

PHYTOTOXIC, ANTIOXIDANT, ANTIBACTERIAL, AND ANTILEISHMANIAL ACTIVITIES OF  
*EUPHORBIA HIRTA* FROM CHITWAN DISTRICT NEPAL

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## ABSTRACT

**Objectives:** The aim of this study is to investigate the antimicrobial, antioxidant, antileishmanial, and phytotoxic activities and estimation of total phenolic and flavonoid contents in methanol extract of *Euphorbia hirta* collected from Chitwan district of Nepal using *in vitro* studies.

**Methods:** Methanol extract of the plant was prepared by cold percolation method. Screening of secondary metabolites was performed by color differentiation methods. The *in vitro* antioxidant potential of the plant extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl assay in which percentage scavenging and inhibitory concentration ( $IC_{50}$ ) were calculated. Furthermore, antileishmanial and phytotoxic activity was evaluated by adopting the standard protocols. Phenolic and flavonoid content was estimated using Folin-Ciocalteu phenol reagent and aluminum chloride colorimetric method, respectively. The antimicrobial activity was evaluated by agar well diffusion method.

**Results:** The antileishmanial activity of the methanolic extract of *E. hirta* showed low leishmanicidal activity  $IC_{50}$  68.1±1.2 µg/ml standard deviation (SD) as compared to the standard drugs amphotericin B 0.29±0.05 µg/ml SD and pentamidine 5.09±0.09 µg/ml SD. The radical scavenging activity shows  $IC_{50}$  =29.23±0.21 µg/ml SD which is found potent than the standard ascorbic acid  $IC_{50}$  =45.93 µg/ml. The extract of *E. hirta* contains high value of total phenolic content milligram gallic acid equivalent per gram (138.10±4.90 mg GAE/g SD) and flavonoid content milligram quercetin equivalent per gram (11.54±0.00 mg QE/g SD). The plant extract exhibits weak antibacterial activity against *Staphylococcus aureus* (9.0 mm), *Bacillus subtilis* (11.0 mm), *Escherichia coli* (9.0 mm), and *Salmonella* Typhi (9.0 mm) at a concentration of 10.0 mg/ml in which 50 µl was loaded per well. Plant extract exhibits moderate phytotoxic activity with the number of fronds 3, 10, and 14 against the different concentrations of plant extract.

**Conclusions:** The preliminary results of this study have put forward *E. hirta* into promising herbs with good antioxidant activities, moderate phytotoxic activity, and potential source of antibacterial agent with a significant amount of total phenolic and flavonoid contents. Although the plant extract shows the potent biological activity, further studies are needed to assess its mechanism of action.

**Keywords:** *Euphorbia hirta*, Phytotoxic, Antioxidant, Antimicrobial, Antileishmanial, Methanolic extract.

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## INTRODUCTION

The plant *Euphorbia hirta* is commonly known as dudhe jhar. The plant is very frequent in rice fields, especially in Terai region of Nepal. The plant belongs to the family Euphorbiaceae. The plant has been traditionally used for the treatment of different diseases basically for skin diseases. The phytochemical screening of the methanolic extract of the stem of *E. hirta* revealed that the plant is the rich source of secondary metabolites. The compound teraxerol isolated from this plant showed the antiasthmatic activity carried out on histamine-induced bronchospasm in guinea pigs that significantly inhibited the contractile effect of histamine [1].

Thin-layer chromatography showed the presence of quercetin, ferulic acid, and gallic acid in flavonoid fraction of *E. hirta* whole plant [2]. *E. hirta* showed antimicrobial, antifungal, antiviral, anti-inflammatory, antiarthritic, and antioxidant effects with the presence of polyphenolic and flavonoid compounds lead to perform the wound healing activity of enriched flavonoid fraction [3]. The plant is widely used in the traditional system of medicines for the treatment of respiratory diseases, gastrointestinal disorders, tumors, lactation in women, wound healing, and urinogenital disorders. The biological activities of isolated compounds such as scopoletin, scoparone, isoscopoletin, quercetin, isorhamnetin, pinocembrin, kaempferol, luteolin, and gallic acid from the *E. hirta* were also studied [4]. The extract and active component showed strong inhibition in nitric oxide production that showed antidiabetic and antioxidant effects in mice [4]. Phytochemical screening of *E. hirta* leaf extract revealed the presence of secondary metabolites as reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids, and phenolic

compounds. The methanol extract of *E. hirta* was reported to show the potent antitumor activity [5]. The ethanol extract of *E. hirta* showed a significant decreased in blood glucose level on alloxan-induced diabetic rats [6]. Ethanol extract and ethyl acetate fractions of the plant had  $\alpha$ -glucosidase inhibition activity while n-hexane, chloroform, butanol, and water fractions had no  $\alpha$ -glucosidase inhibitory effect [7]. Antibacterial sensitivity test indicated that the extract of *E. hirta* had little or no zone of inhibition (ZOI) against *Haemophilus influenzae* [8]. The natural compounds quinine and Cephaelis have been used for the management of protozoan parasitic diseases, malaria, and amoebiasis. The compound holamine and hydroxyl holamine showed potent antileishmanicidal activity against *L. donavari* [9].

The present study focus on the antimicrobial, antioxidant, phytotoxic, and antileishmanial activities of the methanolic extract of *E. hirta* collected from Chitwan district of Nepal.

## MATERIALS AND METHODS

## Plant materials

The whole plants (leaves, stems, and roots) of *E. hirta* were collected from Chitwan district of Nepal in 2014. The plant was identified by Rita Chhetry, Research Officer, National Herbarium and Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Nepal.

## Extraction

The plant was washed with tap water to remove the soil and other debris, dried under shade and kept at 2°C until extraction. The whole

plant was powdered then weighed (50.0 g), soaked in methanol (about 400 ml) for 72 h and filtered using Whatman No 1 filter paper. The filtrate obtained was concentrated under reduced pressure in a rotatory evaporator at 45°C to obtain the crude extract.

#### Phytochemical screening

The methanolic extracts of plant sample were analyzed for the presence of secondary metabolites such as polyphenols, alkaloids, flavonoids, tannin, carotenoids, saponins, reducing sugars, cardiac glycosides, steroids, terpenoids, glycoside, and anthraquinone by adopting the standard procedures of analysis [10,11].

#### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was measured using DPPH radical scavenging assay [12-14]. Different concentrations of test samples (5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml) were prepared while the concentration of DPPH was 0.2 mM in the reaction mixture. These reaction mixtures were taken in tubes and incubated at 37°C for 30 min. Discolorations were measured at 517 nm using a ultraviolet (UV)-visible spectrophotometer. Percent radical scavenging activity was calculated by comparison with the methanol treated control group; ascorbic acid was used as a positive control. Measurement was performed at least in triplicate. The percentage scavenging of the DPPH free radical was calculated using the equation. The inhibition curve was plotted for the triplicate experiments and represented as the percentage of mean inhibition ± standard deviation (SD) and the inhibitory concentration (IC<sub>50</sub>) values were obtained.

#### Total phenolic content determination

The total phenolic content was determined using the Folin-Ciocalteu phenol reagent. 0.1 ml of each extract (2.5 mg/ml) was separately mixed with 1.0 ml of Folin-Ciocalteu phenol reagent and 0.8 ml of aqueous 1M Na<sub>2</sub>CO<sub>3</sub> solution. The reaction mixture was allowed to stand for about 15 min, and the absorbance of the reactant was measured at 765 nm using the UV-visible spectrophotometer. The calibration curve was constructed using the methanolic solution of gallic acid as standard with the concentration ranging from 25 to 250 µg/ml. With the help of this calibration curve of gallic acid, the concentration of the sample was calculated. The total polyphenol content was expressed in milligrams of the gallic acid equivalent per gram of the dry mass (mg GAE/g) [15].

#### Total flavonoid content determination

The total flavonoid content was estimated using the aluminum chloride (AlCl<sub>3</sub>) colorimetric method. In this method, 0.25 ml extract (10 mg/ml) was separately mixed with the 0.75 ml of methanol, 0.05 ml of the 10% aluminum chloride, 0.05 ml of the 1M potassium acetate (CH<sub>3</sub>COOK), and 1.4 ml of the distilled water. The reaction mixture was allowed to stand for about 30 min at room temperature. The mixture was subjected to measure the absorbance at 415 nm in UV-visible spectrophotometer. The calibration curve was constructed with the help of standard quercetin solutions in methanol with concentration ranging from 10 to 100 µg/ml. The total flavonoid content was expressed in milligram of quercetin equivalent per gram of the dry mass (mg QE/g) [15].

#### Statistical analysis

Antioxidant activity, total phenolic content, and flavonoid content reported as the mean ± SD. A significant difference for multiple comparisons was calculated using a one-way analysis of variance. p < 0.05 was considered statistically significant.

#### Antimicrobial activity

The antibacterial activity of the methanol extract of *E. hirta* was tested against the test organisms by the agar well diffusion method. Sterile Muller-Hinton agar plates of approximately 6 mm thickness were prepared. The inoculated plates were left to dry for a few minutes at room temperature with the lid closed. Five different wells were made in the inoculated media plates with the help of sterile cork borer no. 6 and labeled properly. Hence, the diameter of a well was 6 mm. Then, 50 µl of the plant extract was introduced into the respective well. In one well

pure methanol was filled as a negative control. The plates were then left for ½ h with the lid closed so that the extract diffused into media. The plate was incubated overnight at 37°C. After proper incubation (18-24 h), the plates were observed for the ZOI around well.

#### In vitro phytotoxic bioassay

The phytotoxic bioassay was performed by adopting the standard protocol in which E-Medium was prepared by mixing various constituents in 1000 ml distilled water, and pH was adjusted between 6.0 and 7.0 by adding potassium hydroxide pellets. Plant growth inhibitors and promoters serving as negative and positive controls, respectively. The number of fronds was counted and recorded per flasks on day 7. Results were analyzed as growth percent with reference to the negative control [16,17].

$$\text{Percent regulation} = 100 - \frac{\text{Number of fronds in test}}{\text{Number of fronds in negative control}} * 100$$

#### Leishmanicidal assay

Leishmanicidal assay was performed by adopting the standard protocol in which *Leishmania* promastigotes were grown in bulk early in modified Novy-MacNeal-Nicolle biphasic medium using normal physiological saline. *Leishmania* parasite promastigotes were cultured with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. The plate was incubated at 22-25°C for 72 h. The culture was examined microscopically and IC<sub>50</sub> values of fractions possessing antileishmanial activity were calculated [18,19].

## RESULTS AND DISCUSSION

#### Phytochemical analysis

The result of phytochemical screening is presented in Table 1. Plant extract of *E. hirta* showed a good source of secondary metabolites such as flavonoids, terpenoids, polyphenols, glycosides, reducing sugars, cardiac glycosides, anthraquinone, carotenoids, and saponin. It shows that the plant extract can be used to isolate the active compounds for different biological activities.

The plant extracts containing secondary metabolites are known to be biologically active and, therefore, aid the antimicrobial activities through a different mechanism.

#### Antimicrobial activity

The result of antimicrobial activity is shown in Table 2. The plant extract is the rich source of secondary metabolite with mild antibacterial activity against Gram-positive and Gram-negative organisms. The plant extract possess weak antibacterial effects against Gram-positive bacteria *Staphylococcus aureus* (9.0 mm) and *Bacillus subtilis* (11.0 mm) and Gram-negative bacteria *Escherichia coli* (9.0 mm) and *Salmonella* Typhi (9.0 mm) at a concentration of 10.0 mg/ml in which 50 µl was loaded per well. Plant extract revealed the most significant antibacterial activity and broad antimicrobial spectrum against the strains of *S. aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, with moderate zones of inhibition 26.00 mm, 20.33 mm, and 17.00 mm, respectively. The ethyl acetate extract presented a slightly lower antimicrobial activity than the ether extract [20].

#### Phytotoxic activity

Plant extract exhibits moderate phytotoxic activity with the number of fronds 3, 10, and 14 against the concentration of 1000, 100, and

Table 1: Phytochemical analysis of plant extract

Polyphenols	+	Reducing sugar	-
Steroids	-	Tanning	-
Alkaloids	-	Cardiac glycoside	-
Flavonoids	+	Anthraquinone	+
Terpenoids	-	Carotenoids	+
Glycosides	-	Saponin	-

+: Presence, -: Absence

Table 2: Antimicrobial activity of plant extract

Plant extract	Concentration	<i>Staphylococcus aureus</i>	ZOI, mm		
			<i>Bacillus subtilis</i>	<i>Salmonella Typhi</i>	<i>Escherichia coli</i>
<i>Euphorbia hirta</i>	10 mg/ml	9	11	9	9
	15 mg/ml	9	11	9	9
	20 mg/ml	10	12	10	10

ZOI: Zone of inhibition

Table 3: *In vitro* phytotoxic activity

Name of the plant	Concentration of plant extract (µg/ml)	No of fronds		% growth regulation	Concentration of standard drug (µg/ml)
		Sample	Control		
<i>Lemna minor</i>	1000	3	20	85	0.015
	100	10		50	
	10	14		30	

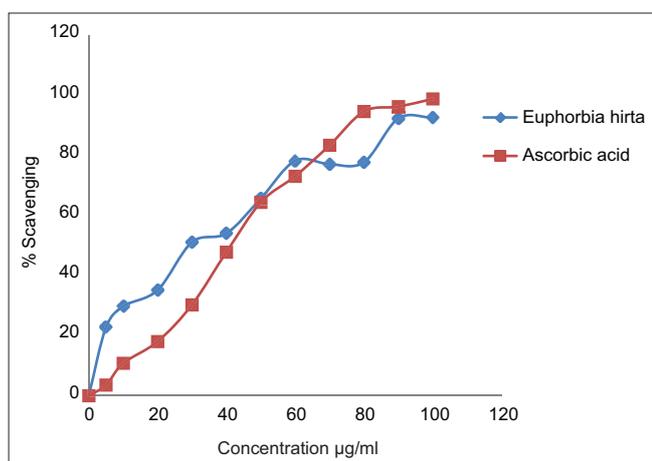
Standard drug paraquat, incubation condition=(28±1°C)

Table 4: Antileishmanial activity

Leishmanicidal activity	IC <sub>50</sub> (µg/ml)±SD
Test compound (plant extract)	68.1±1.2
Standard drugs	
Amphotericin B	0.29±0.05
Pentamidine	5.09±0.09

Incubation period 72 h, incubation temperature 22°C. IC<sub>50</sub>: Inhibitory concentration, SD: Standard deviationTable 5: DPPH scavenging (IC<sub>50</sub>) value, total flavonoid, and phenolic content

Total flavonoid content	11.54±0.00 mg QE/g SD
Total phenolic content	138.10±4.90 mg GAE/g SD
DPPH scavenging (IC <sub>50</sub> )	29.23±0.21 µg/ml SD

DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC<sub>50</sub>: Inhibitory concentration, SD: Standard deviationFig. 1: Percentage scavenging of 2,2-diphenyl-1-picrylhydrazyl free radical of *Euphorbia hirta* and ascorbic acid

10 µg/ml whereas the standard drug paraquat has 20 fronds at a concentration of 0.015 µg/ml. The moderate phytotoxic activity was found in the 100 µg/ml (50% inhibition). Significant phytotoxicity was shown in 100 and 1000 µg/ml with 50% and 85% growth inhibition (Table 3). The result obtained in this study was found comparable to the previously reported results where the phytotoxicity of the investigated samples on *Lemna minor* was detected in the n-hexane fraction with

12.5% and 18.7% inhibition at 10 and 100 µg/ml, respectively. Moderate phytotoxic activity detected in the methanol extract (40.0% inhibition) at 1000 µg/ml. Good phytotoxic activity was detected in the chloroform fraction (68.7% inhibition) at 100 µg/ml and n-butanol fraction (62.6% inhibition) at 1000 µg/ml [21].

#### Antileishmanial activity

The results of antileishmanial activity are shown in Table 4. The methanolic extract of *E. hirta* showed moderate antileishmanial activity with IC<sub>50</sub> 68.1±1.2 SD as compared to the standard drugs. The result found comparable to the previously reported in which the aqueous extract of the stem bark of *Enantia chlorantha* gave a powerful effect with an IC<sub>50</sub> 10.08 µg/ml [22]. Antileishmanial activity of aqueous and methanol extract of *Aloe nyeriensis* leaves against *Leishmania major* showed moderate activity with percent mortality 53.30±5.10 and 68.40±6.30 in concentration 1 mg/ml, respectively [23]. These difference results of the antileishmanial activity could be attributed to the chemical composition of plants by active molecules such as phenols, flavonoids, and terpenes.

#### Antioxidant activity

The result of antioxidant activity is shown in Fig. 1 and Table 5. Antioxidant minimizes the oxidative stress by scavenging the free radicals inhibiting lipid peroxidation. Antioxidants terminate the chain reaction by converting the unpaired electrons to paired ones. The concentration-dependent inhibition of DPPH free radical indicates that plant extract causes a reduction of DPPH radical in a quantitative weight relation manner. The plant extract of *E. hirta* was found as the source of potent antioxidants with the strongest DPPH radical scavenging activity (IC<sub>50</sub>=29.23±0.21 µg/ml SD) whereas standard ascorbic acid has IC<sub>50</sub> of 45.93 µg/ml. The result of the antioxidant activity of this plant extract found comparable to *Nauplius graveolens*, *Picris asplenoides*, *Reichardia tingitana*, and *Urospermum picroides* with radical scavenging activity 14.3%, 6.25%, 7.73%, and 4.14%, respectively [24].

The result showed significant total phenolic and flavonoid content in the plant extract. The extract of *E. hirta* contains high value of phenolic (138.10±4.90 mg GAE/g SD) and flavonoid content (11.54±0.00 mg QE/g SD) exhibited the greatest antioxidant activity.

#### CONCLUSIONS

From this study, it is concluded that the plant extracts are the potent source of phytoconstituents and could be used for the isolation of active natural compounds. The susceptibility of various microbial agents to different concentration of plant extract indicated that plant is the potential source for the antimicrobial compound. DPPH scavenging activity showed that the methanol extract is the potent source of antioxidant natural

compounds with  $IC_{50}$  29.23±0.21 µg/ml SD with respect to the standard ascorbic acid  $IC_{50}$  45.93 µg/ml. Phenolic and flavonoids have been reported to be associated with antioxidant action in the biological system, acting as scavengers of singlet oxygen and free radicals. The plant extract showed moderate phytotoxic activity 85%, 50%, and 30% growth regulation with the number of fronds 3, 10, and 14, respectively, which is lower than the standard drug paraquat with the number of fronds 20 at a concentration of 0.015 µg/ml. Methanolic extracts of *E. hirta* showed moderate phytotoxic activity. The plant extract showed weak leishmanicidal activity 68.1±1.2 µg/ml SD against *L. major*. Plant extract showed mild activity against Gram-positive and Gram-negative bacteria. The plant extract was found a rich source of total phenolic and flavonoid content.

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#### AUTHOR'S CONTRIBUTIONS

The author declares that this work done by the author mentioned in this article.

#### CONFLICTS OF INTEREST

There are no conflicts of interest the author alone responsible for the content and writing of this manuscript.

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