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Research Article

# ANTIMICROBIAL AND ANTIOXIDANT PROFILE OF SOLVENT EXTRACTS OF FRUITS OF *RUBUS ELLIPTICUS*

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## ABSTRACT

Plants are utilized as a source of bioactive molecules for a long time. The traditional knowledge of local healers and Vaidyas is dependent on these plants and their parts along with its pharmacological knowledge. They are the sources of active molecules that are commonly utilized to treat different pharmacological ailments, infections, allergy, or any disorder. The extracts and decoctions prepared from these plant parts are being used by the natives and villagers almost routinely. The present study was performed on the fruits of *Rubus ellipticus*, a common herb of the Himalayas for antimicrobial and antioxidant activities. The polar and non-polar extracts viz. 80% methanolic, hydro-alcoholic, hexane, and chloroform extract showed antimicrobial and antioxidant potential. The polar extracts were found to be most effective and significant in comparison to non-polar extracts. The results were found to be significant ($p<0.05$). The studies are in progress to determine the active principles responsible for these activities.

**Keywords:** *Rubus ellipticus*, Fruits, Solvent extracts, Polar and non-polar extracts, Antimicrobial activity, Antioxidant activity, Active principles, Pharmacological activities.

## 1. INTRODUCTION

The Central Himalayan region covers the new states of Uttarakhand, which includes the divisions of Kumaun and Garhwal. The region supports about 1,386 medicinal plant species, out of which 1,338 are used to treat several human diseases and disorders, and about 364 plant species are used for veterinary diseases by the people of Uttarakhand [1]. The hilly state has its unique geography and diverse climatic conditions. It harbors the highest number of plant species known for medicinal properties among whole of the Indian Himalayan region [2]. The people of Uttarakhand disintegrated from the mainstream as per traditional knowledge, they believe in Vaidyas [3-5]. The flora of Garhwal was explored by several researchers and botanists [6-7]. Recommending this seemingly large number of plants would be impracticable and considerable discretion would have to be applied for selecting herbs for cultivation. The present study was performed on different solvent extracts of fruits of *Rubus ellipticus* for the evaluation of antimicrobial and antioxidant properties. The plant belongs to the family *Rosaceae*. It is locally known as Hisalu. It occurs in the forest from 800 to 2400 meters above sea level [8]. *Rubus ellipticus* is a common shrub species of the Kumaun Himalayan region. It is a 1-3 m tall thicket-forming thorny shrub. Branchlets are pubescent and purplish-brown or brownish with sparse, curved prickles and dense, purplish-brown bristles or glandular hairs. Flowering occurs from March to April, and the fruiting period is from April to May when it produces aggregate golden-yellow fruits [9].

## 2. MATERIAL AND METHODS

The fresh fruit samples of *Rubus ellipticus* were collected from the forest area (about 1370 meters above sea level; latitude and longitude 29.3461°N, 79.5519°E) of Bhimtal, District-Nainital, Uttarakhand, India. The collection took place in the flowering season of the year

2018-2019. The fruits were collected in sterilized bags and were transported to the laboratory with storage at 4°C till further use.

### 2.1. Preparation of solvent extracts of plant material

The fruits were washed aseptically, dried and ground to form fine powder. Further, the powdered material was soaked in polar and non polar solvents viz. methanolic (80% v/v), hydro-alcoholic (50% v/v), hexane and chloroform for 72 hours. The extracts were further filtered with Whatman filter paper No. 1, filtrates were further concentrated in vacuo at ambient temperature and humid conditions [10, 11].

### 2.2. Determination of antimicrobial activity

#### *2.2.1. Culture media and Inoculum*

The antimicrobial activity was determined by well diffusion using Nutrient agar for antibacterial activity determination and potato dextrose agar for antifungal activity determination. The bacterial cultures were grown at 37 °C for 18 h separately while fungal cultures were grown at 28°C for 48-72 h and suspension were checked to provide approximately, $10^5$ CFU/ml.

#### *2.2.2. Pathogenic cultures used in the study*

The fungal test organisms viz. *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404); bacterial strains viz. *Micrococcus luteus* (ATCC 9341), *E. coli* (ATCC 8739), *Salmonella abony* (ATCC 6017) and *Staphylococcus epidermidis* (ATCC 12228) were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

#### *2.2.3. Determination of diameter of zone of inhibition by well diffusion method*

The antimicrobial activity of fruits extracts against the concerned pathogens was determined by modified well diffusion method [12]. The sterilized media plates were poured with bacterial and fungal cultures pre suspended in nutrient broth and potato dextrose broth separately. The plates were then after allowed for solidification. Each of the wells were filled with fruit extracts (1 mg/ml) prepared in 80% v/v methanol, 50% v/v hydro-alcohol, hexane and chloroform separately. The solvent blanks were used in the form of solvents which were used for preparation of extract while Azithromycin (1 mg/ml) was used was used as a positive control against bacterial strains and Fluconazole (1 mg/ml) was used as a negative control against fungal strains. The bacterial culture plates were incubated at 37 °C for 18 h while fungal culture plates were kept at 28°C for 48-72 h. The well diffusion method was performed for the extracts against each of the test organism in triplicates to determine the mean values of diameter of zone of inhibition.

### 2.3. Determination of antioxidant activity

#### *2.3.1. Determination of DPPH Free radical scavenging activity*

The solvent extracts of fruits of *Rubus ellipticus* were screened for DPPH assay as per the modified method [13]. The working solution of DPPH was prepared (0.025g/liter) in methanol solvent while solutions of dried fruit extracts were prepared using 0.2 g of each of the fruit extract per 10 ml of the specific solvent. The reaction was carried out by mixing each of the fruit extract (40 µl) with 2 ml of DPPH solution for incubation for 30 minutes at ambient room temperature. The reaction was observed as absorbance taken at 515 nm using Systronics UV-VIS spectro-photometer.

The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

Percent Inhibition= {[AbsT (0 min) – AbsT (30 min)]/ AbsT (0 min)} x 100

Where AbsT=0 min was recorded as absorbance of DPPH at zero time and AbsT=30 minutes was recorded as the absorbance of DPPH after 30 minutes of incubation.

The standard was used as ascorbic acid dissolved in methanol at 0.5 mM concentration. The IC50 values were determined and recorded.

#### *2.3.2. Determination of total antioxidant activity*

The solvent extracts of fruits of *Rubus ellipticus* were determined for total antioxidant activity determination as per the standard method for determination of total antioxidant activity [14, 15]. The fruits extracts (0.1 ml) were prepared in respective solvents, prepared in 1 ml of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate separately. The test tubes were covered and incubated at 95°C for 90 minutes at 25°C. The blank consisted of 1 ml of reagent solution without the sample. The absorbance of the samples was measured at 695 nm against reagent blank which contained no test samples/ extracts and the values determined the total antioxidant activity. The higher absorbance value indicates greater antioxidant activity.

### 2.3.3. Determination of superoxide anion radical scavenging activity

The modified method of superoxide anion radical scavenging activity was adopted [16]. The fruit extracts prepared in specific solvents of *Rubus ellipticus* were mixed with 3 ml of reaction buffer solution (pH, 7.4) which contained 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction was enhanced after exposure to 30 W fluorescent lamps for 20 minutes. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control. The absorbance was determined at 560 nm using Systronics UV-VIS spectrophotometer. The Superoxide anion radical scavenging activity (%) was calculated as:

$$[(A_0-A_s)/A_0] \times 100$$

Where, $A_0$=absorbance of positive control; $A_s$=absorbance of sample

### 2.3.4. Determination of scavenging activity of hydrogen peroxide ($H_2O_2$)

The percent scavenging activities of solvent extracts of fruits of *Rubus ellipticus* were determined as per the method [17]. The solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and fruit extracts prepared in specific solvents were mixed homogeneously. $H_2O_2$ concentration was determined spectrophotometrically from absorbance at 230 nm after 10 minutes against a reagent blank solution containing phosphate buffer without $H_2O_2$. Ascorbic acid was used as a positive control.

The % scavenging $H_2O_2$ was determined as:

$[(A_0-A_s)/A_0] \times 100$

Where, $A_0$ = the absorbance of positive control; $A_s$ = the absorbance of sample.

## 3. RESULTS AND DISCUSSION

The results of the study suggest that, polar and non-polar solvent extracts *viz.* 80% methanolic extract, hydro-alcoholic extract, hexane and chloroform extracts of fruits of *Rubus ellipticus* were found to have significant antimicrobial and antioxidant activity. The antimicrobial activity of the polar extracts was determined by well diffusion method. These extracts showed significant antimicrobial potential in comparison to non-polar solvent extracts. Amongst the polar extracts, 80% methanolic extract possessed significant antimicrobial activity in comparison to hydro-alcoholic extract while hexane amongst the non-polar extract possessed significant antimicrobial activity in comparison to chloroform extract. The chloroform extract didn't show any activity against *Micrococcus luteus*. The results are shown in Table 1; Fig. 1 (a) and (b). The antioxidant activities were determined by conventional procedures. The results of antioxidant activity also showed similar pattern as that of antimicrobial activity. The results are shown in Table 2 and Fig. 2. The results of the present study are found to be in correlation with the previous findings [18-21].

**Table 1: Antimicrobial activity of solvent extracts of *Rubus ellipticus***

| Plant extracts/Positive Control/Solvent Blanks | Diameter of Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Test organisms | | | | | |
| | *M.s luteus* | *E. coli* | *S. abony* | *S. epidermidis* | *A. niger* | *C. albicans* |
| **80%Methanolic extract** | 25.0± 0.027 | 35.0± 0.023 | 35.0± 0.018 | 37.0± 0.018 | 34.0± 0.023 | 28.0± 0.036 |
| **Hydro-alcoholic extract** | 17.0± 0.067 | 25.0± 0.034 | 28.0± 0.035 | 35.0± 0.023 | 32.0± 0.025 | 25.0± 0.037 |
| **Hexane extract** | 15.0± 0.078 | 22.0± 0.028 | 19.0± 0.058 | 23.0± 0.087 | 23.0± 0.089 | 11.0± 2.06 |
| **Chloroformic extract** | NA | 12.0± 1.67 | 12.0± 1.85 | 8.0± 1.83 | 13.0± 1.56 | 9.0± 2.23 |
| **Positive control Azithromycin (1mg/ml)** | 27.0± 0.038 | 26.0± 0.037 | 35± 0.023 | 38.0± 0.015 | NT | NT |
| **Positive Control Fluconazole (1 mg/ml)** | NT | NT | NT | NT | 36.0± 0.015 | 32.0± 0.023 |
| **Methanol** | NA | NA | NA | NA | NA | NA |
| **Hydro-alcohol** | NA | NA | NA | NA | NA | NA |
| **Hexane** | NA | NA | NA | NA | NA | NA |
| **Chloroform** | NA | NA | NA | NA | NA | NA |

** NA, No activity; NT, No Tested; $p<0.05$ (level of significance)*

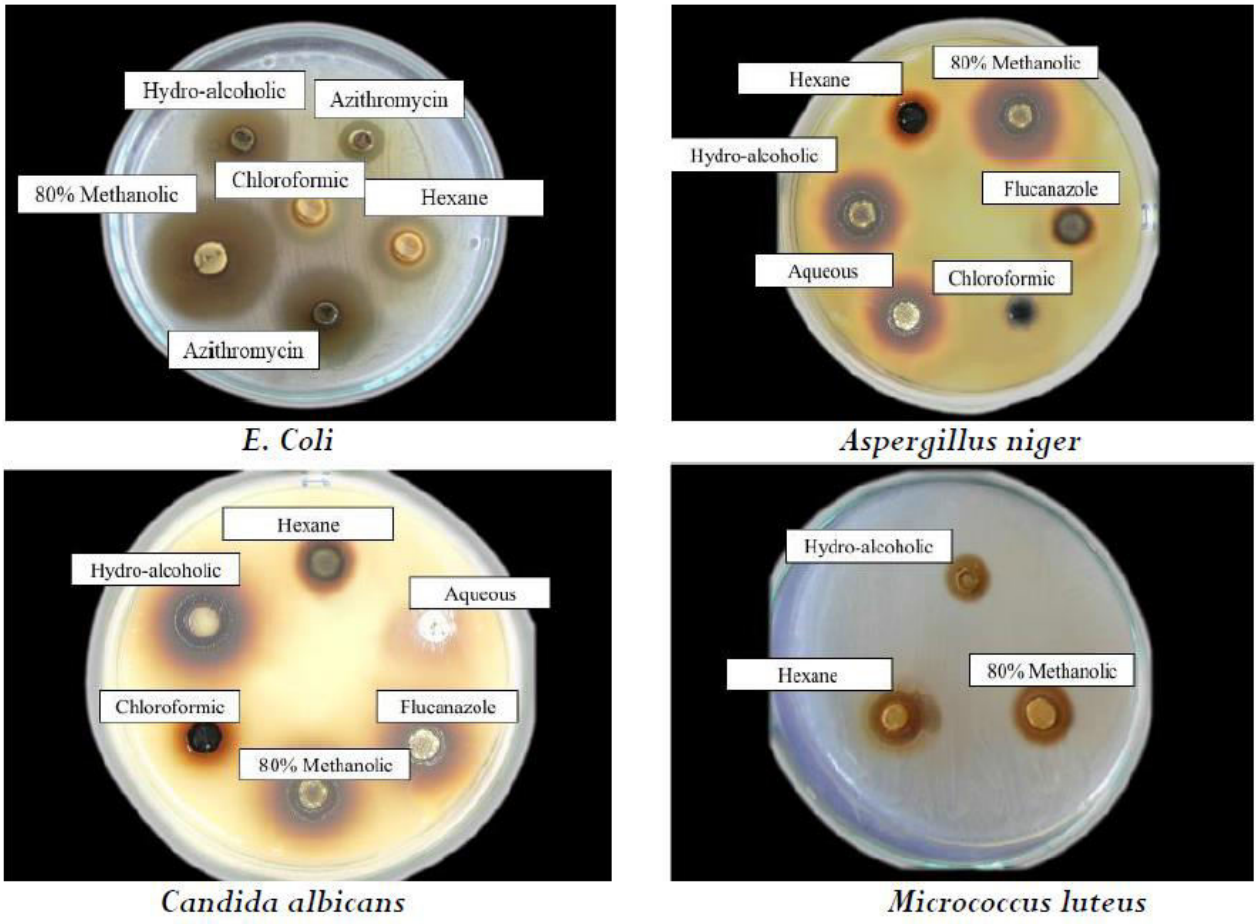


**Fig.1 (a): Antimicrobial activity of solvent extracts of *Rubus ellipticus***

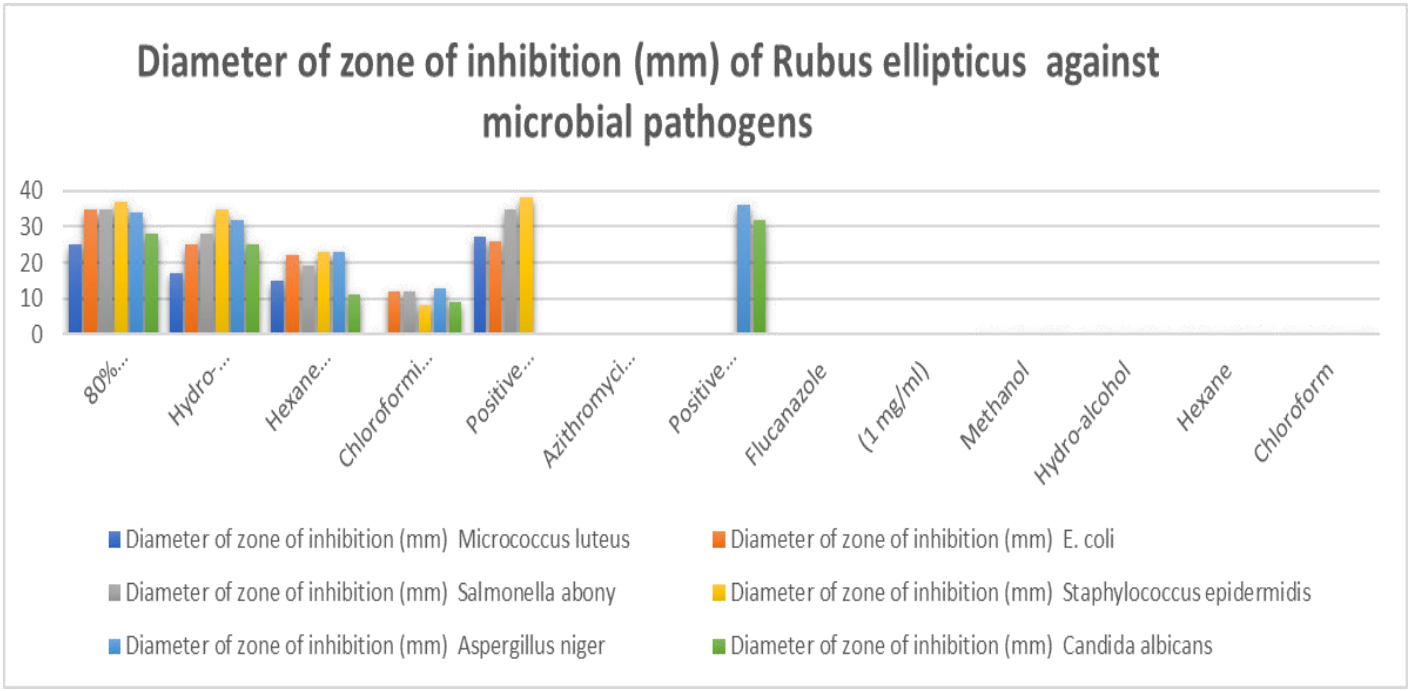


**Fig. 1 (b): Graphical representation of antimicrobial activity of solvent extracts of *Rubus ellipticus***

**Table 2: Results of antioxidant activity of solvent extracts of *Rubus ellipticus***

| Extracts and Standard (1 mg/ml) | Assays of determination of antioxidant activity | | | |
|---|---|---|---|---|
| | DPPH free radical scavenging activity (IC50) mol/L | Total antioxidant activity (A695) | Superoxide anion radical scavenging activity (% inhibition) | Hydrogen peroxide Scavenging (% inhibition) |
| 80% v/v Methanolic extract | 12.46±0.028*** | 2.08±0.035*** | 86.38±0.027*** | 74.37±0.025*** |
| Hydro-alcoholic extract | 15.87±0.035* | 1.23±.0.056** | 72.45±0.053** | 56.24±0.045** |
| Hexane extract | 23.12±0.085* | 0.86±0.067** | 56.45±0.092** | 45.58±0.078** |
| Chloroformic extract | 36.77±1.23* | 0.12±.1.56* | 23.45±1.06* | 18.34±1.82* |
| Standard (Ascorbic acid) | 11.08±0.015** | 1.85±0.062* | 86.56±0.024** | 85.23±0.038** |

*±SD; Level of significance, p<0.05; ***, highly significant; **, medium significant; *, significant*

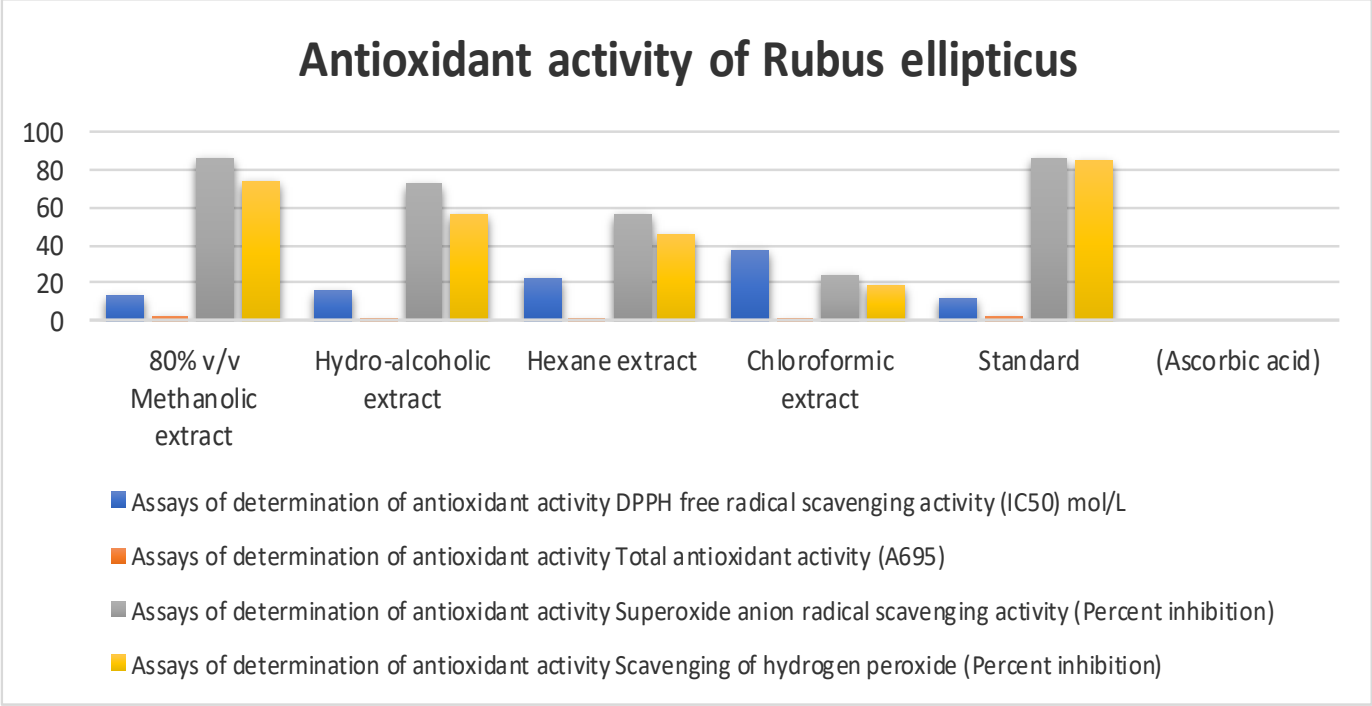


**Fig. 2: Graphical representation of antioxidant activity of solvent extracts of *Rubus ellipticus***

## 4. CONCLUSION

The results of the study conclude that, the extracts of fruits of *Rubus ellipticus* are a great source of antimicrobial and antioxidant agents. The findings reveal that, these extracts are the source of polar and non-polar compounds that can be utilized for formulation of drug or can be utilized as active constituents in the composition of antimicrobial drugs and nutraceutical agents. Further studies are however needed to explore the molecules/active principles in these extracts responsible for antimicrobial and antioxidant activity.

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