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STUDY OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SOLVENT EXTRACTS OF FRUITS OF *MYRICA ESCULENTA*

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ABSTRACT

Plants are found to be the significant bioreactors of versatile phytochemicals and nutraceuticals responsible for significant antimicrobial, antioxidant and other pharmacological activities. These molecules are thus an exemplary source of formulation of drugs and thus the ayurvedic extracts of plant (s) or plant part (s) are in traditional use to combat any infection and immunomodulating activity in the living being. In the present study, the fruit extracts of *Myrica esculenta* were prepared in different solvents viz. 80% methanol, hydro-alcohol, hexane and chloroform. The results of the study revealed significant antimicrobial and antioxidant activities as determined by conventional procedures. The results showed, extracts viz. 80% methanolic extract, hydro-alcoholic extract and hexane extract at 1 mg/ml concentration had significant antimicrobial potential against *E. coli*, *Salmonella abony* and *Staphylococcus epidermidis* while no antimicrobial activity was determined against *Micrococcus luteus*, *Aspergillus niger* and *Candida albicans*. The chloroformic extracts possessed no activity against any of the organism studied. antioxidant activity of the solvent extracts follows the order viz 80% methanolic extract>hydro-alcoholic extract>hexane extract>chloroformic extract.

***Keywords*:** Myrica esculenta, fruits, solvent extracts, antimicrobial activity, antioxidant activity, phytochemicals, nutraceuticals.

Introduction

Plants have been an integral part of the ancient culture of India, China and Egypt as medicine, and their importance even dates back to the Neanderthal period. The use of plant derived medicines in the treatment and prevention of disease has been documented over five millennia. Medicinal plants have been used throughout the world; however, their wide usage had been limited to China, India, Japan, Pakistan, and Sri Lanka, Thailand and a number of African countries (Hoareau *et al*., 1999). The Indian Himalayas spread out approximately 2,500 km in length and 250 km in width casing an area of approximately 590 thousand sq. km where about 51 million populace lives. Even though the region corresponds to only 18% of the geographical area of India, it is home to more than 50% of the country's forest cover and some 40% of the species endemic to the Indian subcontinent. Significance of high-altitude biodiversity is best exemplified by the availability of few rare, endemic and aromatic medicinal herbs (Dhar *et al.,* 1996; Dhyani *et al.,* 2007). The Central Himalayan region covers the new states of Uttarakhand, which includes the major divisions of Kumaun and Garhwal. The region supports about 1,386 medicinal plant species, out of which 1,338 are used to treat human diseases and disorders and about 364 plant species are used for veterinary diseases by the people of Uttarakhand (Pande *et al.,* 2004). The hilly state has its unique geography and diverse climatic conditions. It harbours the highest number of plant species known for medicinal properties among all the Indian Himalayan states (Kala *et al.,* 2004). The inhabitants of Uttarakhand are still dependent on the V*aidhyas* (traditional herbal practitioners) for treating disease due to isolation and relatively poor access to modern medical facilities (Maikhuri *et al.,* 1998; Kala, 2002, 2005). The present study was performed on solvent extracts of fruits of *Myrica esculenta*. The solvent extracts screened for antimicrobial and antioxidant activities. It belongs to the family *Myricaceae* is locally referred to as Kaphal. It occurs in forests from 1600 to 2400 meters above sea level. *M. esculenta* fruits are wild edible species of the Indian Himalayan Region. *M. esculenta* fruits are succulent drupe with small ellipsoidal or ovoid to globose in shape, initially green, and become reddish during ripening. It is perishable, and their shelf life does not exceed 2-3 days, and ripe fruits are made available from April to June (Bergali *et al*., 2011; Kumar *et al.,* 2020).

Materials and Methods

The fresh fruit samples of *Myrica esculenta* were collected from the forest area of Bhimtal, District-Nainital, Uttarakhand, India. The collection area was about 1370 meters above sea level (latitude and longitude 29.3461° N, 79.5519° E). The collection took place in the flowering season of the year 2018-2019. The fruits collected in

sterilized bags were transported to the laboratory and stored at 4^0C till further use.

Preparation of solvent extracts of plant material

The fruits of the plant were washed, dried and pulverized. Further the powdered material was soaked separately in different solvents viz. hexane, chloroform, 80% v/v methanolic, and 50% v/v hydro-alcohol for 72 h. with stirring for every 24 hours followed with filtration. The filtrates obtained were concentrated in vacuo at 30 ^{0}C (Alade and Irobi, 1999; Mathur *et al.,* 2010).

Determination of antimicrobial activity

(i) Growth medium and Inoculant

The growth medium used for growth of bacterial pathogens was nutrient broth and nutrient agar medium while for growth of fungal pathogens, Sabouraud's dextrose agar/broth was used. The bacterial pathogens (10^5 Cfu/ml) were inoculated in nutrient broth followed with nutrient agar medium at 37^0C for 18 h while fungal pathogens (10^5 Cfu/ml) were inoculated in Sabouraud's dextrose broth followed with Sabouraud's dextrose agar at 28^0C for 48 h.

(ii) Pathogenic cultures used

The pathogenic strains used in the study were *Micrococcus luteus* ATCC 9341, *E. coli* ATCC 8739, *Salmonella abony* ATCC 6017, *Staphylococcus epidermidis* ATCC 12228, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. These strains were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

(iii) Antimicrobial activity determination via well diffusion method

The antimicrobial activity was determined by well diffusion method with slight modifications (Perez *et al*., 1993). The pathogenic bacterial cultures pre suspended in sterilized nutrient broth were poured into pre sterilized petri plates with warm nutrient agar medium while fungal cultures were pre suspended in sterilized Sabouraud's dextrose broth till solidification. The specific sized diameter wells were punched into the agar and 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 respectively. The solvents were used as negative controls. For antibacterial activity, Azithromycin (1 mg/ml) was used as positive control, while for antifungal activity, Fluconazole (1 mg/ml) was used. The bacterial plates were kept for incubation for 37^0C for 18-24 h, while fungal cultures were kept for incubation at 28^0C. The wells having antibacterial/antifungal extract was considered as a potent antimicrobial fraction.

Determination of antioxidant activity

(i) DPPH Free radical scavenging activity

The antioxidant activity was determined by DPPH assay as per the modified protocol of Fargere *et al*., 1995. The testing solution was prepared of each of the extracts; mixing 0.2 g of each of the extract in 10 ml of specific solvent. The working solution of DPPH was utilized and testing solution was mixed and incubated for half an hour at room temperature. The absorbance was calculated at 515 nm. The percent inhibition of 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.

Ascorbic acid (0.5 mM), prior dissolved in methanol and was used as a standard; IC50, concentration of the sample required to scavenge 50% of DPPH free radicals was also determined.

(ii) Determination of total antioxidant activity

Total antioxidant activity determination was performed of fruit extracts in reference to standard antioxidant, ascorbic acid as per the modified method (Prieto *et al*., 1999; Mathur *et al*., 2011). The process of this activity starts with using 0.1 ml of each of these extracts and 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in separate test tubes. The tubes further incubated at 95 °C for 90 minutes followed with cooling at 25 °C, followed by measuring the absorbance at 695 nm against reagent blank consisting 1 ml of reagent solution without the sample. Higher absorbance value indicates higher antioxidant levels.

(iii) Determination of superoxide anion radical scavenging activity

The method for determination of superoxide anion radical scavenging activity was performed with some modifications (Duan *et al*., 2006). The reaction was performed by mixing the fruit extracts with 3 ml of reaction buffer solution (pH 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT followed with exposure to 30W fluorescent lamps for 20 minutes. The absorbance was taken at 560 nm using a spectrophotometer. The standard reference control was used as Ascorbic acid (1 mg/ml) while different solvent extracts were used as negative controls. The Superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{Ao - As}{Ao} \times 100$$

Where, Ao = absorbance of positive control; As = absorbance of sample

(iv) Determination of scavenging activity of hydrogen peroxide (H_2O_2)

The percent scavenging activities of fruit extracts prepared in different solvents were determined with some modifications (Duan *et al*., 2006). The solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH, 7.4) and was used in each of the fruit extracts prepared in different solvents separately. The absorbance of the samples was taken after 10 minutes of reaction at 230 nm using UV-spectrophotometer against a reagent blank containing only phosphate buffer without using H_2O_2. The readings of absorbance were determined using Ascorbic acid (1 mg/ml) as a positive control. The % scavenging H_2O_2 was determined as:

$$\frac{Ao - As}{Ao} \times 100$$

where, A0 = the absorbance of positive control; As = the absorbance of sample.

Results

The results of the study suggest that, extracts (1 mg/ml); 80% methanolic extract, hydro-alcoholic extract and hexane extract had significant antimicrobial potential against *E. coli*, *Salmonella abony* and *Staphylococcus epidermidis*. There was no antimicrobial activity observed against *Micrococcus luteus*, *Aspergillus niger* and *Candida albicans*. The chloroformic extracts were found to have no activity against any of the organism studied. The results are shown in Table 1; Figure 1 (a) and Figure 1 (b). The antioxidant activity of the extracts was determined by DPPH free radical scavenging activity, Superoxide anion radical scavenging activity, Hydrogen per oxide free radical scavenging activity and Total antioxidant activity. The results showed that, antioxidant activity of the solvent extracts follows the order viz 80% methanolic extract>hydro-alcoholic extract>hexane extract>chloroformic extract (Table 2; Figure 2).

Discussion

The results of the present study revealed that, fruits of *Myrica esculenta* possessed significant antimicrobial and antioxidant activity. The current studies are in correlation with the previous findings (Martins *et al*., 2019; Purohit *et al*., 2013; Chandra *et al.,* 2012; Bhatt *et al*., 2011).

Conclusion

The investigations revealed that, fruit extracts of *Myrica esculenta* possessed potent antimicrobial and antioxidant activities. The extracts possessed significant antimicrobial and antioxidant agents which should be explored further to develop effective antimicrobial and antioxidant agents or can be utilized as significant antimicrobial and antioxidant drug.

Table 1: Antimicrobial activity of solvent extracts of *Myrica esculenta*

Diameter of Zone of inhibition (mm)						
Plant extracts (1 mg/ml)/Positive Control (1 mg/ml)/Solvent Blanks	**Test organisms**					
	Micrococcus luteus	***E. coli***	***Salmonella abony***	***Staphylococcus epidermidis***	***Aspergillus niger***	***Candida albicans***
80% Methanolic extract	NA	43.0± 0.032	45.0± 0.025	48.0± 0.045	NA	NA
Hydro-alcoholic extract	NA	38.0± 0.049	33.0± 0.038	25.0± 0.082	NA	NA
Hexane extract	NA	27.0± 0.065	20.0± 0.056	15.0± 1.23	NA	NA
Chloroformic extract	NA	NA	NA	NA	NA	NA
Positive control Azithromycin (1mg/ml)	45.0± 0.018	45.0± 0.024	32± 0.062	38.0± 0.056	NT	NT
Positive Control Flucanazole (1 mg/ml)	NT	NT	NT	NT	36.0± 0.034	32.0± 0.045
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

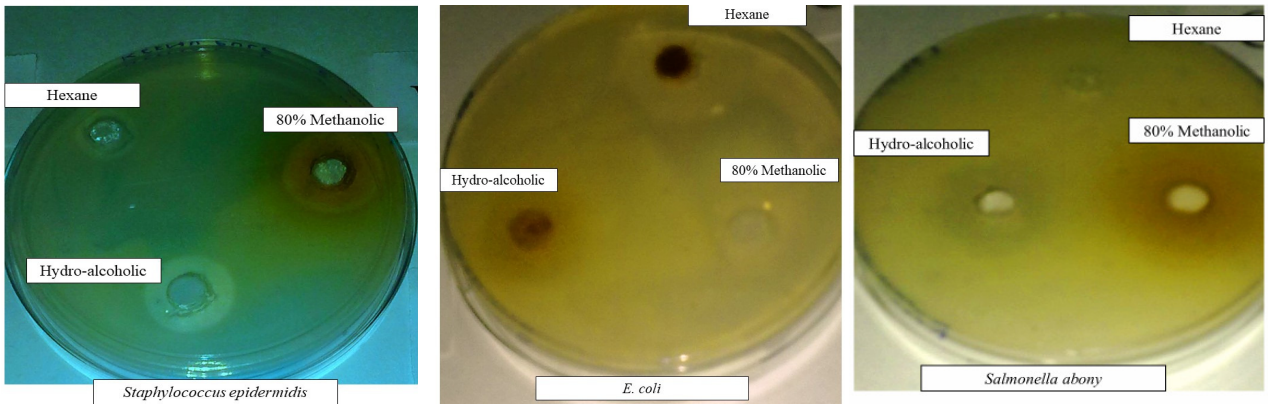

Fig. 1 (a): Antimicrobial activity of solvent extracts of *Myrica esculenta*

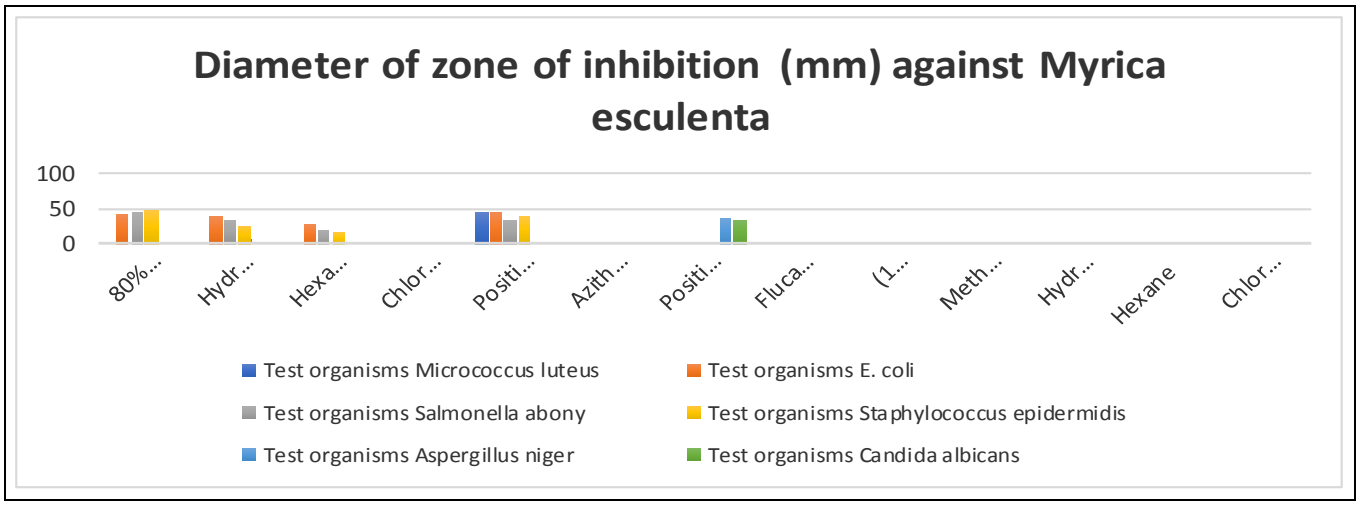

Fig. 1(b): Graphical representation of antimicrobial activity of solvent extracts of *Myrica esculenta*

Table 2: Results of antioxidant activity of solvent extracts of *Myrica esculenta*

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 (Percent inhibition)	Scavenging of hydrogen peroxide (Percent inhibition)
80% v/v Methanolic extract	18.45±0.035***	1.86±0.025***	65.38±0.025***	62.15±0.035***
Hydro-alcoholic extract	23.18±0.042	1.78±.0.037**	46.58±0.046**	53.46±0.027**
Hexane extract	32.78±0.056*	0.94±0.056**	36.45±1.34**	41.08±0.056**
Chloroformic extract	42.13±1.68*	0.15±.1.76*	18.27±1.56*	26.34±1.45*
Standard (Ascorbic acid)	11.08±0.012**	1.85±0.056*	86.56±0.024**	85.23±0.025**

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

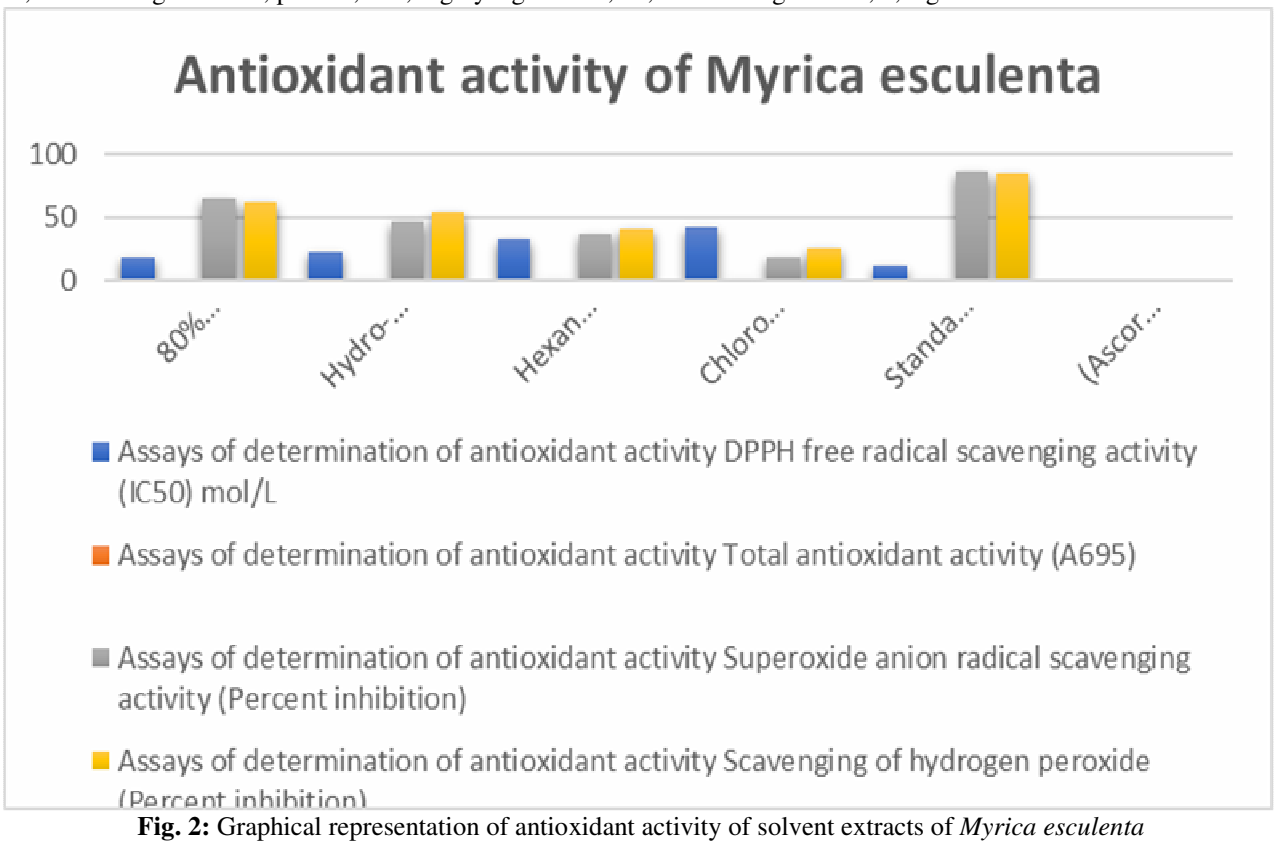

Fig. 2: Graphical representation of antioxidant activity of solvent extracts of *Myrica esculenta*

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