Phytochemical and biological evaluation and isolation of quercetin from hypericum cordifolium flower

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Abstract: *Hypericum cordifolium* is a medicinal plant used in the treatment of various ailments in Nepalese traditional medicine. The medicinal values and bioactive constituents of *Hypericum perforatum* have been well investigated and is used as an alternative medicine in the treatment of depression, cancer, inflammation, bacterial and viral infections. However, scientific reports on Nepalese *H. cordifolium* are very limited. Here we report about the chemical screening of extracts, quality analysis of *H. cordifolium* extract by thin layer chromatography, TLC using *H. perforatum* extract and hyperoside as the standards, estimation of phenolics, flavonoids, tannins and sugars as well as antioxidant, antibacterial and cytotoxic activities of flower extracts. The ethyl acetate extract was fractionated and some phytochemicals were isolated. In our findings, TLC analysis of *H. cordifolium* extract showed the presence of chlorogenic acid, hyperoside and quercetin. Hypericine and rutin were not detected which indicated that the chemotaxonomy of *H. cordifolium* is different from *H. perforatum*. In phytochemical analysis, ethyl acetate extract showed the greater amounts of flavonoids, methanol extract showed the greater amounts of tannins and sugars and 50% aqueous methanol extract showed the greater amounts of phenolics. In DPPH free radical scavenging assay, ethyl acetate (18.79±0.98 µg/ml) and 50% aq. methanol (18.98±0.81 µg/ml) extracts showed the lower IC<sub>50</sub> values than the methanol extract (27.00±1.03 µg/ml) due to the presence of high amounts of flavonoids and phenolics in these extracts. In antibacterial assay, all extracts showed moderate antibacterial activity against *Staphylococcus aureus* except 50% aqueous methanol extract. In cytotoxicity assay on human bladder carcinoma cell line 5637, ethanol extract showed IC<sub>50</sub> value of 41.35±5.01 µg/ml. Quercetin was isolated from ethyl acetate extract and characterized by UV and FTIR spectroscopy. The present findings indicated that the flowers of *H. cordifolium* could be the rich source bioactive compounds with antibacterial, cytotoxic and antioxidant activities.

Keywords: Antioxidant; Antibacterial; Cytotoxicity; Phytochemical analysis.

Introduction

The genus *Hypericum* (Hypericaceae) comprises 484 species, distributed in a variety of habitats from tropical, temperate to alpine regions. Many species of *Hypericum* are reported from Nepal. Among them, *H. cordifolium* is the most abundant species distributed around Kathmandu valley at an altitude between 900-2000 m on open slopes. Locally it is called Areli, Areto or Ghod Jatra Phool as it is offered in the religious places in Ghoda Jatra festival. The yellow flowers bloom from March to April. In Nepalese traditional medicine, the juice of *H. cordifolium* is given to menstrual disorder, root juice is used to treat diarrhea, bark juice is applied in the case of back pain and dislocation of bone, flower paste, fresh or dried is eaten with warm water to cure dysentery. The fresh young shoot is eaten as medicine to relieve throat pain and root and flower juice is used to treat fever, pneumonia, diarrhea, dysentery, cough and cold. In our previous study, we have screened the flower extracts for wound healing activities. It was also included in antiviral screening. The leaf extracts were screened for cytotoxicity against Brine shrimps and total phenolic, flavonoid and antibacterial activities were evaluated.
Among the different species of *Hypericum*, *H. perforatum* L. commonly known as St. John’s wort is one of the thoroughly investigated species because of its wide medicinal values. Extracts of this plant are applied to treat minor burns, wounds, inflammation of the skin and nerve pain externally while internally the extracts are used for the treatment of anxiety and depressive episodes. It is used widely in Germany for the treatment of depression as an alternative medicine and prescribed approximately 20 times more often than fluoxetine chloride. It is also sold in Europe and North America to treat mild to moderate depression. Besides its antidepressant activity, several additional pharmacological effects such as anti-inflammatory, hepatoprotective, antiviral, antimicrobial, antioxidant, antitumoral and wound-healing activities have been reported due to the presence of phytochemicals like naphthodianthrones, acyl-phloroglucinols, flavonoids, phenolic acids and xanthones.

The quality and quantity of *Hypericum* secondary metabolites are responsible for their pharmacological activities and they depend on ecological and environmental conditions where they grow as well as physiological and genetic factors. *H. cordifolium* of Nepalese origin that grows in unique climatic condition is still under explored. Therefore, the aim of the present investigation is the quality analysis of flower extract by TLC, quantification of phytochemicals in different flower extracts, isolation and characterization of phytochemicals by UV, FTIR spectroscopy and evaluation of antioxidant, cytotoxic, antibacterial activities of different flower extracts.

### Materials And Methods

#### Plant materials

The plant, *H. cordifolium* was collected from the pine forest of Nagarkot area of Bhaktapur district in April 2021. It was authenticated by comparison with the herbarium species deposited at Central Department of Botany, Tribhuvan University. The voucher specimen (HC-21-MR) was deposited at Research Center for Applied Science and Technology, Tribhuvan University. The collected materials were separated into flowers, leaves and stems, shade dried separately and crushed to fine pieces and stored in an air tight container for further use.

#### General experimental procedure

All the solvents and chemicals used were of analytical grade and purchased from local vendor. Gallic acid and precoated Silica gel 60 GF were purchased from Merck, Daenemstadt, Germany, (+)-catechin and DPPH was purchased from Sigma Chemical Company, USA. Sephadex LH-20 was purchased from Pharmacia Biotech, Uppasala, Sweden. Absorbance was measured using Chemito UV-VIS Spectrophotometer. UV spectrum was measured on SHIMADZU UV-VIS UV-1900i spectrophotometer. FTIR was measures using High performance SHIMADZU Fourier Transform Infrared Spectrophotometer.

#### Extraction

The crushed flower of *H. cordifolium* (50 g) was extracted successively with hexane (300 ml) dichloromethane (300 ml), ethylacetate (200 ml) and methanol (200 ml) by using Soxhlet extractor. The remaining residue after extraction with methanol was dried and refluxed with 50% aqueous methanol (100 ml) for 2 hours then allowed to cool and filtered. The solvents were evaporated under reduced pressure using rotavapour. The concentrated solid or semi-solid mass was kept in freeze for further use. Similarly, 100 mg flower was percolated with 2 ml ethanol at room temperature for 24 hours. The solvent was evaporated and the dried extract was used for the cytotoxicity assay as well as for TLC analysis.

#### Phytochemical screening

The presence of different classes of natural constituents in different extracts were analyzed by using different specific reagents according to the standard protocol.

#### Quality analysis of ethanol extract

Extracts of *H. cordifolium* and *H. perforatum* (each 50 mg/ml in ethanol) were prepared and the standard hyperoside solution (1 mg/ml ethanol) was prepared for TLC analysis. The extracts were visualized in day light. The extracts and hyperoside were loaded and the TLC plate (Silicagel GF) was developed with the mobile phase, EtOAc-MeOH –H₂O (100:13.5:10 v/v/v) and spots were visualized under UV light at 254. After that, the plate was sprayed with 1% methanolic diphenyl borylxy ethylamine followed by 5% ethanolic polyethylene glycol, incubated for 30 minutes and visualized under 366 nm.
**Estimation of total phenolic content**

The total phenolics content in different extracts was estimated by colorimetric method using Folin-Ciocalteu reagent\(^1\). The diluted extract (1 mL) was mixed with 5 mL of Folin-Ciocalteu reagent (10% v/v) and 4 mL of sodium carbonate (7% w/v). The mixture was shaken and incubated for 30 min at 40°C in a water bath before absorption was measured at 760 nm. Total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram extract (mg GAE/g extract). All extracts were analyzed in triplicate. This method gives a general measurement of phenolic content, as it is not completely specific for phenolic compounds and not all phenolic compounds exhibit the same level of activity in the assay (Öztürk et al, 2009).

**Estimation total flavonoid content**

The total flavonoid content in different extract was estimated by Aluminium chloride colorimetric assay\(^1\). The diluted extract (1 mL) was mixed with 4 mL of double distilled water and 0.3 mL of \(\text{NaNO}_2\) (5 % w/v). After 5 minutes, 0.3 mL of \(\text{AlCl}_3\) (10% w/v)and after 1 minute 2 mL of 1 M NaOH was added. The total volume of the mixture was made up to 10 mL by the addition of 2.4 mL double distilled water and mixed thoroughly. Absorbance of the pink color mixture was determined at 510 nm. Total flavonoids content was expressed as catechin equivalents (CE) in milligrams per gram extract (mg CE/g extract). All extracts were analyzed in triplicate.

**Estimation total tannin content**

The total tannin content in different extracts was estimated by using Folin-Ciocalteu method\(^2\). The diluted extract (1 mL) was mixed with 8.4 mL of double distilled water, 0.5 mL of FC reagent (10% v/v) and 0.1 mL of sodium carbonate solution (7% w/v). The mixture was then shaken well and the allowed to stand for 30 minutes and then absorbance was taken at 700 nm. Total tannins content of the extracts was expressed as mg tannic acid equivalents (TAE) per gram of dry extract (mg/g). All extracts were analyzed in triplicate.

**Estimation total sugars content**

The total carbohydrate/sugar content indifferent extracts was estimated by colorimetric assay using anthrone reagents\(^3\). The diluted extract (2 mL) was mixed with 8 mL of freshly prepared anthrone reagent (200 mg of anthrone in 100 mL ice-cold 95% conc. \(\text{H}_2\text{SO}_4\)). The mixture was shaken well and heated for 8 minutes at boiling water bath. Then cooled rapidly and the absorbance was measured at 630 nm. Total sugar content of the extracts was expressed as mg glucose equivalents (GE) per gram of dry extract (mg/g). All extracts were analyzed in triplicate.

**Determination of antioxidant activity**

Antioxidant activity of extracts was assessed using DPPH free radical\(^4\). A mixture of 2.5 ml of DPPH (0.10 mM in methanol) and 0.5 ml of extract was kept in the dark for 30 min. Its absorbance was then read at 517 nm. A control was prepared by mixing 0.5 ml methanol and 2.5 ml 0.1 mM methanolic DPPH solution. DPPH radical scavenging activity (%) was calculated as follows:

\[
\text{% of radical scavenging} = \frac{A_c - A_s}{A_c} \times 100
\]

Where, \(A_c\) = Absorbance of control and \(A_s\) = Absorbance of solution.

\(\text{IC}_{50}\) values is the concentration of sample required to scavenge 50% of DPPH radical. It was calculated from the plotted graph of radical scavenging activity against the concentration of extracts. The percentage scavenging was then plotted against concentration and \(\text{IC}_{50}\) value of the extracts determined graphically.

**Determination of cytotoxic activity**

The ethanol extract of \(H.\ cordifolium\) was evaluated against Human bladder carcinoma cell line 5637 (ATCC HTB-9) cultured in RPMI (Roswell Park Memorial Institute) 1640 medium with 10% FCS (fetal calf serum). Cell viability was analyzed using the colorimetric WST-8 (water soluble tetrazolium salt-8) Cell Viability kit from Promokine (PromoCell GmbH, Heidelberg, Germany). Briefly 100 µl of a cell suspension was seeded into 96 well microtiter plate with 3000 cells/well. After twenty-four hours of incubation at 37°C, the cells were treated with ethanol extract of \(H.\ cordifolium\) of different concentrations (250, 125, 62.5, 31.25 and 15.625 µg/ml) and incubated for next 72 hours. Etoposide was used as a positive control and DMSO was used as a solvent control. At the end of the incubation time, the medium was removed and the cells were washed with PBS and
stained with 10 µl WST-8 solution and again incubated for four hours at 37 °C. All experiments were carried out twice with six replicates for each tested concentration. The absorbance was measured at 450 nm with an ELISA plate reader. The percentage growth values were calculated by the equation,

\[ \text{Percentage Growth} = \frac{\text{OD}_t - \text{OD}_c,0}{\text{OD}_c - \text{OD}_c,0} \times 100 \]

where OD<sub>t</sub> is the mean absorbance of the treated cells, OD<sub>c</sub> is the mean absorbance of the control, OD<sub>c,0</sub> was the mean absorbance at the time the extract was added. The IC<sub>50</sub> value was calculated by linear regression using MS Excel.

**Determination of antimicrobial activity**

The antimicrobial activity of the extracts were evaluated by agar well diffusion method<sup>23</sup> against one Gram positive bacteria *Staphylococcus aureus* (ATCC 25923), two Gram negative bacteria *Escherichia coli* (ATCC 25922) and *Klebsiella pneumonia* (ATCC 14028) and an yeast *Candida maltosa* by agar well diffusion method. An aliquot of 10 µL of sample of each concentration (200 mg/mL prepared in 50% DMSO) was introduced into each well (diameter 4 mm) so that the exact amount of extracts in each well were 2 mg. Negative control experiments were performed using equivalent volume of 50% DMSO and positive control experiments were performed by use of a standard antibiotic, neomycin (10 µg/well). At the end of the incubation period, the clear inhibition zones of bacterial growth around the wells were observed in the presence of different extracts. Inhibition of the bacterial growth in the presence of extracts was measured in the form of zone of inhibition (ZOI).

**Fractionation of ethyl acetate extract**

The ethyl acetate extract (2 g) was chromatographed on a sephadex LH-20 column (18cm×0.9cm I. D, filled with 50 g Sephadex) and eluted with methanol. Sub-fractions of 4-5 mL were collected. Each sub-fraction was monitored by TLC (Silica gel GF<sub>254</sub>) using solvent system, toluene-ethyl acetate-formic acid (5:4:1 v/v/v). The sub-fractions were pooled into four major fractions, F-4-A, F-4-B, F-4-C and F-4-D on the basis of TLC character. The fractions F-4-A and F-4-D showed single spot on TLC. They were purified by re-crystallization and purity of F-4-D was checked by TLC in solvent systems, ethylacetate-methanol-water, EMW (100:13.5:10) chloroform-acetic acid-water, CAW (10:9:1) and toluene-ethylacetate-formic acid, TEF (10:8:1). The UV and FTIR spectra were recorded.

**Results**

**Extractive values in different solvents**

Extraction with solvents of different polarities provided different amounts of extracts. The yields of the various extracts are shown in Table 1. The yield depends on the nature of the solvent applied. The highest amount of extract was obtained with methanol. As methanol is a highly polar solvent, the hot methanol extracted almost all polar compounds.

**Phytochemical screening**

The results of the phytochemical screening of the hexane, dichloromethane, ethyl acetate, methanol and 50% aqueous methanol extracts of flowers of *H. cordifolium* are shown in Table 1 which revealed that all the tested phytochemicals were absent in hexane extract. As hexane is a non-polar solvent, it can extract only non-polar compounds like hydrocarbons and fatty acids. In our screening, dichloromethane extract showed the presence of phenolics. It is well known that the *Hypericum* species are rich in acylphloroglucinols<sup>24</sup> which are non-polar phenolic compounds. Only terpenoids, phenolics, flavonoids and tannins were present in ethyl acetate extract. In methanol and 50% aqueous methanol extracts, in addition to terpenoids, phenolics, flavonoids and tannins, phytochemicals like saponins, glycosides and reducing sugars were also present. The results of chemical screening of the extracts are in agreement with the results reported for other species of *Hypericum*<sup>25,26</sup> with phenolics and flavonoids were the main constituents.
Table 1: Yield and screening of *H. cordifolium* extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Hexane</th>
<th>CH₂Cl₂</th>
<th>EtOAc</th>
<th>MeOH</th>
<th>50% aq. MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yields in g</td>
<td>1.92</td>
<td>4.92</td>
<td>3.26</td>
<td>9.35</td>
<td>2.00</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R.Sugars</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Quality of analysis of *H. cordifolium* and *H. perforatum* ethanol extracts

There was a visual difference between two extracts. The extract of *H. perforatum* looked reddish brown and *H. cordifolium* looked pale yellow (Fig 1 A). The presence of chlorogenic acid (blue fluorescence spot, R₀.12), hyperoside (yellow spot, R₀.46) and quercetin (yellow spot, R₀.75) in ethanol extract of *H. cordifolium* was confirmed by comparing the TLC with standard hyperoside along with *H. perforatum* extract which is known to contain chlorogenic acid, hyperoside and quercetin. Hypericin, a red fluorescence spot (R₀.62) was not detected in *H. cordifolium* extract. The TLC plates visualized under UV lamp at 254 nm and after spraying with 1% methanolic diphenyl boryloxy ethylamine followed by 5% ethanolic polyethylene glycol and visualized under 366 nm are shown in Fig 1 B and C.

Phytochemical information is necessary to assess the quality of the herb. Presence of hypericin and pseudohypericin result in red solutions with organic solvents²⁷. The present study intended to determine the quality of *H. cordifolium* ethanol extract by simple and quick TLC techniques which allowed the detection of key marker compounds. For routine quality control, detection of major compounds seems to be sufficient. In TLC, the phytochemical compositions of two species were found to be different (Fig 1B, C). According to the European Pharmacopoeia 8.0 Hyperici herba monograph²⁸, on chromatogram, blue band of chlorogenic acid, two yellow bands of rutin and hyperoside, two red bands of pseudohypericin and hypericin and a yellow band of quercetin have to be visible. Absence of hypericin in indicated that the quality of *H. cordifolium* is different from *H. perforatum*. European Pharmacopoeia²⁹ specifies that the total hypericin content of Hyperici herba should be 0.08%, the flavonoids should be minimum 6% expressed as rutoside and hyperforin should be maximum 6%. Hypericin is present only in those *Hypericum* species whose aerial parts bear dark glands³⁰ and the level of accumulation depends on species and plant parts. It was reported that in many *Hypericum* species like *H. calycinum*, *H. cardiophyllum* and *H. xylosteiifolium*, hypericin was absent³⁰. However, the TLC assay only provides qualitative information about the phytochemicals.

Total phenolic content

The total phenolic content in different extracts was calculated from regression equation of calibration curve (Y = 0.011x + 0.021; R² = 0.994) and was expressed as mg of GAE per gram of dry extract. The TPC values ranged from 158.22 (dichloromethane extract) to 228.19 (50% aq. methanol extract) mg GAE/g dry extract. The ethyl acetate extract (227.17 mg GAE/g extract) and 50% aqueous methanol extracts contained nearly the same amounts of phenolics. The results are shown in Table 2.

![Figure 1](image-url)
Total flavonoid content

The total flavonoid content in different extracts was calculated from the regression equation of calibration curve \( Y = 0.002x + 0.002; R^2 = 0.999 \) and expressed as mg catechin equivalent (CE) per gram of dry extract. The total flavonoid content ranged from 227.4 (50% aq. methanol extract) to 306.41 (ethyl acetate extract) mg CE/g dry extract. However, methanol extract contained nearly the same amounts of flavonoids (231.5 mg CE/g extract) as 50% aq. methanol extract. The total flavonoid content of plant extracts are shown in Table 2.

It was reported the total phenolic content of the methanol extract of the aerial parts of \( H. \) foliosum was 174.13±2.90 mg GAE/g extract\(^31\). The ethanol-water extract of the flowers of \( H. \) perforatum contained total phenolics 371±49 mg GAE/g extract and total flavonoids 160±7 mg CE/g extract\(^32\). From the reported data on other species, it was found that the extracts of \( H. \) cordifolium contained relatively greater amounts of phenolics and flavonoids compounds.

Total tannin content

The total tannin content in different extracts was calculated from the regression equation of calibration curve \( y = 0.003x + 0.008; R^2 = 0.996 \) and expressed as mg tannic acid equivalent (TAE) per gram dry extract. The total tannin content in different extracts ranged from 278.08 (50% aq methanol extract) to 621.32 (methanol extract) mg TAE/g dry extract. The results are shown in Table 2.

Table 2: Total phenolic, flavonoid, tannin and sugar in various extracts, antioxidant and cytotoxic activity.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>CH(_2)Cl(_2)</th>
<th>EtOAc</th>
<th>MeOH 50% aq. MeOH</th>
<th>EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content (mg GAE/g dry extract) (Mean ± S.D) (n=3)</td>
<td>158.22 ± 0.74</td>
<td>227.17 ± 0.736</td>
<td>199.28 ± 0.576</td>
<td>228.19 ± 0.639</td>
</tr>
<tr>
<td>Total flavonoids content (mg CE/g extract) (Mean ± S.D) (n=3)</td>
<td>-</td>
<td>306.41 ± 0.954</td>
<td>231.5 ± 0.935</td>
<td>227.4 ± 0.993</td>
</tr>
<tr>
<td>Total tannins content (mg TAE/g extract) (Mean ± S.D) (n=3)</td>
<td>-</td>
<td>298.72 ± 0.89</td>
<td>621.32 ± 0.537</td>
<td>278.08 ± 0.712</td>
</tr>
<tr>
<td>Total sugar content (mg GE/g extract) (Mean ± S.D) (n=2)</td>
<td>-</td>
<td>-</td>
<td>178.08 ± 0.712</td>
<td>105.71 ± 0.531</td>
</tr>
<tr>
<td>IC(_{50}) µg/ml against DPPH free radical assay (Mean ± S.D) (n=2)</td>
<td>-</td>
<td>18.79±0.98</td>
<td>27.00±1.03</td>
<td>18.98±0.81</td>
</tr>
<tr>
<td>IC(_{50}) µg/ml against bladder carcinoma (Mean ± S.D) (n=2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41.35 ± 5.01</td>
</tr>
</tbody>
</table>

DPPH free radical scavenging activity

In DPPH radical scavenging assay, ethyl acetate and 50% aqueous methanol extracts showed nearly similar IC\(_{50}\) values (18.79 and 18.89 µg/ml) and methanol extract showed somewhat higher IC\(_{50}\) value (27.0 µg/ml) but these values are relatively higher than the IC\(_{50}\) value of ascorbic acid (11.0µg/ml). The results are presented in Table 2. The IC\(_{50}\) values of ethyl acetate (2.63±0.01
µg/ml), methanol (3.63±0.01 µg/ml) and methanol-water (4.71±0.04 µg/ml) extracts of *H. perforatum* in DPPH free radical scavenging assay were reported. In general, extracts with higher radical scavenging and antioxidant activities showed a higher phenolic content. This is in agreement with our findings and a strong correlation was observed between the phenolics and flavonoids content and antioxidant activities.

**Cytotoxic activity**

In cytotoxicity assay, it was observed that the ethanol extract at 250, 125, 62.5, 31.25 and 15.625 µg/ml concentrations significantly decreased the viability of human bladder carcinoma cell line 5637 after 72 hours. Etoposide, a semi-synthetic derivative of podophyllotoxin from the rhizome of *Podophyllum peltatum* was used as a positive control. The methanol extract showed the IC\(_{50}\) value of 41.35 ± 5.01 µg/ml whereas the standard etoposide has IC\(_{50}\) value of 0.67±0.03 µM.

It is well known that *Hypericum* species are rich in acylphloroglucinols which showed significant cytotoxicity on different cancer cell lines. Hyperforin inhibits tumor cell growth in vitro. It was reported that phenols, anthraquinonoids, xanthones, benzophenone, phloroglucinol and flavonoids isolated from *H. sampsonii* showed selective cytotoxic activities against cancer cells. The cytotoxicity of ethanol extract of *H. cordifolium* could be due to the presence of various classes of phytochemicals as shown by chemical screening.

**Antimicrobial activity**

In antibacterial assay, all the tested extracts showed only narrow spectrum of activities. Except 50% aq. methanol extract, other extracts showed weak to moderate antibacterial activity against *S. aureus* with inhibition zone ranged from 9-18 mm. The methanol extract also showed moderate activity against *E. coli*. The tested extracts did not show activity against *K. pneumonia* and *C. albicans*. The results are shown in Table 3.

It was reported that the main antibacterial component from *H. perforatum* was hyperforin. *H. perforatum* and other species have been investigated for their antimicrobial activity and found that the extract was active against gram-positive bacteria but not against

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**Table 3. Antibacterial activity of extracts.**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Bacteria</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td><em>S. aureus</em></td>
<td>17.0 mm</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td><em>S. aureus</em></td>
<td>9.0 mm</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td><em>S. aureus</em></td>
<td>18.0 mm</td>
</tr>
<tr>
<td>Methanol</td>
<td><em>E. coli</em></td>
<td>14.0 mm</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>14.0 mm</td>
</tr>
<tr>
<td>Ampicilin</td>
<td><em>S. aureus</em></td>
<td>33.0 mm</td>
</tr>
<tr>
<td>10 µg/well</td>
<td><em>E. coli</em></td>
<td>14.0 mm</td>
</tr>
</tbody>
</table>

**Characterization of compounds**

Repeated column chromatograph of the ethylacetate extract over Sephadex LH 20 yielded fraction F-4-A, yellow amorphous solid (A). It was purified by re-crystallization. It showed single spot on TLC (Rf \(0.57\) in TEF). The UV spectrum showed the absorption bands at 268 and 340 nm (Fig 2). This is the characteristics UV-VIS absorption profile of flavonoid structure. The FTIR spectrum showed the broad peak between 3400-3000 cm\(^{-1}\), a strong peak at 1650 cm\(^{-1}\) and a peak at 1600 cm\(^{-1}\).

**Figure 2:** UV spectrum of compound A (above) and compound D (below).
Similarly, fraction F-4-D yielded yellow amorphous solid (D). It was purified by recrystallization and purity was checked by TLC, R, 0.87 in CAW; 0.46 in TEF and 0.76 in EMW. The UV spectrum showed the absorption bands at 255 and 375 nm (Fig 2). The FTIR spectrum showed the broad peak between 3400-3000 cm⁻¹, a strong peak at 1650 cm⁻¹ stretching and a peak at 1600 cm⁻¹.

The UV-VIS absorption spectrum of both compounds were characteristic of flavonoid structure. In compound D, Band I, which appeared at 375 nm is considered to be associated to the B-ring cinnamoyl system. Band II, which appeared at 255 nm is caused by electron transition of benzoyl group of A-ring. These λmax values and IR stretching frequencies with λmax values, λ 35-46. Thus, by comparing the Rf values, λmax values and IR stretching frequencies with quercetin, compound D was identified.

Conclusions

The quality of *H. cordifolium* ethanol extract was determined by simple and quick TLC technique which allowed the detection of key marker compounds. The phytoconstituents of *H. cordifolium* was found to be different from *H. perforatum*. Estimation of the contents of total phenolics, flavonoids, tannins and sugars indicated the extracts are rich source of these phytochemicals. Evaluation of antioxidant, cytotoxicity and antibacterial activities revealed that the extracts exhibited good biological activities and supports the traditional use of *H. cordifolium* in the treatment of infections and inflammation. Isolation of some phytochemicals and characterization of quercetin from the ethyl acetate extract indicated that *H. cordifolium* could be the potential source of bioactive compounds. As there is a great chemical diversity, detailed phytochemical investigation on *H. cordifolium* of Nepalese origin is necessary.

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