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Research Article

Botany for better natural therapy

Screening of Antioxidant, Antimicrobial and Phytochemicals Composition of Various Plant Parts of *Cichorium Intybus*

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Abstract: The present study was conducted to evaluate the antioxidant and antimicrobial potential as well as the phytochemical composition of root, seed and leaves of *Cichoriumintybus*. The antioxidant activity was analyzed by detecting the 2, 2-Diphenyl-1-picrylhydrazyl DPPH radical scavenging potent of the constituents found in various plant parts of *Cichorium intybus*,whereas for analyzing in-vitro antimicrobial activity, agar well diffusion method has been opted. The antimicrobial activity of plant was tested against both pathogenic as well as non-pathogenic species of bacteria by analyzing the corresponding inhibition zone exhibited by various extracts of plant parts against the bacterial growth. Apart from this, the present study also involves the quantitative phytochemical evaluation of total ascorbic acid in root, seed and leaves of *Cichoriumintybus*. The antioxidant potential of the plant was found to be comparatively lower in seed and leaves of plant than in root in accordance to the determined IC50 value . From the antimicrobial activity analysis, it was concluded that majority of times the non-polar extracts of all plant parts were found to be more effective in inhibiting growth of bacteria. Most significant activity has been exhibited against gram negative bacteria *Pseudomonas aeruginosa*. The ascorbic acid content in plant was found in the range of 2.253±0.867 to 12.594±2.05µg/ml. From the results of the conducted research, the importance of plant as an antimicrobial and anti-oxidative agent has been established. So, the plant can further be introduced as a potential drug for herbal medicine in the treatment of human disorders.

Keyword: *Cichorium intybus*, Antioxidant, DPPH, Agar Well Diffusion Assay, Ascorbic Acid

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1. INTRODUCTION

Antioxidants are basically the compounds that have the capability to terminate the oxidative damage imposed upon the human body by the free radicals or highly reactive oxygen species and reduce the risk of various detrimental diseases.[1] Free radical/reactive oxygen species forms as a result of either endogenous process that occurs inside the body (biotic stress) or due to deleterious and damaging effects caused by exogenous chemicals (abiotic stress). The radicals can oxidize the bio-molecules i.e. proteins, lipids, nucleic acids that can result in serious degenerative diseases like cancer, arthritis, cirrhosis, emphysema, neurological disorders, chronic diseases, atherosclerosis.[2,3] All the organisms have developed a certain internal defensive mechanism to save from the effect of oxidative damage caused by these radicals by the help of antioxidant components like flavonoids, ascorbic acid, tocopherol, glutathione, phenols, etc. or the antioxidant enzymes viz. catalase, peroxidase, dismutase, etc. Antioxidants can be provided to the body through dietary antioxidant supplements. Most of such dietary sources of antioxidants were derived from plant and have various physical and chemical properties.[4] The primary derivative sources for antioxidants such as vegetables, fruits, food grains, etc. [5] in the form of vitamin C, vitamin E, carotenes, and phenolic acids have a great potential in reducing the risk of disease occurrence.[6] It has become a necessity that drugs and pharmaceutical products from the medicinal plants must be identified, isolated and characterized for their pharmacological potential so as to replace and expand the available sources apart from the traditional system of medicines that are used till date. *Cichorium intybus*is, a herbaceous perennial plant that belongs to *Cichorium* genus of family Asteraceae. The genus comprises 6 species that were used as traditional medicine in Europe and Asia. *Cichorium intybus* is a plant traditionally used as food and medicinal crop in temperate parts of the old world [7,8] and as carminative, and against other cardiac ailments as well.[9] Chicory has been found to be a potential anti-cancer,[10] anti-malarial, anti-microbial[11], anti-diabetic[12] and is considered as a potent source of natural antioxidants [13]. Chicory has also been found to be very effective in case of severe jaundice, gout, asthma, and rheumatic conditions.[14] Inulin extracted from the root of plant is being used as a substrate of fiber in health and functional foods.[15] Inulin is also used as pre-biotic that helps in gastrointestinal infection inhibition and boosts the immune system.[16] Besides, being popularly cultivated and used widely, not much research on phytochemical analysis and antioxidant potential of cultivated chicory has been done. The present research was designed to fulfill the lack of scientific evidences on the pharmacological potential of the cultivated cichory plant. In the present study the antioxidant and antimicrobial activities of sequential (non-polar to polar) extract of root, stem and leaves of *C. intybus*. The phytochemical screening for the determination of total ascorbic acid content of different plant parts of *C. intybus* was also done phenolic content. The current research was done for providing further step on providing a clear justification of the ayurvedic and traditional folklore use of this plant in medicinal practices.

2. MATERIAL AND METHODS

2.1 *Chemical Reagents*

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), BHT (Butylated hydroxytoluene), ascorbic acid, methanol, acetate buffer (pH-3.6), Folin-ciocalteau phenol reagent, sodium carbonate, hydrogen peroxide, phosphate buffer, Nutrient Agar, Pet ether, Chloroform, Acetone, Spirit, DMSO (dimethyl sulfoxide), metaphosphoric acid, 2,4-dinitrophenylhydrazine, Thiourea, Copper sulphate, Ascorbic acid.

2.2 *Plant material*

Dry roots and whole plant of *C. intybus* were collected from semi-arid areas of Gujarat and was deposited for identification and authentication at Herbarium Botany Department, University of Rajasthan, Jaipur, Rajasthan, provided the voucher specimen number RUBL-211705. Fresh samples of whole plant have been collected. Collected plants were then allowed to dry under shade at room temperature. Different plant parts were then separated and were mashed into fine powder with the help of grinder.

2.3 *Extraction Preparation*

About 50 gm of powdered material of root, leaf and seed were taken and soaked in 150 ml of each of the following solvents one after the other in order of their increasing polarity sequentially- Pet Ether, Chloroform, Acetone, Spirit, and Water for 2 weeks. It is then shaken well 2-3 times a day and then filtered. The filtrate was then allowed to dry or evaporate in incubator at 40°C. All the extracts were dissolved in DMSO making the concentration of the extract 100 µg/ml, and then they were stored in clean and sterile glass vials. The concentration of the DMSO used was tested for its ineffective nature on the growth of any bacteria tested for the analysis

2.4 *Bacterial Specimen*

Four bacterial strains *E.coli*(MTCC 1652), *Bacillus subtilis*(MTCC 0121), *Pseudomonas aeruginosa*(MTCC 4646),*Staphylococcus aureus*(MTCC 0087)have been taken against which the antimicrobial potential of various plant extracts were tested. These bacterial strains were cultured and stored in nutrient broth. All the bacterial strains were obtained from S.M.S. Medical College, Jaipur.

2.5 *Antibacterial Assay*

Different solvent extracts of *C. intybus* were tested for their antimicrobial potential via disk diffusion method by measuring the inhibition zone exhibited. Soluble solvent fractions were subjected for screening of antimicrobial potential against four different bacterial strains. Extract/fractions were dissolved in 10% sterile dimethyl sulfoxide. Agar Well diffusion method was used for determining the antimicrobial bioassay. The bacterial colonies were inoculated in nutrient broth. Bacterial specimens were spread over the solidified and cooled agar plates after appropriate dilution with saline solution. On the inoculated agar plates wells of 5mm were made by using sterile borer. The discs (6 mm in diameter) were filled with 50 mg/mL concentration extract/fractions (60µL/disc) placed on the inoculated agar. The plates were then incubated at 40°C. After 24 hrs the bacterial growth was checked and the antibacterial activity were measured as the diameter of the zone of inhibition exhibited by different solvent extracts and compared with the zone of inhibition exhibited by the standard antibiotic.

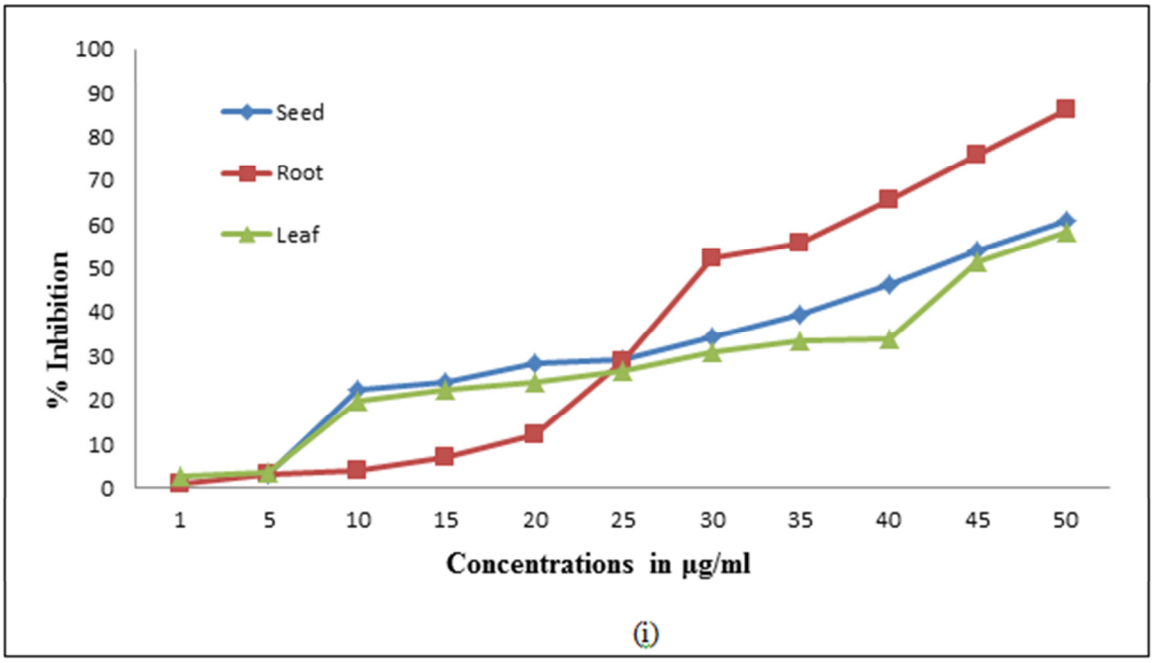
2.6 *Antioxidant Potential Analysis*

2.6.1 *DPPH Assay*

DPPH assay has been used for evaluation of the free radical scavenging activity of methanolic extracts of seed, root and leaves of *Cichorium intybus* plant. In this method, 0.1 mM solution of DPPH was prepared in ethanol. This solution (1 ml) was added to 1 ml. of different concentrations (1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/ml) of extracts in methanol. Various concentrations of the extracts were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 20 min. Then the absorbance of the extract was measured at 517 nm via a UV-Vis spectrophotometer.[17] Standard compound being used as reference was ascorbic acid. The radical scavenging activity change in pattern on the basis of increasing concentration expressed by extracts of different plant parts and standards like BHT- Butylated hydroxy toluene and ascorbic acid have been shown in figure 1. The IC50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using interpolation method from the obtained regression curve. The lower the absorbance of the reaction mixture indicated the higher will be the free radical activity exhibited. The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%) or Percent inhibition} = \frac{A0 - A1}{A0} \times 100$$

A0→ the Absorbance of control reaction
A1→Absorbance of test or standard sample.



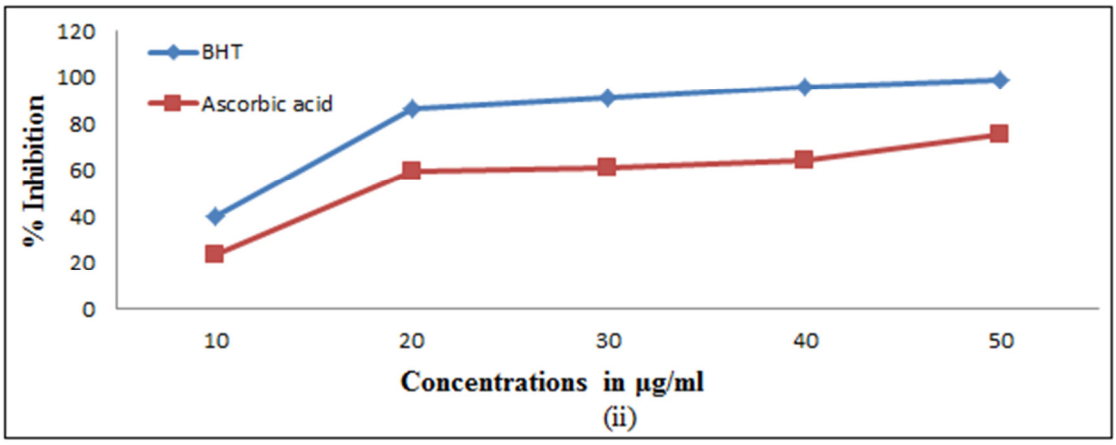


Fig1.Free radical scavenging activity of different concentrations of (i) Methanolic Extract of *Cichorium intybus* (ii) Standards- BHT and Ascorbic acid

2.7 *Total Ascorbic acid content*

Total ascorbic acid in the plant extract has been estimated by applying slight modifications in the colorimetric method[18]. According to this method, homogenization of plant extract was done in 20 ml of freshly prepared metaphosphoric acid. Afterwards, the homogenized extract was centrifuged at 2500 rpm for 10 minutes. Supernatant was collected of which 1.2 ml was taken and 0.4 ml of 2,4-dinitrophenylhydrazine-Thiourea-Copper sulphate reagent was added. The mixture was incubated for 2 hours at 37° C and then chilled for 10 min. To each test tube 6 ml of cold sulfuric acid (12mol / l) was mixed. Then the solution was divided into three separate test tubes of 3.6 ml each and the optical density of the mixture was measured at 520 nm through spectrophotometer. 1.2 ml of metaphosphoric acid 6.0 gm/dl concentration was stated as blank. The concentration of the test samples were computed from the standard calibration curve of the ascorbic acid given in figure 2.

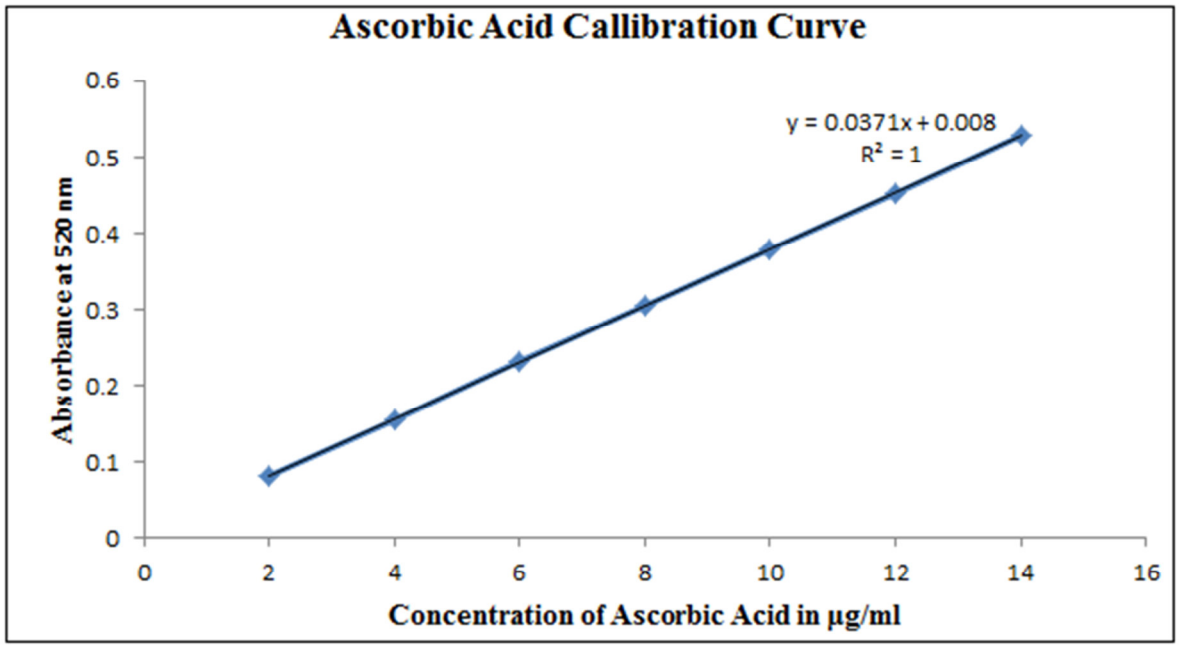


Fig2. Standard Calibration curve of Ascorbic Acid

3. STATISTICAL ANALYSIS

The amount of extract required to inhibit 50% of the free radicals concentration (IC50) was estimated graphically using a linear regression analysis. Data for minimum inhibitory concentration (MIC) were expressed as mean ± SD. All other data were expressed as mean of three independent analyses.

4. RESULTS

4.1 *Antibacterial Activity*

Root, stem and seed extract of *C. intybus* were tested for the antimicrobial potential found in the extract against various pathogenic and non-pathogenic gram-positive and gram negative bacteria. The activity was analyzed on the basis of potential of an extract in inhibiting the growth of tested bacteria via agar well diffusion method. The zone of inhibition exhibited by the sequential extracts of different plant parts was measured. The antimicrobial potential of Acetone and water extract of leaf, chloroform, spirit and water extract of root, and pet ether extract of seed against *E. coli*; acetone extract of leaf and seed, chloroform extract of root and seed and pet ether extract of seed against *P. aeruginosa*; chloroform extract of leaf, pet ether and water extract of root against *B. subtilis*; and chloroform extract of root and seed against *S. aureus* were found to have highly significant antimicrobial potential. The results of antimicrobial activity has been tabulated in table.1

Table 1. Antibacterial activity of various extracts of different plant parts of Cichoriumintybus

Name of the bacteria	Zone of Inhibition and Activity Index of the extracts				
	Pet ether	Chloroform	Acetone	Spirit	Water
	ZI	ZI	ZI	ZI	ZI
Leaves					
E.coli	15	18	20	14	21
P. aeruginosa	12	17	20	18	18
B. subtilis	+/-	20	12	18	14
S. aureus	15	12	16	10	+/-
Root					
Name of the bacteria	**Zone of Inhibition and Activity Index of the extracts**				
	Pet ether	**Chloroform**	**Acetone**	**Spirit**	**Water**
	ZI	**ZI**	**ZI**	**ZI**	**ZI**
E.coli	14	20	15	20	20
P. aeruginosa	19	22	18	17	15

B. subtilis	20	16	17	16	20
S. aureus	12	20	22	18	12
Seed					
Name of the bacteria	**Zone of Inhibition and Activity Index of the extracts**				
	Pet ether	**Chloroform**	**Acetone**	**Spirit**	**Water**
	ZI	**ZI**	**ZI**	**ZI**	**ZI**
E.coli	20	16	14	14	+/-
P. aeruginosa	22	20	18	20	15
B. subtilis	15	12	16	10	+/-
S. aureus	18	23	16	12	12

4.2 *MIC Determination*

MIC is the minimum concentration of the sample which is required for exhibiting any type of inhibition activity against the microbes. The MIC of chloroform and methanol extract of the root, seed and leaf were tested. The highly significant MIC was found in chloroform extract of root and methanol extract of seed against *E. coli*; root methanol extract against *P. aeruginosa*; methanol extract of leaf against *B. subtilis* and chloroform extract of root and leaf against *S. aureus*. The results of MIC analysis have been tabulated in table 2.

Table 2MIC determination of Chloroform and Methanol samples of roots leaves and stem at concentrations ranging from (50 to 200 µg/ml)

Bacteria	**Minimum Inhibitory Concentration**					
	Root		**Seed**		**Leaves**	
	Methanol [µg/ml]	**Chlorofom [µg/ml]**	**Methanol [µg/ml]**	**Chloroform [µg/ml]**	**Methanol [µg/ml]**	**Chloroform[µg/ml]**
E. coli	122.75±3.39	74.3±9.25	80.4±1.26	125±7.70	145±6.57	98.4±1.115
P. aeruginosa	88.2±5.793	104.8±4.46	98.5±8.87	92.46±6.64	95.8±3.4	89.5±2.91
B. subtilis	156.25±3.37	188±5.87	194±1.35	193±7.98	64.23±0.9	169±4.87
S. aureus	108±2.512	79.36±2.33	160±5.56	110±1.76	89±0.87	153±2.26

Values are mean± S.D. (standard deviation) of 3 observations (p<0.05)

4.3 *Antioxidant Activity Analysis*

The potential to eradicate the antioxidant radical found in different plant parts of plant *C. intybus* was measured and expressed in terms of their respective IC50 values(the concentration of extract at which the radical scavenging potential reaches half the potential activity). The IC50 value in plant ranges from 29.54±0.83µg/ml to 44.55±1.365µg/ml. The leaves show higher IC50 value, referring to the higher concentration of extract required for reaching the 50% of antioxidant potential whereas the root extract was found to be the most effective due to the least IC50 values (table 3). Apart from that all the extracts show lower DPPH scavenging activity than the standard BHT and ascorbic acid. All the remaining extracts show only a moderate amount of activity against the activity exhibited by standard antioxidants.

Table 3 *IC50 value of DPPH radical scavenging assay and total ascorbic acid content*

Sample Material	**IC50 value (µg/ml)**	**Total AscorbicAcid Content (in µg/ml)**
Root	29.54 ± 0.83	12.594±2.05
Leaves	44.55 ± 1.365	9.7096±1.799
Seed	42.30 ± 0.45	2.253±0.867
BHT	19.15 ± 1.12	-
Ascorbic Acid	17.37± 0.278	-

Values are mean± S.D. (standard deviation) of 3 observations (p<0.05)

4.4 *Ascorbic Acid Content*

The total ascorbic acid concentration of the sample material was computed from standard calibration curve of Ascorbic acid prepared in different ascorbic acid concentration at 520 nm wavelength in a UV-Vis spectrophotometer. The highest amount of ascorbic acid concentration was exhibited by root extract of *C. intybus*. The results of total ascorbic acid concentration exhibited by different plant parts of *C. intybus* were tabulated in table 3.

5. DISCUSSION

From the present investigation, it was concluded that *C. intybus*root extracts exhibit comparatively higher radical scavenging activity, with IC50 (the concentration of extract providing 50% inhibition) values of 29.54 ± 0.83 µg/mL.when compared with the synthetic antioxidant BHT (IC50=19.15±1.12 µg/mL) and ascorbic acid (IC50=17.37± 0.278µg/mL), all the extracts offered slightly lower antioxidant activity.These research findings were in contrast to the previous research result conclusions of methanol extract to be a greater antioxidant potential than ascorbic acid.[19,20] From the results obtained from the current research work, a positive correlation has been found between the total ascorbic acid content and the antioxidant activity attributed to the hydroxyl groups present in these compounds due to their hydrogen donating ability.Phenolic compounds and ascorbic acid majorly plays an important role in imparting potential antioxidant activity in preventing oxidative cell damage to the extract.The antioxidant potential of *C. intybus* is attributed to the presence of different

phytochemicals in the plantsuch as sterols, volatile oils andphenolic acids. With the variations in the concentration of phenolic compounds, DPPH scavenging activity varies accordingly hence does the antioxidant activity exhibited by the extract of the plant[21]. In addition, conducted research suggested that polar extracts of leaves and root are found to be effective against gram positive bacteriawhereas the non-polar extracts like petroleum ether and chloroform extracts of *C. intybus* seeds exhibit comparatively higher inhibitory activity against growth of both gram positive and gram negative bacterial. The difference in less effective antimicrobial effects towards the gram negative bacteria is due to the difference and complexity of the cell wall morphology of the gram negative bacteria.The results obtained were in agreement to the research findings that the antimicrobial activity of the methanol extract *C. intybus* exhibits broader antimicrobial spectrum compared with the standard antibiotics gentamicin and tobramycin., the leaves and root extracts have significant activity against S. aureus, *B. subtilis* and *P. aeruginosa* and also the comparative exhibited zone of inhibition was found lower against *E. coli*[22]. The research results may further suggests potential of plant to be used as a source of natural antimicrobial agent. Significant antibacterial activity of root extracts of *C.intybus* has been reported against *E. coli*, *B. subtilis* strains where all the tested extracts showed antibacterial activity[23]. These results are in agreement with our analysis. Overall *C. intybus* seeds showed moderate antibacterial activity. The antimicrobial activity and the medicinal importance of *Cichorium intybus* has been experimentally proved due to presence of a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins[24]. These bioactive compounds have already been reported to impart antimicrobial potential against thetested microbes[25-27].

6. CONCLUSION

The methanolic extract of root exhibited significant antioxidant activity rather than other plant parts. In conclusion, all the *C. intybus* extracts showed significant potential antimicrobial activity against tested microorganism, but *E. coli and P. aeruginosa* were found to be most sensitive and had the widest zone of inhibition. It was observed that the non- polar extracts of seed showed significantly higher antimicrobial activity against all tested microbes except against *B. subtilis* for which it showed moderate effect. Aqueous extract of leaves shows significant activity against *E.coli* and *P. aeruginosa* whereas aqueous extract of root shows activity against *E. coli and B. subtilis.* The results obtained from the current research work contributes to the findings that, chicory contains higher concentration of water soluble compounds (inulin, flavonoids, etc.) that may be responsible for showing significant inhibitory effect on microbial growth which was not exhibitedby other extracts.A comparison among the results leads us to the conclusion that constituents of this plant extract may serve as a source of drugs useful in the chemotherapy of some infections caused by bacteria and also as an antioxidant agent.

7. ACKNOWLEDGEMENT

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8. AUTHORS CONTRIBUTION STATEMENT

Miss. Maitry Choudhary conceptualized and gathered all the results and data by performing all the required experiments by her related to this work. Dr. R. A. Sharma provided necessary inputs and guidance for the concerned work. All the authors discussed the methodology, results and data interpretation and analysis method and contributed the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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