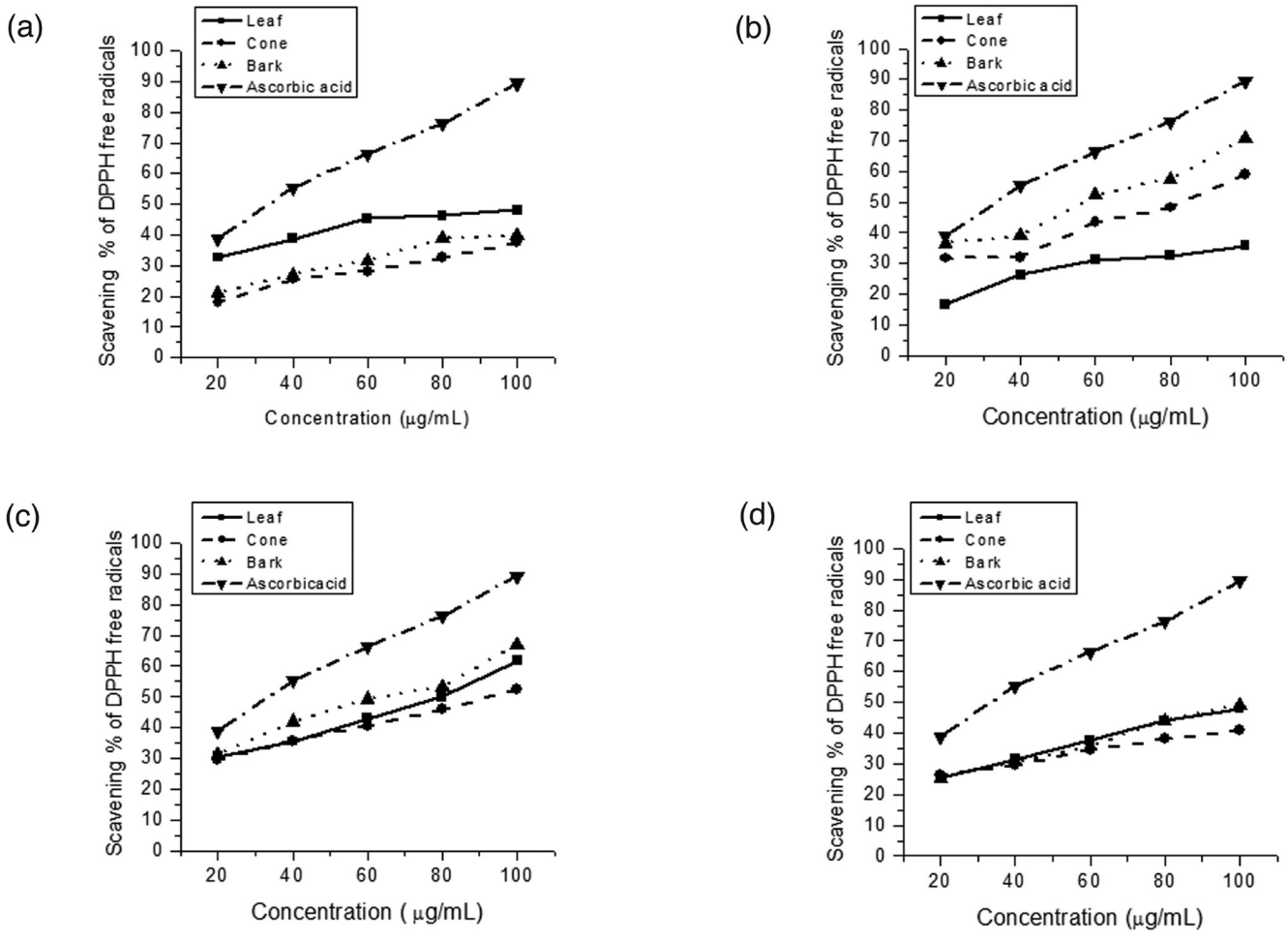


**Figure 1.** DPPH radical scavenging activity of *Pinus roxburghii*. (a) Water extract, (b) Methanol extract, (c) Acetone extract, and (d) Hexane extract.

standard. However, the lowest antioxidant activities were observed in the case of aqueous and hexane extract.

Similarly, total reducing power of medicinal extracts and their $EC_{50}$ values (effective concentration when absorbance is 0.5) are summarized in Figure. 2 and Table 3. In general, lower the $EC_{50}$ values higher the reducing ability. It was observed that the bark crude methanol extract revealed the highest antioxidant activity with $EC_{50}$ 410.1 ± 0.42 µg/ml. On the other hand, hexane crude needle extract showed lowest $EC_{50}$ value 970.25 ± 1.2 µg/ml. This higher reducing power of methanol extract is attributed to the higher extraction efficiency of bioactive phytonutrients.

## Antimicrobial Activity

Antimicrobial activity of *P. roxburghii* extracts was tested against four strains both gram positive and gram negative and the results are summarized in Table 4. The extracts showed a zone of inhibition ranging from 9 to 12.5 mm and compared with standard ampicillin and kanamycin antibiotics. It can be expected that these crude extracts have unique phytochemicals which are responsible for the inhibition of microbial metabolism. Comparison of the antibacterial activity of cone, needle, and bark in four different solvent extracts, it was observed that methanol and acetone crude extracts revealed good antimicrobial activity with a clear zone of inhibition. The cone extracts revealed higher antibacterial activities against *Bacillus substilis* in comparison to needle and bark. On the other hand, needle extract showed relatively lower antibacterial activities in all solvent extracts. Furthermore, hexane and water extracts showed the least activity. It shows that polarity of solvent and compound to be extracted plays a vital role in the extraction of high biologically important compounds. The higher antibacterial activity of methanol and acetone extracts could possibly due to the higher extraction efficiency of

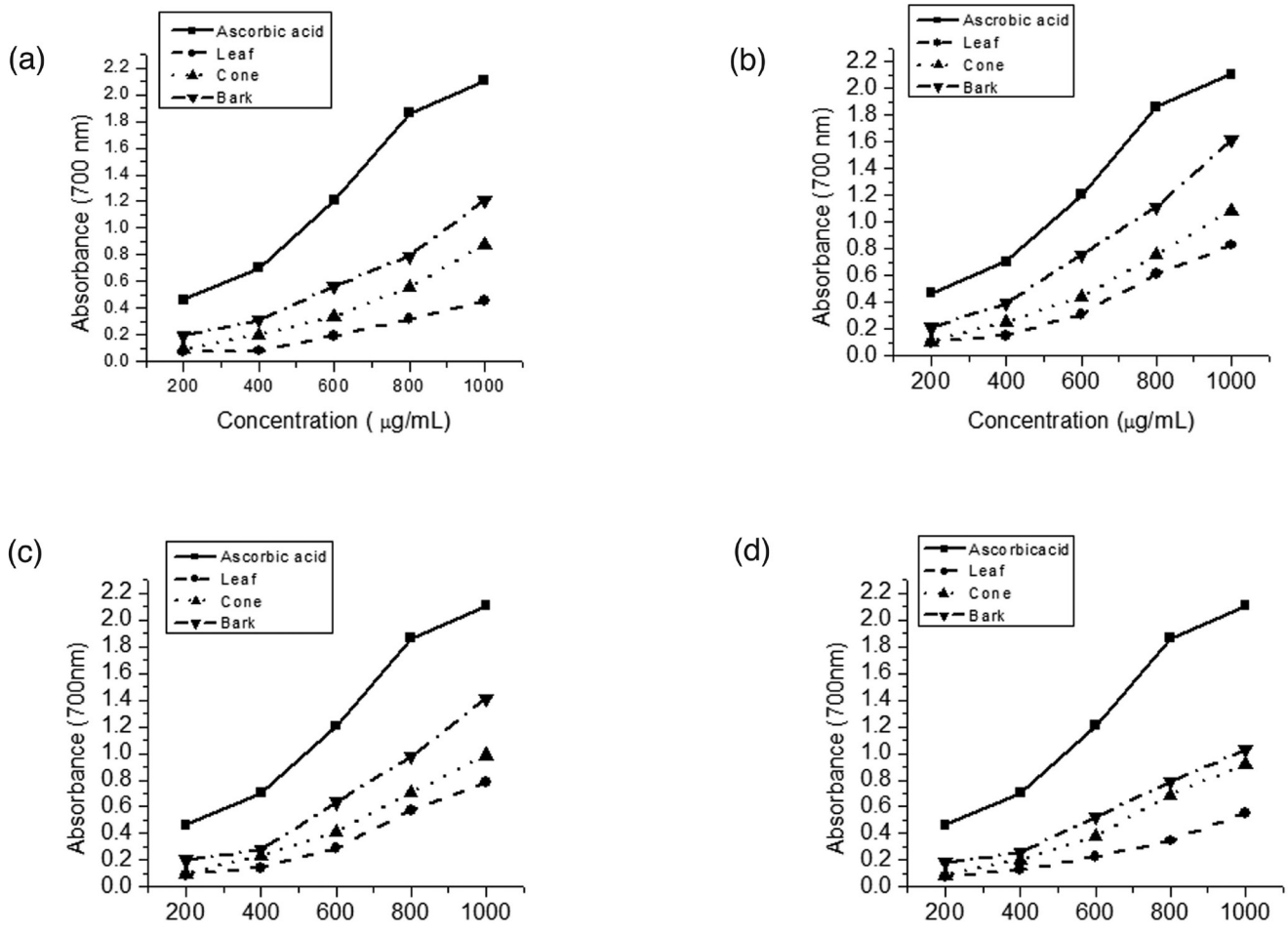


**Figure 2.** Reducing power assay of *Pinus roxburghii*. (a) Water extract, (b) Methanol extract; (c) Acetone extract, and (d) Hexane extract.

polyphenolic and flavonoids compounds. It is well established that polyphenol and flavonoids possess higher antibacterial activities.

## GC-MS Profiling of Chemical Constituents

Gas chromatography-mass spectrometry (GC-MS) analysis of acetone crude extract of *P. roxburghii* bark revealed the presence of 14 different compounds (Fig. 3). The compounds were identified based on the mass fragmentation pattern and the comparing the peak area and retention time of the NIST database. The chemical composition of the crude acetone extract is summarized in Table 5. The most of the compounds are monoterpene and hydrocarbons such as 1,8 cineole, linalool, beta-thujone, chrysantheone, camphor, terpinen-4-ol, *n*-dodecane, *n*-pentadecane, *n*-tetradecance, and *n*-hexadecance. Furthermore, longifolene, diethyl phathalate, and 2-ethylhexanoic acid were also identified through the GC-MS analysis.

## Discussion

Plants produce diverse phytochemicals known as secondary metabolites. It is well known that plants produce these metabolites to protect themselves from pathogenic attacks. These secondary metabolites possess several biological activities such as antimicrobial, antifungal, anticancer, and anti-inflammatory activities [16]. Owing to the biological activities of the plant-derived metabolites, it is of great scientific interest. The different parts of the plants revealed different quantities of these metabolites. The phytochemical analysis of bark, needle, and cone extracted with four different solvents (water, methanol, acetone, and hexane) displayed promising phytonutrients such as flavonoids, phenol, alkaloid, saponin, xanthoprotein, quinone, sterol, and cardiac glycoside. The most of these phytochemicals were extracted with methanol, acetone, and water; however, least were observed with

**Table 4.** Antibacterial activities of *Pinus roxburghii* crude extract. Ampicillin and Kanamycin were used as standard antibiotics, where ND = not detected.

| Solvent | Organism | Zone of bacterial growth inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Antibiotics | | Pinus roxburghii | | |
| | | Ampicillin | Kanamycin | Cone | Bark | Needle |
| Methanol | *Staphylococcus aureus* | 12.0 | 11.5 | 9.0 | 11.5 | 10.0 |
| | *Bacillus subtilis* | 13.0 | 12.5 | 12.5 | 12.0 | 11.5 |
| | *Klebsiella pneumoniae* | 14.0 | 13.5 | 9.5 | 11.5 | 10.0 |
| | *Enterococcus spp.* | 12.5 | 12.5 | 9.0 | 11.5 | 10.0 |
| Water | *Staphylococcus aureus* | 12.0 | 11.5 | 9.5 | 10.0 | ND |
| | *Bacillus subtilis* | 13.0 | 12.5 | 11.0 | 9.5 | ND |
| | *Klebsiella pneumoniae* | 14.0 | 13.5 | ND | ND | ND |
| | *Enterococcus spp.* | 12.5 | 12.5 | 10.0 | 11.5 | 10.0 |
| Acetone | *Staphylococcus aureus* | 12.0 | 11.5 | 10.0 | 11.0 | 9.5 |
| | *Bacillus subtilis* | 13.0 | 12.5 | 10.5 | 11.5 | 10.0 |
| | *Klebsiella pneumoniae* | 14.0 | 13.5 | 10.0 | 11.5 | 9.5 |
| | *Enterococcus spp.* | 12.5 | 12.5 | 9.5 | 11 | 10.0 |
| Hexane | *Staphylococcus aureus* | 12.0 | 11.5 | ND | ND | ND |
| | *Bacillus subtilis* | 13.0 | 12.5 | ND | ND | ND |
| | *Klebsiella pneumoniae* | 14.0 | 13.5 | 9.5 | 10.0 | 9.0 |
| | *Enterococcus spp.* | 12.5 | 12.5 | ND | 10.5 | ND |

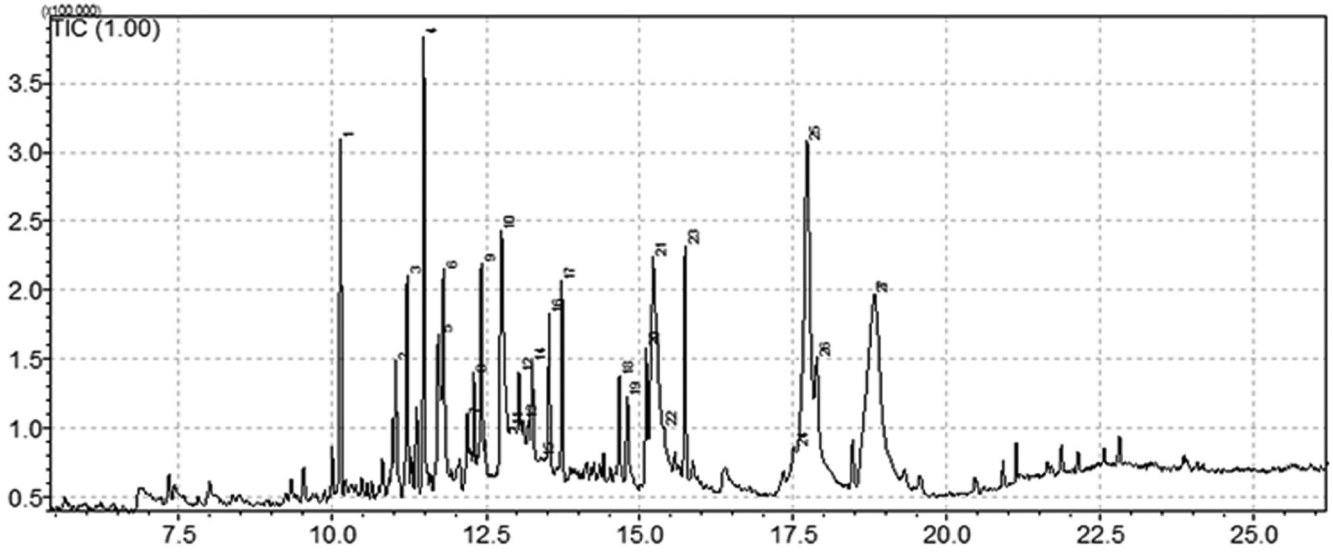


**Figure 3.** GC-chromatogram of the crude acetone extract from *Pinus roxburghii* bark.

hexane crude extracts. Phenolic and flavonoids are the largest category of phytochemicals and the most widely distributed in plants. It has been reported that polyphenol and flavonoid molecules displayed a high radical scavenging activity as well as anti-inflammatory activities [17]. The higher TPC and TFC contents were observed in the bark crude extract followed by cone and needle. Least amounts of TPC and TFC were observed in the needle crude extract. Among the four different solvent extracts, methanol revealed higher amount of TPC and TFC, followed by acetone and water extracts. On the other hand, least amounts of TPC and TFC were observed with hexane crude. This might be due to the poor extraction efficiency of polyphenolic compounds by hexane. Major flavonoid compounds reported from the bark of *P. roxburghii* were quercetin, catechin, kaempferol, rhamnetin, and gallocatechin [18]. Hence, it is justifiable that bark contains the higher amount of total flavonoids.

Reactive oxygen species (ROS) are essential for life of aerobic metabolism. In normal cells, these ROS are neutralized due to the presence of natural defense mechanism in the human body. However, under certain conditions, ROS production exceeds

**Table 5.** GC-MS profiling of chemical constituents of the *Pinus roxburghii* acetone crude extract.

| Chemical compounds | Molecular weight (g/mol) | Retention time (min) | Chemical formula |
|---|---|---|---|
| 1,8-cineole | 154.249 | 10.125 | $C_{10}H_{18}O$ |
| Linalool | 154.250 | 11.025 | $C_{10}H_{18}O$ |
| beta- thujone | 152.237 | 11.208 | $C_{10}H_{16}O$ |
| Chrysanthenone | 150.220 | 11.483 | $C_{10}H_{14}O$ |
| Camphor | 152.230 | 11.800 | $C_{10}H_{16}O$ |
| Terpinen-4-ol | 154.250 | 12.192 | $C_{10}H_{18}O$ |
| n- dodecane | 170.340 | 12.300 | $C_{12}H_{26}$ |
| (Z)-3-hexenyl tiglate | 182.263 | 12.892 | $C_{11}H_{18}O_2$ |
| *n* - pentadecane | 212.420 | 13.092 | $C_{15}H_{32}$ |
| *n*- tetradecane | 198.390 | 13.525 | $C_{14}H_{30}$ |
| *n* - hexadecane | 226.450 | 14.667 | $C_{16}H_{34}$ |
| Longifolene | 204.360 | 15.108 | $C_{15}H_{24}$ |
| Diethyl phthalate | 222.240 | 17.517 | $C_{12}H_{14}O_4$ |
| 2-ethyl hexanoic acid | 144.210 | 18.817 | $C_8H_{16}O_2$ |

the natural ability of cells to eliminate them from the organism; hence, leads to oxidative stress and causes several diseases such as cancer [19]. To prevent such deleterious action of ROS, antioxidants come in action which scavenges these radicals. DPPH radical scavenging model is a widely used method to determine the antioxidant activity of plants natural products. Our study revealed the highest antioxidant activity of bark extracted with methanol. This might be due to the presence of high polyphenolic and flavonoid contents in *P. roxburghii* bark crude extract [20]. This higher radical scavenging activity reveals *P. roxburghii* as promising natural source of antioxidants and opens new insight for exploitation of its secondary metabolites for medication purposes. Furthermore, antioxidant activity of the extract was confirmed through the reducing power assay. Reducing agent causes the reduction of the $Fe^{3++}$/ferricyanide complex to the ferrous with color changing to green and blue indicating the reducing ability of the extract. Reducing ability of bark crude methanol extract was found to be highest in comparison with the acetone, hexane, and water extracts. This higher value of reducing power indicates its higher antioxidant activity. Both DPPH and reducing assay revealed bark methanol extract as potent antioxidant agents.

One of the objectives of our research was to investigate the chemical constituents of *P. roxburghii*. Since bark extract revealed the higher antioxidant activity hence we investigated the volatile components of the bark acetone extracts using GC-MS. GC-MS chromatogram revealed the presence of different chemical constituents constituents that is eluted as a function of retention time. Although chromatogram revealed 28 different peaks, we were able to identify 14 different compounds through the careful analysis of the mass fragmentation patterns and NIST library data analysis. The compounds 1,8 cineole, linalool, beta-thujone, chrysanthenone, camphor along with *n*-dodecane, *n*-pentadecane, *n*-tetradecane, *n*-hexadecane, and longifolene have been identified in the bark crude extract. The identified compound terpinen-4-ol were were reported to have antibacterial and antifungal activities. Although essential oils from *P. roxburghii* have been researched elsewhere in the world, there are little information available in the chemical constitutens extracted with different solvents. Our results shed light that bark extract contained several biologically important compounds.

Antimicrobial activites of various plants extracts are being researched in many parts of the world in search of nartural compounds as a potential source of antimicrobial agents. In this study, needle, cone, and bark acetone and methanol crude extracts revealed higher antimicrobial activities in comparision to water and hexane indicating that most of the bioactive constituents are extracted with methanol and acetone as a extracting solvent. The results were compared with standard antibiotic ampicillin and kanamycin. The presecne of bioactive flavonoid, phenolic compounds as well as terpenoid may be responsible for the biological activities.

## Conclusion

The present study revealed that *P. roxburghii* needle, cone, and bark are the potential source of diverse bioactive phytonutrients. This is supported by the promising antioxidant activity and antimicrobial activity of the crude extract. Our results also showed that *P. roxburghii* bark, cone, and needle contain a significant amount of flavonoids and phenolic contents. Our analysis further revealed that the bark methanolic extract contained the highest amount of TFC, TPC contributing to greater antioxidant and reducing power activity compared to cone and needles. This encourages the use of bark as a potential source of various phenols and flavonoids for medical application, food industry as well as to the cosmetic product. Furthermore, GC-MS profiling of the bark extract revealed the presence 14 different compounds consisting of monoterpene, hydrocarbon, and ester compounds. Our findings suggest that *P. roxburghii* is the huge source of bioactive compounds. Plenty of rooms left to investigate the potential bioactive flavonoids and phenolic compounds and its impact on human health.

## Acknowledgement

The authors are grateful to Department of Chemical Science and Engineering, Kathmandu University for the support to carry out this research. We also like to extend our gratitude to Department of Microbiology, School of Medical Science, Kathmandu University. Furthermore, we also like to acknowledge Mrs. Jyoti Joshi, Deputy Director General and Mr. Kesav Paudel, Department of Plant Resource, Government of Nepal for research support.

## Competing Interests

The authors declare that they have no competing interest.

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