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## Phytochemical analysis, antioxidant and antibacterial efficacy of methanol and hexane extract of *Centella asiatica*

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### ABSTRACT

*Centella asiatica* is well known for its anti-inflammatory, anticancer, antioxidant, antimicrobial, analgesic, diuretic properties. Its hexane and methanol extracts were screened for the presence of phytoconstituents as well as their antibacterial and antioxidant activity. The phytochemical screening showed the presence of carbohydrate, glycosides, flavonoids, phenolic compound, alkaloids, and saponins. The methanol extract showed more effective antibacterial activity against *Salmonella typhi*. Antioxidant activity of methanol extract was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity showed potent antioxidant activities with IC<sub>50</sub> value 2.57 µg/mL slightly higher than standard ascorbic acid (IC<sub>50</sub> = 3.74 µg/mL). The present study indicates that the tested plant can be an important source of antibacterial agents and recommends that the active phytoconstituents be isolated, identified, and screened individually for activities and also subjected further for *in vivo* and toxicological studies.

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## 1. Introduction

*Centella asiatica* (family Umbelliferae) commonly referred to as water pennywort or Indian pennywort or Asiatic pennywort or Ghodtapre in Nepal. It is a common perennial herbaceous creeper flourishing abundantly in moist areas and distributing widely in tropical and subtropical countries including Bangladesh, Nepal, South Africa, India etc [1]. The plant of *Centella asiatica* is a quite aromatic, prostrate, perennial, stoloniferous, creeper herb, which height up to 15 cm. Stem is striated, glabrous, rooting at the nodes. Several pharmaceutical

companies are utilizing such plant based formulations in treatment of various diseases and disorders world around [2].

A large number of people in developing countries depend on medicinal plants as their primary source of medication [3]. *Centella asiatica* has been used by Ayurvedic medical practitioners for almost 3000 years [4] to treat wounds, mental and neurological disorders, atherosclerosis, microbial infections, and cancer [5]. Traditionally it is used to treat a broad range of diseases such as diarrhea, hepatitis, measles, toothache, syphilis, leucorrhoea etc [6]. It also possesses ulcer-preventive [7, 8a, b],

antioxidant, and antidepressive effects and improves venous insufficiency [9, 10]. Pittella reported that *Centella asiatica* prevents the oxidative damage that takes place in neuropathological disorders including stroke, Parkinson's disease and Alzheimer's disease by increasing the antioxidant neurological state related to aging [11]. Therefore, this study was conducted to investigate the bioactive compounds of methanol and hexane extracts, their antioxidant and antibacterial activities against different strains of bacteria.

## 2. Materials and Methods

### 2.1 Plant materials

The plants (*Centella asiatica*) were collected from Hamsapur, Gorkha District, Western Development Region of Nepal in June, 2018 and authenticated by Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. The collected plants were washed, air dried in shade for about a month then ground to powder and stored in clean plastic bag.

### 2.2 Chemicals and equipment

DPPH, Ascorbic acid and Ofloxacin were purchased from Merck, Darmstadt, Germany. DMSO, methanol, n-hexane etc. are of analytical grade reagent were brought from Thermo Fisher Scientific India Pvt. Ltd. (India). Spectrophotometer [SL177] was used for the absorption for DPPH assay.

### 2.3 Extraction

The dried and powdered form of whole plant (65 g) were extracted successively with hexane (300 mL, 8 h), methane (300 mL, 8 h) by Soxhlet extraction. The extracts were concentrated by rotary evaporator and solid and semi-solid mass obtained was kept in freezer for further analysis.

### 2.4 Phytochemical screening

The freshly prepared crude extracts were subjected to analyze the presence of main classes of phytoconstituents in hexane and methanol extract using standard protocol [12].

### 2.5 Antibacterial screening

The antibacterial assay of the crude extract of *Centella asiatica* was carried out by agar well diffusion method. Effectiveness of antibacterial substance was evaluated by determination of zone of inhibition (ZOI) as given in Ref [13, 14]. The microbial strains *staphylococcus aureus cocci* ATCC 25923 (gram-positive) and *Escherichia coli* ATCC 25922 and *Salmonella typhi* (gram-negative) bacteria were obtained from MED-MICRO Nepal Lab, Kathmandu. The 50  $\mu$ L of the working solution of the plant extract, DMSO as negative control (NC) and 25  $\mu$ L of Ofloxacin (antibiotic- ear and eye drop) as positive control (PC) at the same time in separated well (6 mm) were loaded into the respective wells with the help of micropipette. The plates were incubated overnight at 37 °C. After 24 hours of incubation, the plates were observed for the presence of inhibition of bacterial growth that indicated by a clear zone around the wells. The size of the zone of inhibition was measured and the antibacterial activity expressed in term of the average diameter of zone of inhibition in millimeters.

### 2.6 Antioxidant activity

Antioxidant activity of the methanol extract was assessed using DPPH free radical [15, 16]. Initially, 10 mg of the sample to be tested was dissolved in 10 mL methanol to get the stock solution of concentration of 1 mg/mL (1000  $\mu$ g/mL). Different concentrations of test samples of 20, 40, 60, 80 and 100  $\mu$ g/mL were made from stock solutions. Then 2 mL of all the concentration of test solution 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 minutes in dark place at room temperature. The control was prepared as above but without the plant extracts (methanol + DPPH). After 30 minutes, absorbance of the entire sample was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid of same concentration was prepared as a standard and its absorbance was also taken at 517 nm and calibration curve was constructed. Percent radical scavenging

activity by sample treatment was determined by comparison with methanol treated control group, ascorbic acid was used as positive control. The radical scavenging activity was expressed as the radical scavenging percentage using the equation (1) [16]:

$$\text{Radical Scavenging (\%)} = \frac{A_0 - A_s}{A_0} \times 100 \quad (1)$$

where,  $A_0$  = Absorbance of the control

$A_s$  = Absorbance of test sample

The  $IC_{50}$  value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated from the plotted graph of radical scavenging activity against the concentration of extracts.

### 3. Results and Discussion

#### 3.1 Phytochemical screening

Results of the phytochemical screening are summarized in below Table 1. The phytochemical screening of methanol extract of *Centella asiatica* leaves revealed that carbohydrate, glycosides, phenols, flavonoids, and saponin were present but alkaloids, protein and xanthoprotein were absent. Except alkaloid, carbohydrate, glycosides, other tested phytochemicals were found to be absent in hexane extract.

The most important bioactive compounds (phytochemicals) such as alkaloids, saponins, flavonoids, tannins, sterols and phenolic compounds [17, 18] are plant derived metabolites naturally occurring in medicinal plant leaves, stem and roots where they are used as defense mechanism to protect the plant from various diseases. The presence of these phytochemicals in *Centella asiatica* is an indication of its medicinal potential. The results presented in the above table are slightly different than the data present in the literature of plants. Flory Shobana et al. reported that the plant part contains most of the important secondary metabolites like carboxylic acid, flavanoids, saponin, steroids, resins, xanthoprotein, coumarins etc [17]. Saranya et al. reported the presence of carbohydrates, tannins,

steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, saponins etc [18]. The outputs of this study are consistent with Saranya et al. and Flory Shobana et al. with slight different results. These slight differences are due to variation in altitude of plants, different environmental conditions.

#### 3.2 Antibacterial susceptibility assay

Results obtained from the antibacterial assay of methanol and hexane extracts of *Centella asiatica* are tabulated as Table 2. Hexane extracts against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* gives ZOI value 13 mm, 0 mm, and 10 mm respectively in 10% concentration. Hence, the hexane extracts showed more effective towards *E. coli*. Figure 1 (a, b, c) show the results of antibacterial activity of methanol and hexane extract. Hexane extracts against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* showed ZOI value 13, 0, and 10 mm respectively in 10% concentration. However that of 1% hexane extracts against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* was found 11 mm, 0 mm, and 12 mm respectively. Thus hexane extracts was found more effective against *Escherichia coli* and *Salmonella typhi*. On the other hand hexane extract did not show any antibacterial activity against *Staphylococcus aureus*. Methanol (10% concentration) extracts showed moderately effective against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* with ZOI 11, 10, and 14 mm respectively whereas 1% methanol extracts proved more effective against *Salmonella typhi* with 16 mm zone of inhibition (ZOI).

Flory Shobana et al. reported that the methanol leaf extract showed maximum inhibition against *Staphylococcus aureus* 16 mm and the 50% and 75% methanol extract also showed inhibition zone 10 and 11 mm respectively [17]. Zaidan et al. explored in vitro antibacterial activity of the plant extract against *Staphylococcus aureus* showed a zone of inhibition of 5 mm [19]. Therefore, the result of antibacterial assay was also supported by other study [17, 19]. *Staphylococcus aureus* causes infections including

superficial skin lesion, localized abscesses, and food poisoning [19]. *Centella asiatica* has traditional claims for antibacterial activity and this finding is in line with

**Table 1:** Phytochemical screening of different extracts of *Centella asiatica*

S.N	Phytochemical	Hexane	Methanol
1	Alkaloids	+	-
2	Carbohydrate	+	+
3	Glycosides	+	+
4	Phenol	-	+
5	Flavanoids	-	+
6	Proteins	-	-
7	Xanthoproteins	-	-
8	Saponins	-	+

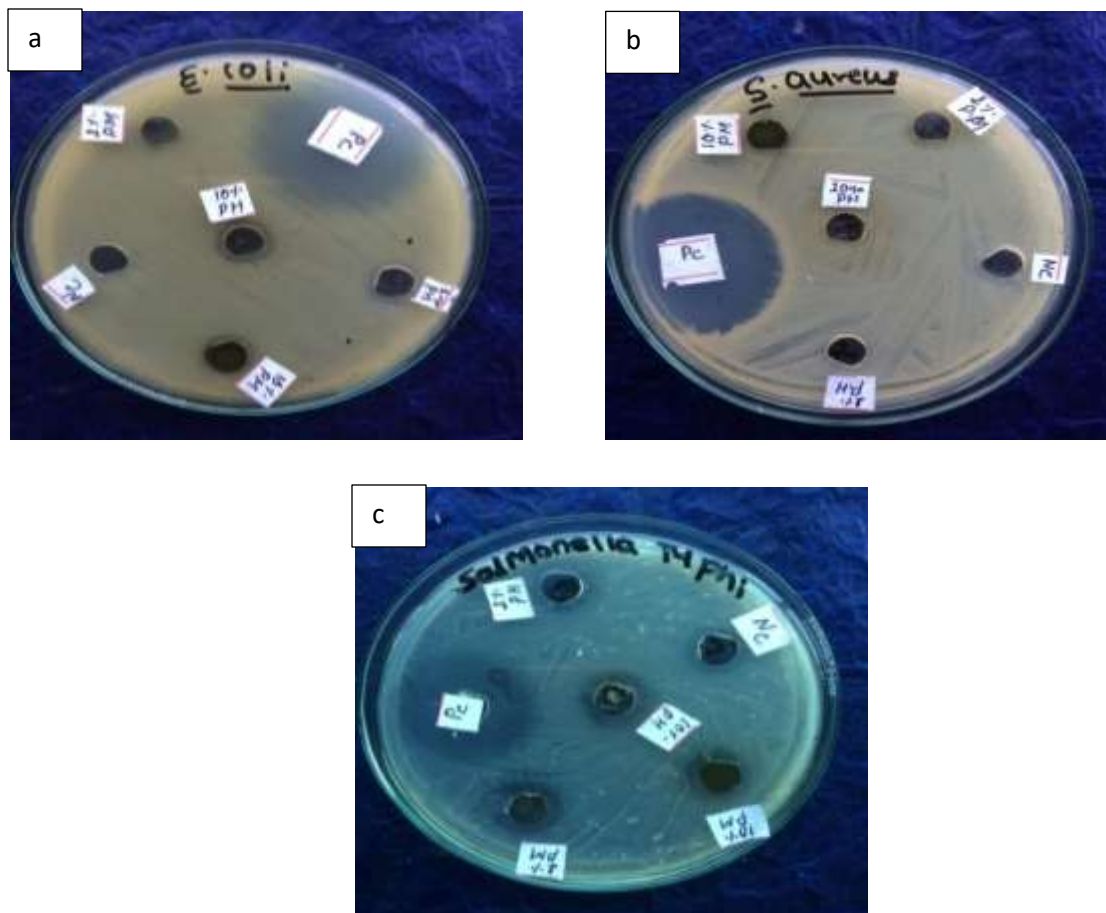
(+) indicates present and (-) indicates absent

**Table 2:** Antibacterial analysis results of methanol and hexane extracts of *Centella asiatica*

S.N.	Plant extract	Bacteria	ZOI (mm) of crude extract		ZOI (mm) of positive control
			10%	1%	
1.	Hexane				
		<i>E. coli</i>	13	11	28
		<i>S. aureus</i>	0	0	30
		<i>S. typhi</i>	10	12	28
2.	Methanol				
		<i>E. coli</i>	11	0	28
		<i>S. aureus</i>	10	0	30
		<i>S. typhi</i>	14	16	28

**Table 3:** Percentage of radical scavenging with different concentration

% of radical scavenging					
Sample	Concentration (µg/mL )				
	20	40	60	80	100
Methanol extract	26.68	34.25	61.98	74.44	88.94
Ascorbic acid	13.21	19.38	27.50	58.66	72.59



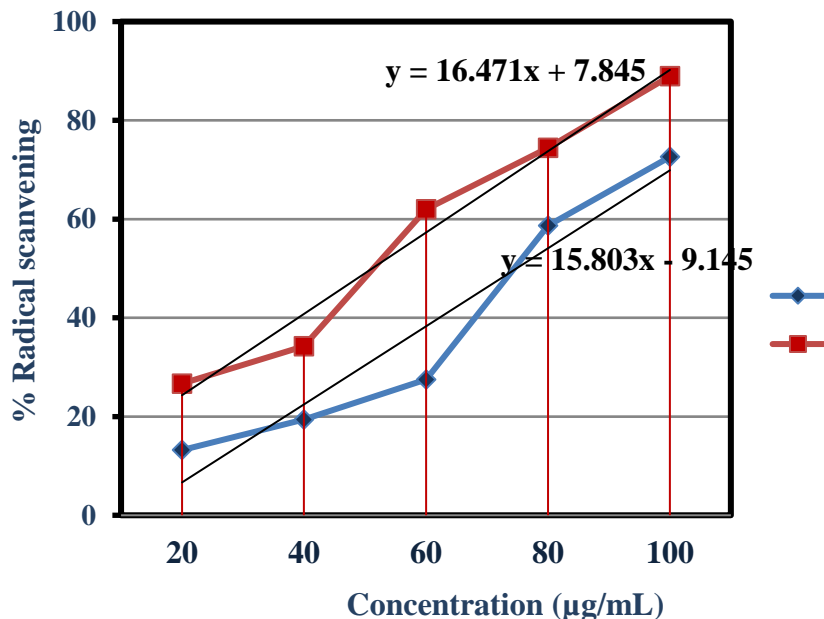
**Fig. 1:** Antibacterial screening of methanol and hexane extract of (a) *Centella asiatica* against (b) *Escherichia coli*, *Staphylococcus aureus* and (c) *Salmonella typhi*.

their indication as curative properties for antibacterial as claims. The result of this study can form the basis for further studies to evaluate against a wider range of bacteria strains. Figure 1 (a, b, c) show the results of antibacterial activity of methanol and hexane extract.

### 3.3 Antioxidant activity

The antioxidant activity of extract was calculated as their capacity to scavenge free radicals of DPPH, which has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources [20]. The DPPH radical assay

was carried out for the methanol extract of *Centella asiatica* by using ascorbic acid as standard. The absorbance values were measured at 517 nm for different concentrations of extracts and the control. These values were used to calculate the percentage inhibitions of DPPH radicals against the samples. IC<sub>50</sub> value was calculated from the calibration curve constructed by measuring the absorbance of ascorbic acid (Table 3). The absorbance with the different concentration of ascorbic acid was taken as the reference from our previous paper [14]. The comparison of percentage radical scavenging at different concentration between plant extract and ascorbic acid as standard was shown in Fig 2.



**Fig. 2:** A plot of percentage radical scavenging activity vs. concentration of methanol extract and ascorbic acid.

**Table 4:** IC<sub>50</sub> values of methanol extract and ascorbic acid

S.N.	Sample	IC <sub>50</sub> (µg/ mL)
1.	Standard Ascorbic acid	3.74
2.	Methanol extract	2.576

The IC<sub>50</sub> value of methanol extract and ascorbic acid was observed 2.576 µg/mL and 3.74 µg/mL respectively supported by Ref [14]. The antioxidant potential is in an inverse relation with IC<sub>50</sub> value, lower value of IC<sub>50</sub> indicates high antioxidant potential. Anand et al. reported 0.07 mg/mL and 500 µg/mL IC<sub>50</sub> values of DPPH and hydroxyl radical scavenging activity of methanol extract respectively [21]. Since the phytochemical screening of methanol extract showed the presence of phenol and flavonoids which are responsible for the antioxidant activity. Singh et al. mentioned antioxidant activity of methanol extracts ranged from 72.0 to 85.7% and

proved that there is a significantly positive correlation with anthocyanin, total phenol, flavonoids, and tannin [22]. Phenolic contents are very important constituents because they act as reducing agent, hydrogen donors and metal chelator. They also act as radical scavenger due to their hydroxyl groups. Funde reported that flavonoids can show their antioxidant action through scavenging or chelating process [23]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [23].

#### 4. Conclusions

The results of this study revealed that the methanol extract of *Centella asiatica* possesses pharmacologically active substances like carbohydrate, glycosides, phenols, flavonoids, saponins. The antibacterial assay of hexane extract showed moderate activity against *Salmonella typhi* and *Escherichia coli* but methanol extract has proved most effective against *Salmonella typhi*. Similarly, antioxidant activity of methanol extract showed potent antioxidant activities with IC<sub>50</sub> value 2.57 µg/mL slightly higher than standard ascorbic acid (IC<sub>50</sub> = 3.74 µg/mL). It is concluded that *Centella asiatica* extract possess pronounced

antioxidant and antibacterial biological properties and recommends that active phytoconstituents can be isolated, identified, and screened individually for activities and also subjected further for in vivo and toxicological studies.

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