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COMPARING ANTIBACTERIAL POTENTIAL AND PHYTOCHEMICAL CONSTITUENTSOF TWO SPECIES OF GENUS *URTICA*

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ABSTRACT

The present study was conducted to evaluate and compare the *in-vitro* bioactive potential of various organic extracts of the root, stem and leaves of *Urtica dioica* and *Urtica urens* on growth of tested Gram Positive (+ve) and Gram negative (–ve) bacteria.The present investigation also includes the comparative chemical constituency of the crude extract of root, stem and leaves of both plants. The comparative bioactive potential of *Urtica dioica* and *Urtica urens* was tested against both pathogenic and non-pathogenic strains of bacteria that are Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Pseudomonas aeruginosa* and *E. Coli*) conducted by using agar well diffusion method .The Minimum Inhibitory Concentrations (MICs)-the lowest concentration of antimicrobial agent that will inhibit any visible growth of microbein chloroform and methanol extracts of root, stem and leaves of both plants was determined using micro-well dilution method. For the phytochemical evaluation of plants metabolites- ascorbic acid, flavonoids, phenols and proteins has been quantitatively estimated. The MIC has been to be most effective in case of methanolic extract of root against both gram positive and gram negative bacteria. Significant antimicrobial activity has been observed against gram negative bacteria *E. coli* and both the gram positive bacteria testedexpressed by *U. dioica* comparatively more effective than *U. urens*. Only a moderate amount of activity is exhibited against *P. aeruginosa* by both the plants. The non-polar extracts were found to have comparatively higher bactericidal potential than the polar extracts. The phytochemicals analysis suggested the presence of ascorbic acid, phenols and proteins to be found in *U. urens* in significantly higher concentration than *U. dioica*. On the other hand, *U. dioica* has been found to be more abundant in flavonoid concentration comparatively.

KEYWORDS: ***Antibacterial efficacy, Urtica dioica, Urtica Urens, Agar well diffusion assay, Minimum Inhibitory Concentration (MIC), Phytochemicals constituency.***

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INTRODUCTION

A vast source of medicinally active plants has been provided for the benefit of human beings by the environment.[1] Thousands of modern drugs are isolated from these vast natural sources from the knowledge and information of medicinal uses of herbs that are scripted in our ancient literature of Ayurveda, Charak samhita.[2] Researchers are continuously exploring the potentials of different plants and their products for expanding the use of natural sources for isolating active biological compounds making them suitable for pharmacological usage.[3] The genus *Urtica* is derived from the word 'euro' which means to burn or 'urere' to sting. [4]*Urtica dioica* (wild inhabitant), and *Urtica urens* (wild as well as cultivated in recent years) belong to family Urticaceae of order Rosales is commonly known as stinging nettle and dwarf or burning nettle, respectively. The names have been provided probably on the basis of characteristic property of being covered with stinging hairs that introduce irritants onto the human skin on coming in contact with the plant resulting in persistent pain and burning sensation afterwards. The potential of this plant for long remained far from acknowledgement and has remained undervalued till now[5]. But now-a-days the plants, especially U. urens has started gaining significance due to its pharmacological potential. [6] *U. dioica* plant is a perennial shrub, erect in nature, 1-1.5 m tall in height armed with stinging hairs. Leaves are found in opposite arrangement and are 7-15 cm long. Stinging hairs of *U. dioica* are trichomes that are usually found distributed along its leaves and stem, that acts as hypodermic needles which on coming in contact with skin injects chemicals like acetic acid, histamine, etc. that causes a stinging sensation.[6-8]*Urtica urens* is an annual herb with erect plant of ascending type up to 2 feet tall about 60 cm in height with a tap root system. Leaves on the plant found are oval with opposite arrangement up to ½ to 2 inch or 4 cms long, sharp, tipped leaves that are densely toothed. The irritant hairs present on the leaves and stem of plant cause irritant dermatitis.[9]Traditionally the plant stinging potential has been used in therapies known as urtication, which is believed to treat the numb arthritic and paralytic limbs by flailing with a fresh plant.[10] This stimulates blood circulation in the area and brings warmth to joints. Stinging nettle is currently being used in the treatment of rheumatism as a diuretic agent. [11] It is utilized in relieving symptoms of seasonal allergies and also found helpful in reducing difficulties associated with urination problems in benign prostatic hyperplasia and also in regulating sugar levels in diabetic patients.[12]Phytochemicals are the metabolites of non-nutrient nature derived from plants found in various dietary substances, food grains, vegetables with an enormous potential of fighting the chronic human and animal disorders. These phytochemicals arise in the plant body through various in-built metabolic processes.[13] The phytochemicals analysis of genus *Urtica* has proved the presence of 100 different compounds like sterols, phenols, fatty acids, lignans, alkaloids, terpenoids and other compounds. Among all the species of the genus *Urtica*, the most important one regarding to its importance in ethno-pharmacological medicines is *U. dioica*.[14]The current investigation has been conducted to evaluate the comparative potential of the two different species of the genus *Urtica* to act against the bacterial growth and both nonpolar and polar extracts of *U. dioica* and *U. urens* were evaluated to compute the MIC exhibited by different plant parts-root, stem and leaves. Apart from screening the comparative bioactive potential of the plant, a comparative study on phytochemical constituency of crude extract of both plants has been carried out.

MATERIALS AND METHODS

Chemical Reagent

Petroleum ether**,** chloroform, acetone, methanol,distilled water, sodium chloride, nutrient agar, ciprofloxacin as standard, metaphosphoric acid, 2,4-dinitrophenylhydrazine, thiourea, copper sulphate, sulfuric acid, ascorbic acid, aluminium chloride, potassium acetate, quercetin, Folin–Ciocalteau reagent, caffeic acid, ethanol, Sodium Carbonate (Na_2CO_3), Tri-chloro Acetic acid (TCA), alkaline solution.

Collection of plant material and extraction of plant sample for antimicrobial potential estimation

Urtica dioica and *Urtica urens* fresh plant material were collected from the Dist.- Palampur (Himachal Pradesh) (July, 2016) and Village –Naggar, Dist-Kullu (H.P.) (May, 2017) respectively, and were authenticated by CSIR, IHBT Herbarium, Palampur(H.P.). Voucher specimen herbarium (PLP-18301) of *Urtica dioica* and (PLP-18302) of *Urtica urens* were deposited at IHBT, CSIR, Palampur (H.P.) herbarium. The plant was thoroughly washed with distilled water without pressing or crushing it to remove dirt and soil

particles. Root, Stem and leaves of plant were separated, shade dried and then powdered. The dried powder (200gm) was then extracted using different solvents- Petroleum ether, chloroform, acetone, methanol and water by soxhlet extraction apparatus. The solvent extract was prepared by dissolving 20 mg of powdered material sequentially in 100 ml solvent each. The solvent was allowed to get evaporated at room temperature spontaneouslyand the remaining dry crude extract from each of the solvent was weighed and then diluted in 100% DMSO at a concentration of 10 mg/ml.

Bacterial Strains

The strains of bacteria against which the effective potential of various solvents of plant parts was tested includes *Staphylococcus aureus* (MTCC 0087), *Pseudomonas aeruginosa* (MTCC 4646), *Bacillus subtilis* (MTCC 0121), *E. coli* (MTCC 1652). These bacterial strains are collected from S.M.S. Medical College, Jaipur, Rajasthan, India. These strains were maintained in sterile nutrient agar (Himedia) slants and were stored at 4ºC until further use .

Agar well diffusion assay

In-vitro antibacterial efficacy of different plant parts were tested using agar well diffusion method.[15] Following the provided protocol the medium used for bacterial growth was Mueller Hinton Agar Medium. The agar media was melted and cooled to 48-50ºC before pouring into plates. Plates were prepared by pouring 25 ml of freshly prepared agar media in sterilized 100mm X 15 mm plates. Plates were allowed to solidify.The solidified agar plates were then inoculated aseptically with various bacterial strains suspensions. The terget bacterial strain suspensions were freshly prepared by diluting the microbial culture to prepare a microbial concentration of 10^8 CFU/ml. Wells of 3mm radius were prepared in the inoculated agar plates.The analyte or the material under testing (60 µl each well) was then introduced in the wells (6 mm) prepared. These plates were then kept under incubation at 37ºC for 24 hours. The antibacterial potential were measured in terms of the diameter of the inhibition zone produced by the chemical composition of the analyte tested for the potential activity in comparison to that of antibiotic, ciprofloxacin of concentration 10 mg/ml taken as standard (40 µl each well). As the incubation period is over, the plates were analyzed for the inhibitory zones (ZI) measured in millimeters (mm). The extracts of *U. dioica and U. urens* exhibiting significant inhibitory effect on microbial growth were shown in figure 1 below.

$$\text{Activity Index (AI)} = \frac{\text{Zone of Inhibition of Sample}}{\text{Zone of Inhibition of Standard}} \quad (1)$$

The activity index of the extract would be determined using the above described formulae.

MIC determination

For the determination of MIC, micro-plate method was used.[16]The MICs of the chloroform and methanol extract of the root, stem and leaves of *U. dioica* and *U. urens* were determined. In this method TTC (2, 3, 5-triphenyltetrazolium chloride) visually indicates the presence of microbial growth. In each well of the 96-well micro plate, 5 µl inoculum and 95 µl of nutrient broth was added, to which 100µl extracts of concentrations 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml and 100 µl of TTC (0.01% w/v). The plates were incubated at 37 ºC for 24 hrs in incubator. In the presence of bacterial growth the TTC converts to red colored formazan which indicates the active viability of cells.

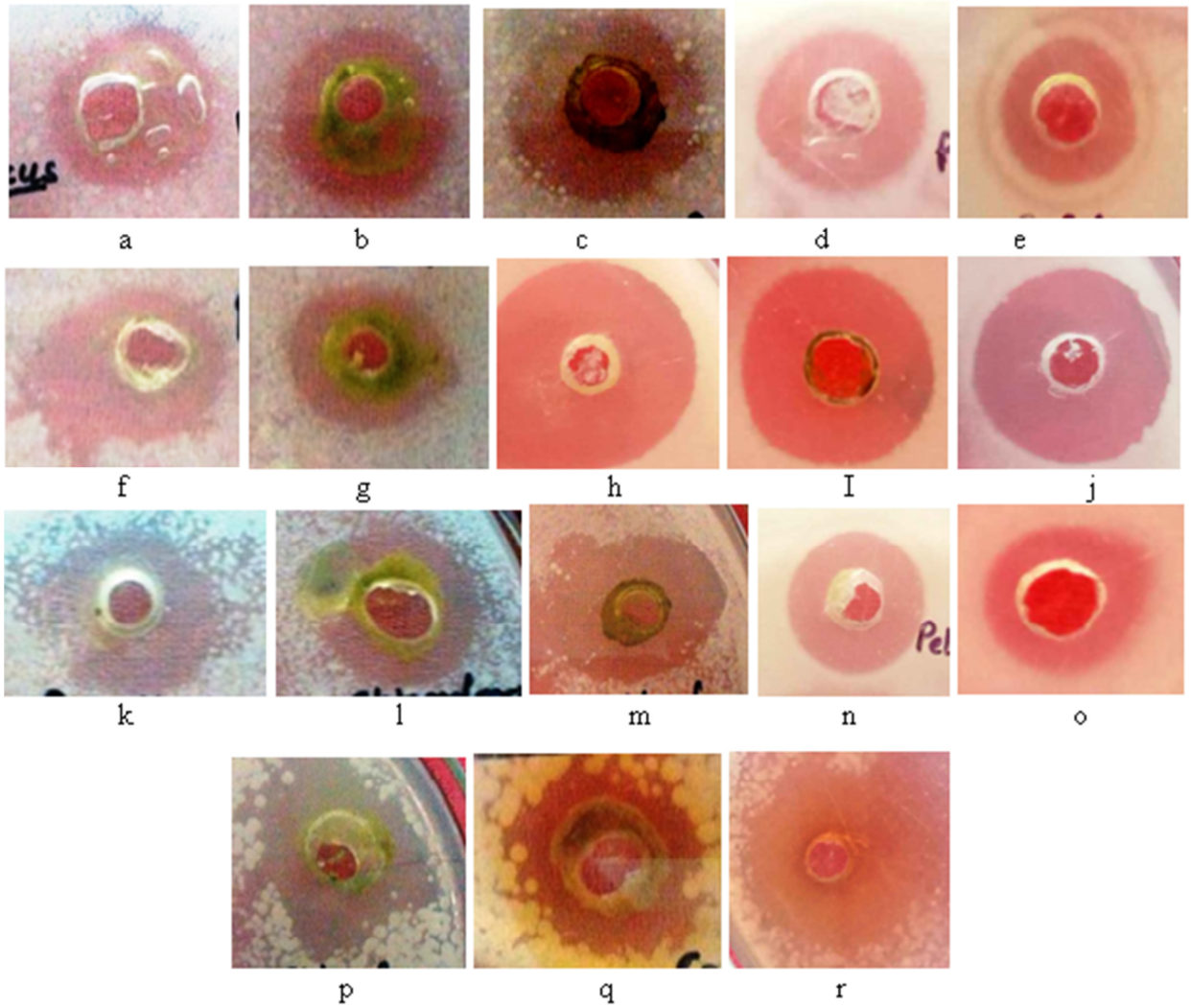


Figure 1

Illustration of zone of Inhibition (selected) exhibited by various organic extracts

a.S. aureus:* UD:S*(P)	***g. B. subtilis: UD: S (C)***	***m. E. coli: UD: L(C)***
b.S. aureus: UD: S(C)	***h. B. subtilis: UU: S(P)***	***n. E. coli: UU: S(P)***
c.S. aureus: UD: L(C)	***i. B. subtilis: UU: S (C)***	***o. E. coli: UU: R(A)***
d.S. aureus: UU: S (P)	***j. B. subtilis: UU: R(P)***	***p. P. aeruginosa:UD:S(C)***
e.S. aureus: U.U: S (M)	***k. E. coli: UD: S(P)***	***q.P. aeruginosa:UD:S(M)***
f. B. subtilis: UD: S(P)	***l. E. coli: UD: S(C)***	***r.P. aeruginosa:UU:R(M)***

***Key:* UD →*U. dioica,* UU →*U. urens* S→ Stem, L→ Leaves, R→ Root, P→ Pet Ether, C→ Chloroform, M→ Methanol, A→ Acetone.**

Phytochemical Analysis

The quantitative estimation of phytochemicals: ascorbic acid content, flavonoids, phenols and protein contentin different plant parts of *U. dioica* and *U. urens* was carried out The detailed procedure were described below.

Total ascorbic acid content

The total ascorbic acid content of powdered plant samples were estimated using a slight modification of the colorimetric method.[17] Each of the powdered plant material (500 mg) was homogenized in 20 ml of freshly prepared metaphosphoric acid in 6.0 gm/dl concentration. After 5 minute homogenized material was centrifuged at 2500 rpm for 10 minutes. Supernatant was collected in separate test tube of which 1.2 ml was taken and 0.4 ml of 2,4-dinitrophenylhydrazine-Thiourea-Copper sulphate reagent was added into it. The mixture was incubated for 2 hours at 37º C and then chilled for 10 min in an ice bath. To each test tube 6 ml of cold sulfuric acid (12mol/l) was mixed. Now 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 Figure 2 (a) of the ascorbic acid.

Total Flavonoid Content

Total flavonoids content of an extract was determined using aluminium chloride colorimetric method.[18] According to this method 200 µg/ml concentration of extract solution was prepared by adding 1 ml of methanol plant extract in 3 ml methanol, 200 µl of 10% $AlCl_3$ solution, 200 µl of 1M Potassium acetate solution and 5.6 ml of distilled water. Then the optical density of each prepared extract was measured at 415 nm. The solution prepared by substituting sample with 0.25 ml ethanol dissolved in 3 ml methanol, 200 µl of 10% $AlCl_3$ solution, 200 µl of 1M Potassium acetate solution and 5.6 ml of distilled water serve as blank. The concentration of the test samples were computed from the standard calibration curve of quercetin (Figure 2 (b)).The total flavonoid concentration is measured in terms of mg/100g QE (Quercetin Equivalent).

$$\text{Total Flavonoid Content} = \frac{R \times D.F. \times V \times 100}{W}$$

R = Concentration computed through standard curve of quercetin

V = Volume of stock Solution

D.F.= Dilution factor

100=for 100 gm dried plant

W= Weight of the plant used in experiment (in gm)

Estimation of total protein content

The total protein of the powdered extract has been estimated by using Lowry method.[19] According to this method, 10 gm of plant sample was weighed properly and homogenized in 50 ml of 10% TCA. After that obtained extract was centrifuged for 10 min at 15000 rpm in 4ºC, of which the supernatant was discarded and further pellet was dissolved in 5% TCA. Mixture was mixed in a vortex mixer and collected in a test tube. The collected mixture was kept in incubator at 80 ºC for 30 minutes. The mixture is allowed to cool at room temperature. From the sample extract mixture 5 ml is separated and to it is added 25 ml of alkaline solution and 2.5 ml of folin-ciocalteu phenol reagent and it is mixed properly. The optical density of sample mixture was taken at 750 nm via a UV-Vis spectrophotometer with 10% TCA taken as blank solution. A standard calibration curve of Bovine Serum Albumin (BSA) of varying concentration with their respective optical density readings at 750 nm has been used for calculating the concentrations of protein in different extracts provided in figure 2 (c).

Total Phenolic Content Determination

Phenolic content present in the samples was determined by using Folin–Ciocalteu reagent using caffeic acid as a standard phenolic compound.[20] According to this method 200 mg of plant sample crushed with 3ml of 80% ethanol was centrifuged for 20 minutes at 1500 rpm at room temperature. From the supernatant 1 ml is taken in the test tube to which 1 ml of one milliliter of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3min, 3ml of Na_2CO_3 was added which then was allowed to stand for 2h with intermittent shaking. The absorbance was measured at 750 nm in a spectrophotometer. The total concentration of phenolic compounds in the ethanol extract was determined as micrograms of caffeic acid equivalent by using an equation that was obtained from standard caffeic acid graph given in figure 2 (d).

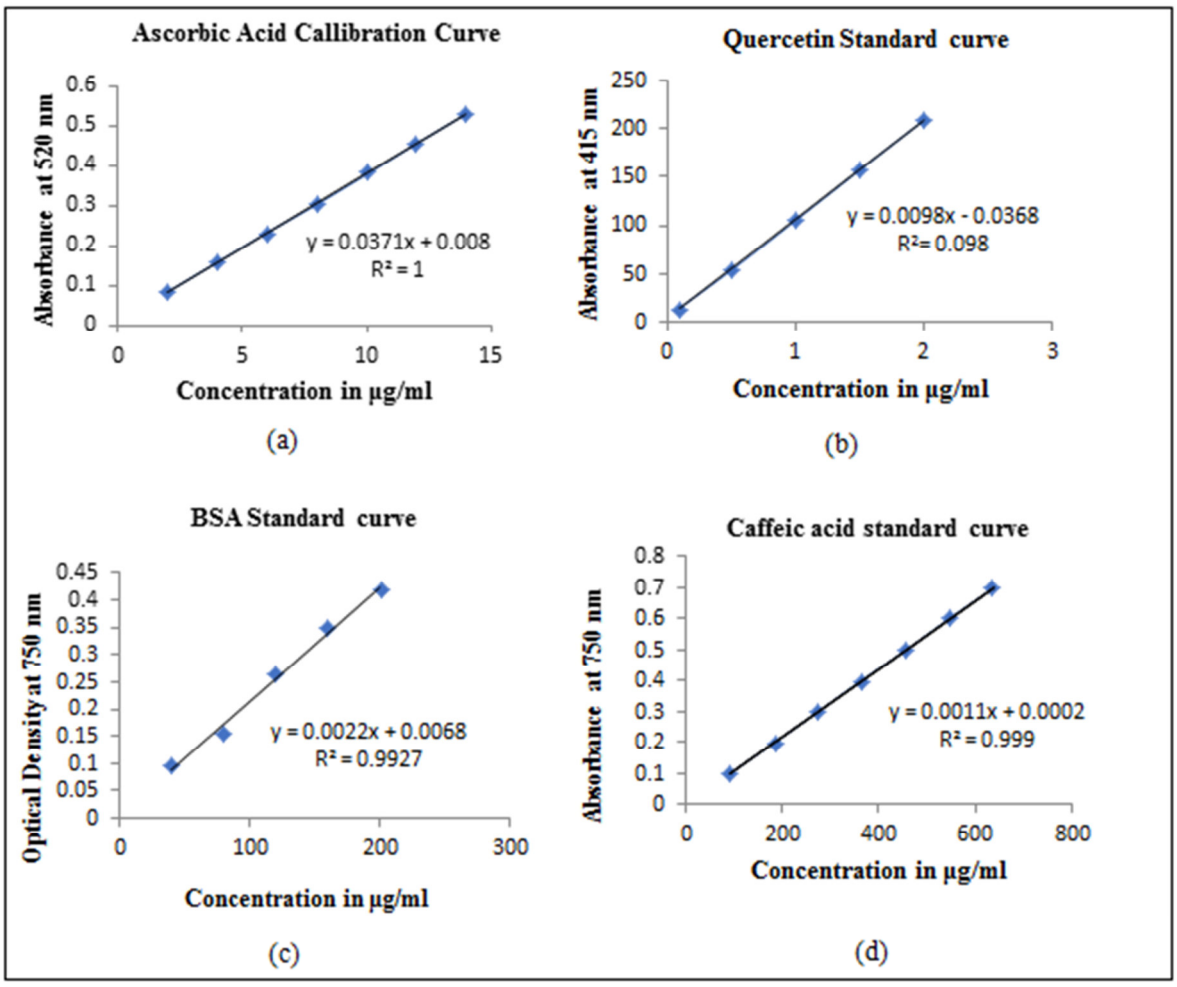


Figure 2

Calibration curves of (a) Ascorbic acid (b) Quercetin (c) BSA (d)Caffeic acid

STATISTICAL ANALYSIS

Statistical analysis of data was performed using minitab SPSS statistical subscription. One way ANOVA (Analysis of variance) was carried out for statistical analysis. Each experiment was repeated 3 times. Mean±Standard error was computed from analysis of each treatment. Data was presented in mean±SE format and were compared via Tukey's test at a level of 5% probability.

RESULTS

Antibacterial screening

The antibacterial assay of different extracts of root, stem and leaves of *U. dioica* and *U. urens* on observation after 24 hr incubation at 38 ºC exhibited various zones of inhibition with their respective activity index compiled in comparative graphs (Figure 3). From the above data obtained via agar well diffusion method, some of the extracts show high inhibitory activity against growth of various gram positive as well as gram negative bacteria.

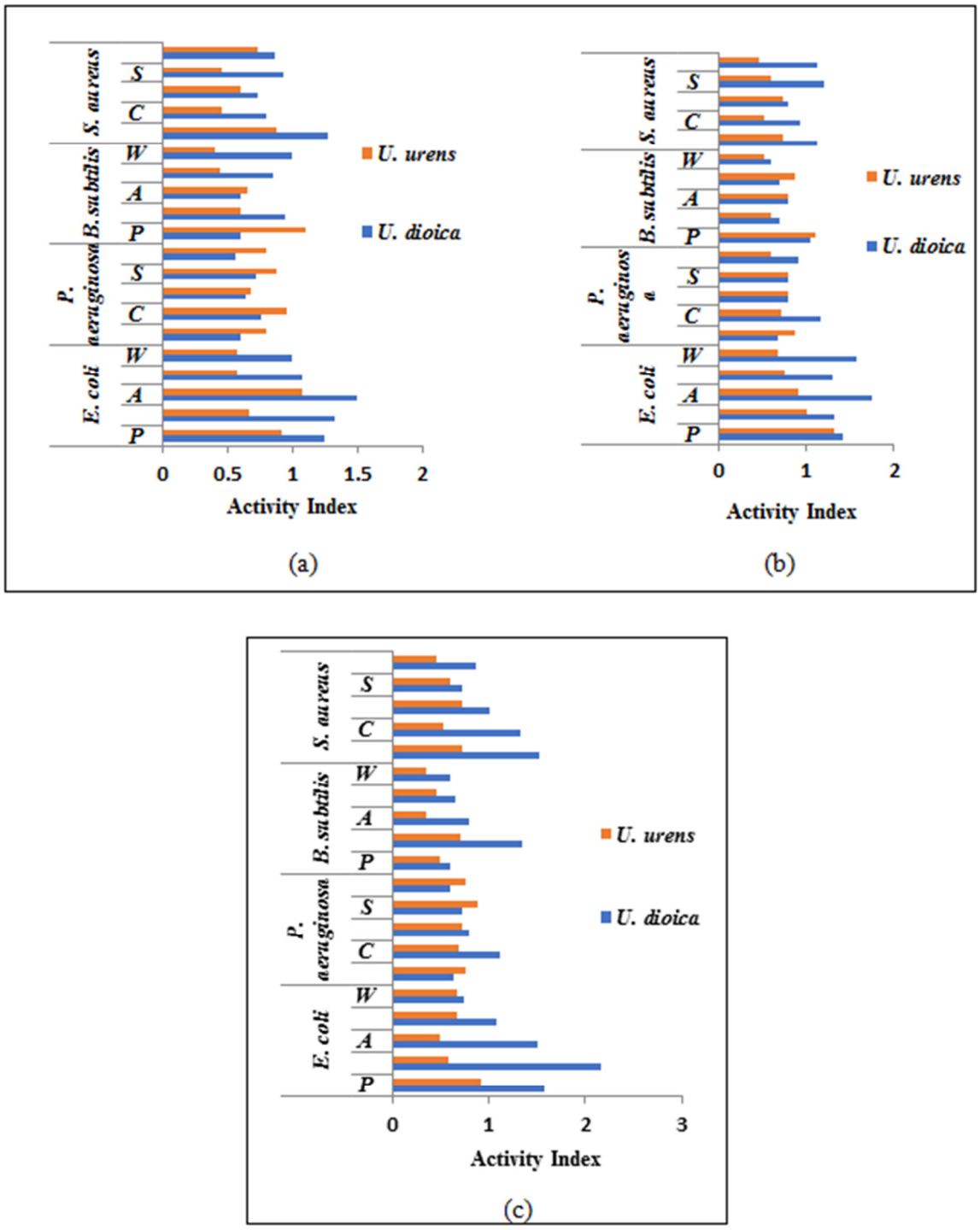


Figure 3

Graphs indicating comparative activity indices of U. dioica and U. urens exhibited by various organic extracts of (a) Root, (b) Stem and (c) Leaves

The significantly higher amount of inhibitory activity has been exhibited by petroleum ether extract of root and leaf of *U. dioica* and stem of *U. urens*, chloroform extracts of root and leaves of *U. dioica*, acetone extract of all the tested plant parts of *U. dioica* and root of *U. urens* while all the leaf extracts of *U. urens* shows moderate effect against gram negative bacteria *E. coli*. Significant inhibition effect was also observed from pet ether extract of root, stem and leaves of *U. dioica*, spirit and water extract of stem and leaves chloroform extract of *U. dioica* exhibits significant inhibitory activity against *S. aureus* (gram positive). The plant is found to be least effective against *P. aeruginosa,* except the chloroform extract of stem and leaves of *U. dioica* plant shows moderate inhibitory activity against the growth of *P. aeruginosa.* Pet ether extract of root, chloroform extract of stem and leaves, methanol extract of root, stem and leaves of *U. dioica* shows least significant inhibitory

potential against gram positive bacteria *B. subtilis*. On the other hand, chloroform extract of root, pet ether and chloroform extract of stem and aqueous extract of the leaves of both *U. dioica* and *U. urens* shows moderate inhibitory potential against both *B. subtilis* and *P. aeruginosa*. Apart from all the above observations an overall comparatively more effective inhibition on growth of all the tested microorganisms were impacted by *U. dioica* rather from *U. urens*.The methanol extract of the root of *U. dioica* showed significant level of inhibitory potential against all gram positive and negative bacteria taken for testing. *B. subtilis* has been found to be sensitive to both methanol and chloroform extract of stem at a concentration of 100 mg/ml. No significant difference has been found in the inhibitory potential of the methanol and chloroform extract of stem and leaves against *P. aeruginosa*.

MIC Determination

The methanol and chloroform found to be the most effective against most of the tested bacteria. Therefore both the extracts were examined for the minimum concentration required for antimicrobial growth. The result of MIC determined that methanol extract of root was active against all four strains of microorganisms at the lowest concentration examined i.e. 6.25 mg/ml as shown in table 1. The extracts that shows significant MIC values were chloroform extract of root against *B. subtilis*, methanol extract of stem and leaves against *E. coli*. The minimum MIC value was exhibited by root chloroform extract against *P. aeruginosa* and against *B. subtilis* by methanol extract of stem and leaves. All the remaining extracts showed an intermediate effect against remaining bacterial strains.

Table 1

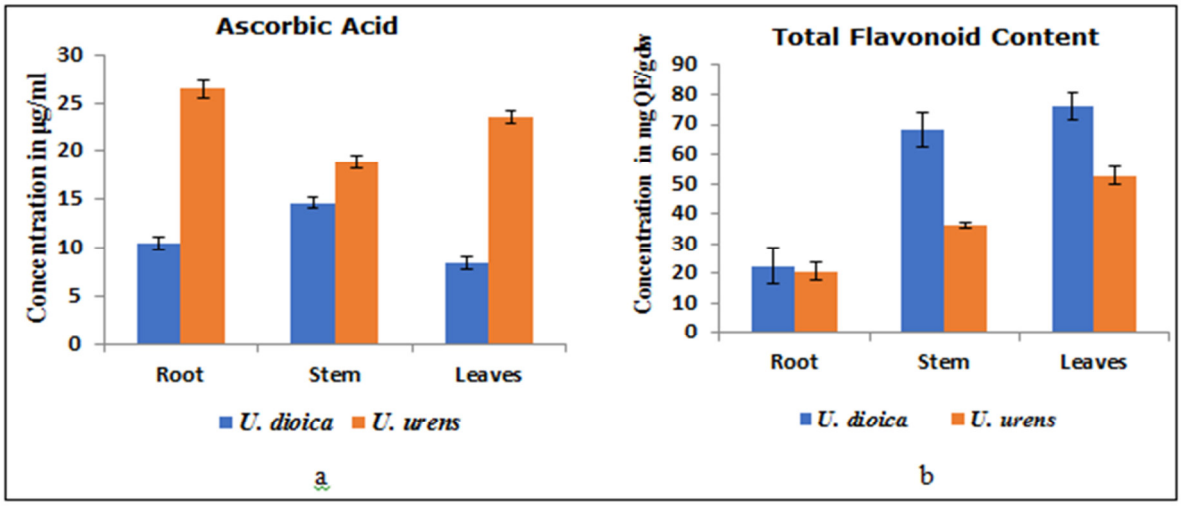
MIC determination of chloroform and methanol samples of roots leaves and stem at concentrations ranging from (6.25 to 100 mg/ml)

Name of the Bacteria	Minimum Inhibitory Concentration											
	Root				Stem				Leaves			
	U. dioica		*U. urens*		*U. dioica*		*U. urens*		*U. dioica*		*U. urens*	
	M	C	M	C	M	C	M	C	M	C	M	C
E. coli	6.25	12.5	NA	50	6.25	12.5	NA	100	6.25	50	NA	NA
P. aeruginosa	6.25	100	NA	25	12.5	12.5	25	NA	12.5	12.5	NA	12.5
B. subtilis	6.25	6.25	12.5	12.5	100	100	NA	NA	50	12.5	NA	NA
S. aureus	6.25	12.5	NA	NA-	12.5	25	NA	25	12.5	50	12.5	12.5

#'NA' → No activity .

Phytochemical analysis

Primary phytochemical investigation was done to determine the chemical composition of the different plant part crude extract. The comparative results of the appropriate quantitative estimation tests conducted were compiled in graphical format below in figure 4.



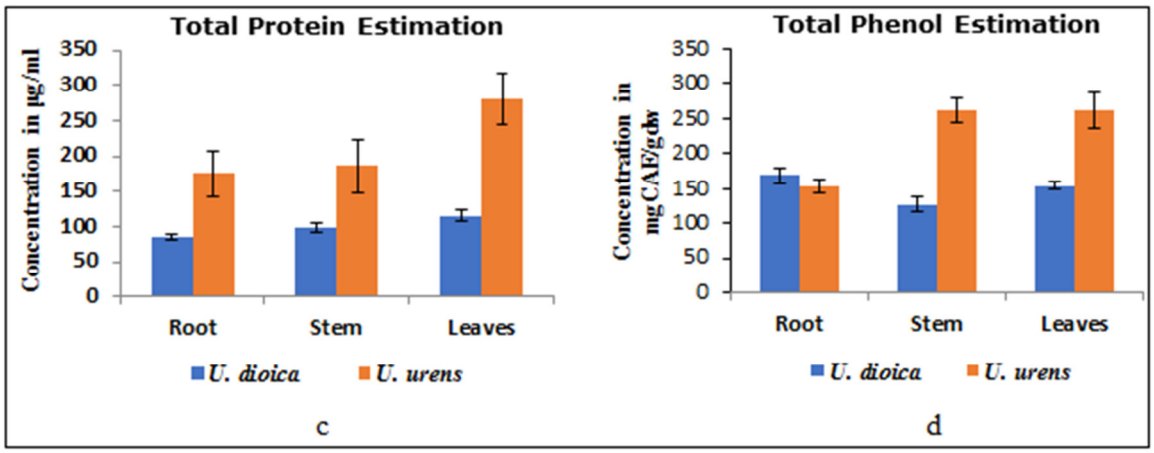


Figure 4

Comparative graphs of quantitative estimation of different plant parts of U. dioica and U. urens (a)Ascorbic acid (b) Flavonoids (c) Protein (d) Phenols

Ascorbic acid estimation

Ascorbic acid content found in crude powdered extracts in roots, stem and leaves of *U. dioica* and *U. urens* computed from the standard ascorbic acid calibration curve formed by observing optical density of standard ascorbic acid at varying concentration at 520 nm wavelengths via a UV-Visible spectrophotometer. The highest amount of ascorbic acid concentration 26.439±0.891 µg/ml was exhibited by root extract of *U. urens*.All the plant parts has higher proportion of ascorbic acid in their chemical composition.The results of total ascorbic acid concentration in different plant part extracts of *U. dioica* and *U. urens* were tabulated in table 2.

Protein Estimation

The total protein concentration of the sample material was computed from standard calibration curve of (Bovine Serum Albumin) BSA prepared in different concentrations at 750 nm wavelength in a UV-Vis spectrophotometer. Comparatively significant amount of protein concentration in their chemical constitution was shown by all plant parts of *U. urens* of which leaves shows the highest amount of 280.4±35.2 µg/ml whereas least amount 8.488±0.671 µg/ml was found to be available in the leaves of *U. dioica.* The obtained results were compiled in table 2.

Table 2

Total concentration of ascorbic acid and protein (in µg/ml)

	Plants	Root	Stem	Leaves
U. dioica	**Ascorbic acid Concentration (µg/ml)**	10.42±0.648^{a}	14.615±0.582^{b}	8.488±0.671^{a}
	Protein Concentration (µg/ml)	85.39±4.37^{b}	98.58±7.53^{a}	115.7±6.81^{c}
U. urens	**Ascorbic acid Concentration (µg/ml)**	26.439±0.891^{b}	18.919±0.642^{c}	23.555±0.628^{b}
	Protein Concentration (µg/ml)	175.4±32.4^{a}	185.5±38.42^{a}	280.4±35.2^{c}

 # Values are mean± S.E. (standard error) of 3 observations (p<0.05). Values marked with similar superscript a, b or c indicates lack of significant difference from each other

Total Phenolic and Total Flavonoid Content

Both the total phenolic and flavonoid concentration of different parts of plant are shown in table 3. The highest concentration of flavonoid has been reported from leaf extract i.e. 76.007±2.483 mg QE/100gdw while highest phenol concentration was reported from root extract i.e. 168.393±5.781 mg CAE/100gdw. The range of concentration of flavonoid and phenols found in *U. dioica* is 22.522±3.515to 76.007±2.483 mg QE/100gdw and 126.75±4.9321 to 168.393±5.781 mg CAE/100gdw, respectively.

Table 3
Total flavonoid and total phenol concentration

Plant Part	Total flavonoid content in mg QE/100g of dried material		Total phenolic content in mg CAE/100g of dried material	
	U. dioica	U. urens	U. dioica	U. urens
Root	22.52±6.09[a]	20.77±2.84[a]	168.39±10.01[a]	154±9.86[b]
Stem	68.211±3.380[b]	36.214±0.972[a]	126.75±10.46[b]	260.97±17.02[c]
Leaf	76.007±2.483[b]	5.280±2.86[c]	153.683±3.424[a]	260.7±25.3[c]

 # Values are mean± S.E. (standard error) of 3 observations (p<0.05). Values marked with similar superscript a, b or c indicates lack of significant difference from each other

DISCUSSION

The results of the conducted study exhibited a greater more significant microbial growth inhibitory activity by organic extracts of *U. dioica*. The bactericidal activity was more active against gram negative bacteria *E. coli* and moderate activity against gram positive bacteria *B. subtilis* and *S. aureus*. The results suggested comparative higher resistance of gram –ve bacteria *P. aeruginosa* against almost all the tested organic extracts of both plants *U. dioica* and *U. urens*.This difference in antibacterial property towards *P. aeruginosa* was attributed to the morphology of bacteria which is the presence of an extra protective membrane of lipopolysaccharide enclosing the cell wall in gram –ve bacteria that help in blocking the penetration of bioactive compounds.[21-23] It has been observed that growth of gram positive bacteria was most sensitive due to the presence of phenols as a predominant active compound in the plants of genus *Urtica*.[24]The various organic extracts of different plant parts of both plants exhibited great spectrum of inhibitory activities by agar well diffusion method. The results indicated that the non-polar extracts like petroleum ether extracts has been significantly more active against microbial growth. Results of some studies shows that non-polar extracts provide better results than polar extract.[25]According to the present research results petroleum ether and chloroform extracts showed higher antimicrobial activity than the other crude organic extracts. A comparatively larger zone of inhibition was observed against *E.coli* (chloroform extract of root of *U. dioica*), with an MIC value of 12.5mg/mL while the methanol extract of the root has an MIC value of 6.25 mg/ml. The plant *U. dioica* has been used in the treatment of B. cereus, V. parahaemolyticus, S. aureus and methicillin-resistant S. aureus (MRSA) infections. As it is well known, MRSA, E. coli and Bacillus species, especially B. cereus, are the potential food poisoning agents. The potential area of applying plant extracts in the reduction in numbers and growth inhibition of food-borne pathogens.[26] For introducing these extracts for future use in pharmaceutical and food industries further research and testing were needed to examine the effectiveness as well as the toxicity of these extracts to achieve the pure bioactive component that attribute to their significant antimicrobial potential. Antimicrobial activity of plant extract was attributed to the presence of numerous bioactive compounds, therefore the phytochemical screening of plant parts has been done along with quantitative determination of total phenols and flavonoid concentration in the sample.Terpenes and phenols of *U. dioica* are found to be one of the major groups of compounds with a potential source for extraction of useful drugs exhibiting the inhibitory activity of microbial infections.[27-30]In accordance to the findings of the present study, research shows that antimicrobial activity was found in *U. dioica* water extract against *E. coli*, while no significant activity was found against *P. aeruginosa*. On the other hand, in contrast to our predicted results a research showed that *U. dioica* exhibits low antibacterial potential against *S. aureus,* whereas no activity against *E. coli*, *P. aeruginosa* and *S. aureus* [31] and another study confirmed that U. urens possess no significant antibacterial activity.[32-34]The significant effect of inhibition on *E. coli* growth can be hypothesized as the effect that an extract exerted on the outer membrane leads to alteration in membrane structure and permeability of the cell. These changes may result in breakage of hydrogen bonds that help in keeping the structure of membrane rigid.[35,36] It is a fact that gram positive bacteria are comparatively

more susceptible to inhibition by the solvent extracts than their gram –ve counterparts.In addition, our findings suggested that the non-polar extracts like petroleum ether and chloroform of *U. dioica* and some polar and non-polar extracts of *U. urens* that are showing good inhibitory effect on growth of pathogenic bacteria may further be suggested as a potential source of natural antimicrobial agent.

CONCLUSION

The results obtained from the agar well diffusion assay has established that the root, stem and leaves of *U. dioica* plant display *in-vitro* antimicrobial potential to varying extent against various bacterial strains. The antimicrobial potential was found better against gram positive bacteria than towards gram negative ones. The positive results of the anti-bacterial screening contribute for the extraction of potent antimicrobial agents. It provides preliminary information for further phytochemical and pharmacological analysis on the chemical constituency of the plant extracts. Further research is needed for the bioactive compounds identification and isolation and also an in vivo antimicrobial activity evaluation along with toxicity testing of the extracts showing significant bactericidal activity should be done before introducing them for usage to commercialize in the form of pharmaceutical medicine.

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AUTHORS CONTRIBUTION STATEMENT

Miss. Priyanka Rajput 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 contributed the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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