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# Antioxidant, Antibacterial and Sun Protection activity of *Centella asiatica* Leaves Extract

**[1] Abhinay Kumar Dwivedi, [2] S Nayak**

[1, 2] Department of Pharmacognosy, Bansal College of Pharmacy, Kokta, Anand Nagar Bhopal, Madhya Pradesh, India

Corresponding Author: **Abhinay Kumar Dwivedi**

**Abstract**

*Centella asiatica* described as an important medicinal herb used from ancient time to relieve various symptoms. The leaves extract of the plant has been evaluated for antioxidant, antibacterial and sun protection potential by DPPH radical scavenging method, well diffusion method and *in vitro* SPF potential determined by UV-VIS Spectrophotometer respectively. DPPH radical scavenging activity of *Centella asiatica* extracts conferred percent inhibition 77.15%, the antibacterial activity has shown maximum zone of inhibition of 9.65 mm against *S. aureus* and 13.66 mm against *E. coli* at 2% concentration. The significant sun protection efficiency was found with the SPF value of 1.275, 1.461, 1.582 against 40 µg/mL, 50µg/mL and 60µg/mL respectively. *Centella asiatica* leaves extract given satisfactory results on the parameter tested and could be used for its anti-ageing, antibacterial and antioxidant potential in pharmaceutical preparations.

**Keywords:** Antioxidant, Antibacterial, Sun protection factor, *Centella asiatica*, SPF

## Introduction

*Centella asiatica* commonly known as mandukparni is a valuable medicinal herb of belonging to family Apiaceae. The plant has been used since ancient time to cure various ailments and sufferings all around the world. The curative properties of the herb have been documented in traditional system of medicine. In Ayurvedic system of medicine *Centella asiatica* has mentioned as one of the main herbs for rejuvenation of nerves and brain cells. As per various texts available the plant could be used for blood purification, treating high blood pressure, brain tonic for memory enhancement, succored longevity, anti-aging and antioxidant potential [1-7]. In the present study *Centella asiatica* leaves extract were evaluated for their antioxidant, antibacterial and sun protective effect to find out the possibility and potential of these agent in skin care.

## Materials and methods

Analytical grade chemicals and reagents have been used for the purpose of study, the chemicals procured from central drug house (P) Ltd. New Delhi, the glass wares used in the study was borosilicate and ASGI mark. UV-VIS Spectrophotometer model UV-1700 Pharmaspec Shimadzu, Japan, used for SPF determination.

## Collection and processing of plant material

The leaves of *Centella asiatica* has been collected from Bansal Pharmacy College, herbal garden Bhopal M.P. India, in the month of May. Plant sample were authenticated by Dr. Suman Mishra, Scientist, Vindhya Herbal Testing and Research Lab, Bhopal, MP, India. The collected plant materials washed with tap water to remove debris and dirt. The plant were subjected to dried in shade for seven days, The shade dried plant material then grounded using electric grinder to get coarse powder, the coarse powder were subjected to extraction with suitable solvent.

## Extraction of plant material

The hydro alcoholic extract has been prepared by maceration method. 250g of coarsely powdered plant sample has been macerated with 75% ethanol for seven days in closed flask. The extract was collected and filtered using whatman filter paper, the filtrate was subjected to evaporation to remove excess solvent to get concentrated residue under reduced pressure at the temperature 35°C in rotary evaporator. The concentrated extracts obtained were placed in the desiccators to wipe off the residual solvent.

### *In-vitro* Anti-oxidant Activity

Antioxidant potential of *Centella* leaves extract has been determined by DPPH radical scavenging using ascorbic acid as standard. 2, 2-Diphenyl-1-picrylhydrazyl, 0.1mM solution was prepared in methanol.

### Preparation of Sample/Standard

One mg dried extract of *Centella asiatica* has been taken to made 1mg/mL stock solution with methanol. 1mg/ml methanolic solution of standard stock solution has been prepared with ascorbic acid. Different dilutions of extract and standard (20 to100 µL) have been made from stock solution and methanol was added to make the volume to 1 ml. 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly, the sample were placed in dark for 30 minutes, the absorbance of the sample were recorded at the wavelength 517 nm[8,9].

### Preparation of control

Three milliliter of 0.1mM DPPH solution has been prepared. Incubated for 30 minutes at room temperature in dark. The absorbance of the control solution was recorded against methanol as blank at 517 nm. The antioxidant activity of sample and standard were calculated by using formula [10,11].
Percentage Inhibition = [(Absorbance of control-Absorbance of sample/Absorbance of controlx100]

### Antibacterial Activity

Antibacterial activity of the extract has been evaluated by well diffusion assay against gram negative *E.coli* MTCC 42 and gram-positive *S. aureus* MTCC 10787; the test microorganism culture for has been procured from PBRI Bhopal.

### Preparation of Nutrient Media

The nutrient media was prepared by dissolving twenty-nine gram of nutrient agar in one litre of distilled water; pH of the prepared media was checked and recorded. The media was autoclaved at 121ºC for 15 minutes at 15 lbs pressure, The sterilized media was allowed to cooled, the media were transferred to the Petri plates before get harden and then placed in the laminar air flow to get solidified[12,13].

### Well diffusion assay

The bacterial cultures were spread on prepared media; the 1% and 2% of test extract has been prepared by mixing with distilled water. One milligram of standard ofloxacin and gentamycin were mixed with 1ml of distilled water to make 1mg/1ml of standard solution. The bacterial inoculum of selected species *E.coli* MTCC 42 and *S. aureus* MTCC 10787 were prepared. The selected strains were inoculated in 10 ml of nutrient broth. The culture was standardized to get $10^8$ CFU/ml. 100 µl of the inoculums was taken and transferred to fresh sterilized agar media**.** Three wells of 6 milli metre were made by sterile cork-borer. The primary two wells were filled with 50 µl of 1% and 2% sample repectevily and third well were filled with 50 µl of standard drug, the sample and drugs allowed to suffuse for 30 minutes at room temperature and then incubated for 24 hours at 37ºC. The clearing zone around the well was the indicator of antimicrobial efficiency of tested compounds. The zone of inhibition of sample and standard were observed and recorded [14,15].

### *In vitro* SPF Determination

The sun protection potential of sample could be determined by evaluating the sun protection factor (SPF). The SPF sun protection factor was defined as the Ultraviolet energy required to trigger a minimal erythemal dose in protected skin by the Ultraviolet energy required generate MED in unprotected skin.

$$SPF = \frac{\text{Minimal erythema dose in sunscreen-protected skin}}{\text{Minimal erythema dose in non-sunscreen-protected skin}}$$

The MED or minimal erythemal dose was the lowest time interval or dosage of Ultraviolet irradiation sufficient to produce a minimal and apparent erythema on unprotected skin [16, 17]. Value of SPF was an indicator for sun protection efficiency.
The *in vitro* SPF was determined by recording absorbance at 290 to 320 nm at 5 nm intervals and the values were calculated by using the formula [18-20].

$$SPF_{Spectrophotometr} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF is correction factor equivalent to 10, EE (λ) Erythmogenic effect of radiation with wavelength (λ), Abs (λ) is spectrophotometric absorbance at wavelength (λ).The values of EE(λ)× I(λ) was constant[21,22].

**Table 1:** Product function used in calculation of SPF

| S. No | Wavelength in nm | EE(λ)XI(λ) (normalized) |
|---|---|---|
| 1 | 290 | 0.0150 |
| 2 | 295 | 0.0817 |
| 3 | 300 | 0.2874 |
| 4 | 305 | 0.3278 |
| 5 | 310 | 0.1864 |
| 6 | 315 | 0.0839 |
| 7 | 320 | 0.0180 |
| Total | | 1 |

### Sample Preparation and SPF Determination

The stock solution has been prepared by using 10 mg of *Centella* leaves extract dissolved in 100 ml of ethanol to get 100 µg/ml then filtered through Whatman filter paper to obtain clear solution. Three dilution 40µg/ml, 50µg/ml and 60µg/ml of the extract were made using stock solution. All the samples were scanned thrice for specified wavelength from 200 nm to 400 nm in UV-Visible spectrophotometer (UV-1700 Pharmaspec Shimadzu). The base line correction has been done by using blank 80% ethanol and then absorbance of the sample has been measured by using one cm quartz cell against blank. The absorbance of *Centella asiatica* leaves extracts on three different concentrations were recorded and SPF has been calculated by using formula [23-24].

### Results

**Table 2:** DPPH radical scavenging activity of ascorbic acid

| Concentration (µg/ml) | Absorbance | % Inhibition |
|---|---|---|
| 20 | 0.447 | 52.74 |
| 40 | 0.341 | 64.05 |
| 60 | 0.261 | 72.42 |
| 80 | 0.186 | 80.23 |
| 100 | 0.118 | 87.41 |
| Control | 0.946 | 0 |
| IC50 | | 10.04 |

The values are mean: n=3

**Table 3:** DPPH radical scavenging activity of *centella* extract

| Concentration (µg/ml) | Absorbance | % Inhibition |
|---|---|---|
| 20 | 0.311 | 44.28 |
| 40 | 0.272 | 51.22 |
| 60 | 0.224 | 59.81 |
| 80 | 0.172 | 69.27 |
| 100 | 0.128 | 77.15 |
| control | 0.56 | 0 |
| IC50 | | 35.32 |

The values are mean: n=3

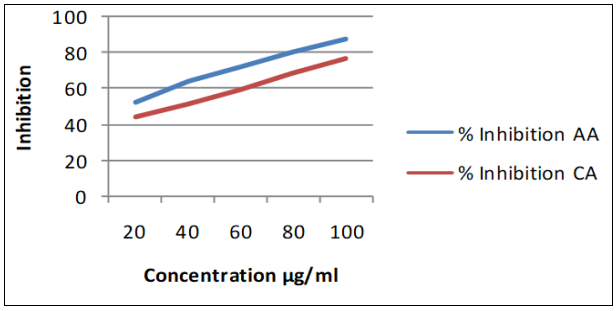


**Fig 1:** Graph represents percentage inhibition against concentration

**Table 4:** Antimicrobial activity of extract against *S. aureus*

| Sample | Plate 1 | Plate 2 | Plate 3 | Mean±SD |
|---|---|---|---|---|
| 1% | 5 mm | 6 mm | 6mm | 5.66±0.533 |
| 2% | 9 mm | 10 mm | 10 mm | 9.65±0.577 |
| Ofloxacin (1mg/ml) | 22 mm | 20 mm | 25 mm | 22.33±3.056 |

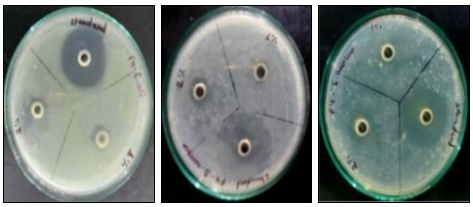

**Fig 2:** Antimicrobial activity against *S. aureus*

The values are mean±SD n=3

**Table 5:** Antimicrobial activity of extract against *E.coli*

| Sample | Plate 1 | Plate 2 | Plate 3 | Mean±SD |
|---|---|---|---|---|
| 1% | 11 mm | 10 mm | 9 mm | 10±1 |
| 2% | 14 mm | 13 mm | 14 mm | 13.66±0.577 |
| Gentamycin (1mg/ml) | 25 mm | 26 mm | 24 mm | 25±1.487 |

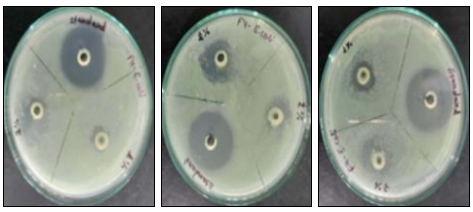

**Fig 3:** Antimicrobial activity against *E.coli*

The values are mean±SD n=3

**Table 6:** *In vitro* SPF Determination

| S. No | Wavelength in nm | EE(λ) X I (normalized) | CA (absorbance) 40µg/ml | CA (absorbance) 50µg/ml | CA (absorbance) 60µg/ml |
|---|---|---|---|---|---|
| 1 | 290 | 0.0150 | 0.1518±0.019 | 0.1782±0.025 | 0.1834±0.061 |
| 2 | 295 | 0.0817 | 0.1435±0.021 | 0.1661±0.016 | 0.1765±0.028 |
| 3 | 300 | 0.2874 | 0.1361±0.032 | 0.1553±0.041 | 0.1675±0.035 |
| 4 | 305 | 0.3278 | 0.1282±0.069 | 0.1437±0.029 | 0.1578±0.061 |
| 5 | 310 | 0.1864 | 0.1135±0.023 | 0.1366±0.056 | 0.1472±0.027 |
| 6 | 315 | 0.0837 | 0.1110±0.051 | 0.1253±0.016 | 0.1362±0.013 |
| 7 | 320 | 0.0180 | 0.1098±0.023 | 0.1216±0.021 | 0.1301±0.011 |
| SPF value | | | 1.275 | 1.461 | 1.582 |

The values are mean±SD n=3

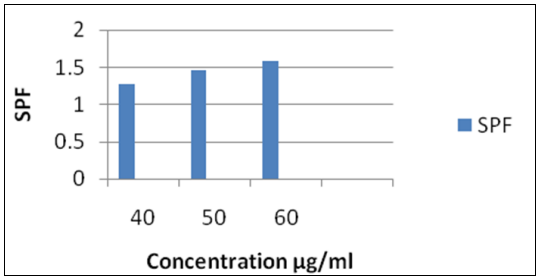


**Fig 4:** *In vitro* SPF value against concentration

## Discussion

The results of DPPH scavenging activity of *Centella asiatica* extracts exhibited percent inhibition 77.15% compared to standard ascorbic acid value of 87.41% and the $IC_{50}$ value was found to be 35.32 µg/ml for *Centella asiatica* compared to ascorbic acid $IC_{50}$ value of 10.04 µg/ml. The antibacterial potential has been evaluated against *S. aureus* and *E. coli* and the results revealed the maximum zone of inhibition of 9.65 mm against *S. aureus* and 13.66 mm against *E. coli* were found at 2% concentration, while the standard drug ofloxacin and gentamycin exhibited the maximum zone of inhibition 22.33 mm and 25 mm 1mg/ml concentration respectively.The sun protection effect (SPF) value of the extracts has been measured at three different concentration 40 µg/ml, 50µg/ml and 60µg/ml has shown the SPF value of 1.275, 1.461, 1.582 respectively. The results of SPF indicated increase in SPF value with increase in the concentration of extracts.

## Conclusion

Increasing demand of herbal products inclined to explore the new potential substance from natural origin that could give better and safer alternative and efficiently used in various formulation. In this study we have tested the efficiency and potential of newer effects of *Centella asiatica* extracts that might be useful to explore the plant in the newer area of herbal formulation. The results indicated that the *Centella asiatica* extracts given satisfactory results on the parameter tested and could be used as anti-ageing, antibacterial and antioxidant potential. Further study needed to refine the extract by using isolated components and some more pharmacological evaluation needed that could broader the effect of drug.

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