Research Article

IN VITRO ANTIMICROBIAL ACTIVITY, NUTRITIONAL PROFILE AND PHYTOCHEMICAL SCREENING OF GARHWAL HIMALAYA MEDICINAL PLANT - *URTICA DIOICA*

Dr. Sarla Saklani, Mr. Subhash Chandra*

[1]Department of pharmaceutical Sciences, H.N.B. Garhwal University (A Central University) Srinagar Garhwal, Uttarakhand, India.

***Corresponding author's E-mail:** subhashkothiyal@gmail.com

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ABSTRACT

In this study, all extract of nettle (*Urtica dioica* L.) was studied for *in vitro* antibacterial, antifungal activity, nutritional evaluation and phytochemical screening. The plant edible leaf content nutrients such as crude protein 1.5%, carbohydrates 21.98%, crude fiber 2.9% and ash content 8.5% and minerals as calcium, magnesium, potassium and phosphorus 0.42, 0.53, 0.75 and 0.086 mg/100gm) respectively. The ethanolic stem extracts of *Urtica dioica* showed significant activity 17±1mm, 16±1mm and 14±1mm against *Escherichia coli* (MTCC 729), *Streptococcus pyogenes* and *Salmonella entericatyphim* against food poisoning bacteria, and phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins. This analysis revealed that, the leaf contained higher value of fat, protein, fiber and minerals as compared to the cultivated medicinal plant leaf with palak and rayi. *Urtica dioica* leaf contain sufficient amount of nutrients, required per day by a person. Consumption of leaf and stem may promote general health and well-being as well as reduce the risk of chronic diseases.

Keywords: Antibacterial, Antifungal, Nutritional value and Phytochemical screening.

INTRODUCTION

Himalaya has great wealth of medicinal plants and traditional and local knowledge. Central Himalaya Region covers the new state of India which comprising the major divisions of Garhwal and Kumaon. Diseases are the bane of humankind ever since its advent on this planet. Humans have been fighting against a variety of diseases since prehistoric periods. Eventually he developed an indigenous pattern of medicines, which tries to resist the effects of the diseases. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in India. The potential of higher plants as source for new drugs is still largely unexplored. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[1]. Plant as sources of medicinal compounds has continued to play a dominant role in the maintenance of human health since ancient times. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. *Urtica dioica* (U.D) has widely been used in traditional medicine for its hypotensive and vasodilatory effects. The different parts used include leaf, root, stem, flower and modified plant organs. Indian people have a tradition of using a number of plant species for the treatment of infectious diseases and various ailments. The effect of plant extracts on microorganism has been studied by a very large number of researchers in different parts of the world. *Urtica dioica* herbs are used to treat stomachache in Turkish folk medicine[2]. In addition, this herb is used to treat rheumatic pain and for colds and cough[3] and is used against liver insufficiency[4].

MATERIALS AND METHODS

Plant Material

The fresh parts of leaf and stem of *Urtica dioica* were collected from adjoining area of Srinagar city (Dist- Pauri Uttarakhand) in the month of June - August. The plant was authenticated by botanist Dr. R. D. Guar, Department of Botany; H. N. B. G. U. Srinagar Garhwal.

Preparation of plant Extract

The plant material was separated into its selected parts (leaf, stem and root) air dried ground to moderately fine powder and Soxhlet extracted with increasing polarity solvent (Petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water)[5]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of leaf stem and root was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 100gm). The various concentrated extracts were stored in air tight container for further studies.

Media

Nutrient broth, Nutrient agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, and sulphuric acid, Distilled water etc all product of Himedia Laboratories Mumbai (India) were used in this study.

Bacterial Strains

Ten bacterial strains were used namely *Escherichia coli, Klebsiella pneumoniae, Enterobacter gergoviae, salmonella entericatyphim, shigella flexneri, Staphyloccus*

aureus, staphyloccus epidermidis, streptococcus pyogenes, and *Bacillus cereus*. The bacterial strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India (Customer no. 3921).

Fungal Strains

Three fungal strains were used namely *Candida albicans*, *Aspergillus flavus* and *Aspergillus parasiticus*. The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Antibacterial assay

The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts[6 & 7]. Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 10mg/ml and 50mg/ml of the each extracts were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

Antifungal assay

The antifungal activity was tested by disc diffusion method[8, 9]. The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hrs. both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45°C respectively, and fungal plates were incubated at 25-28°C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

Nutritional & Mineral assay

The edible portion of plant was analyzed for moisture, ash, fat and Fiber as per method[10]. Total nitrogen was analyzed by micro-kjeldhal method[11] and for crude protein the value was multiplied by 6.25. Total carbohydrates were obtained by subtracting the value moisture, crude protein, crude fat crude fiber and ash from 100%[12]. The total energy value equal to addition of fat, protein and sugars calorie, each gram of fat give 9 kcal, protein and sugar give 4 kcal energy. The minerals analyzed were Potassium using atomic absorption spectrophotometer, Calcium and Phosphorus by flame photometer. Ascorbic acid in fruits was estimated[13].

Phytochemical analysis

The qualitative phytochemical properties of the dried powdered sample were determined using standard methods[14].

Table 1: Antibacterial activity of ten bacterial strains against *Urtica dioica* plant stem extract. Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Bacterial Name		Petroleum ether Extract		Chloroform Extract		Ethyl acetate Extract		Acetone Extract		Ethanol Extract		Water Extract	
		Concentration (Mg/ml)											
Genus /Species/Subspe.	MTCC (Code)	10	50	10	50	10	50	10	50	10	50	10	50
Bacillus cereus	1272	-	-	-	-	-	7	6	8	9	12	8	10
Escherichia coli	729	-	7	-	8	8	10	-	11	11	17	-	9
Enterobacter gergoviae	621	-	-	-	-	-	9	7	9	9	12	-	8
Klebsiella pneumonia	432	-	-	-	7	7	9	8	10	9	10	7	9
Salmonella entericatyphim	98	-	-	-	-	-	8	-	9	10	14	-	8
Shigella flexneri	1457	-	6	-	-	7	9	-	9	8	10	7	9
Staphyloccus aureus	902	-	7	-	7	-	8	-	8	7	9	8	9
Staphyloccus epidermidis	435	-	-	-	-	7	9	-	8	8	10	-	8
Streptococcus pyogenes	1925	-	-	-	6	8	10	-	9	11	16	7	9
Escherichia coli	443	-	6	-	9	-	9	-	-	7	9	-	7

Table 2: Fungal activity of three fungal strains against *Urtica dioica* plant stem extract. Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Fungal Name		Petroleum ether Extract		Chloroform Extract		Ethyl acetate Extract		Acetone Extract		Ethanol Extract		Water Extract	
		Concentration (Mg/ml)											
Genus /Species/Subspe.	MTCC (Code)	10	50	10	50	10	50	10	50	10	50	10	50
Candida albicans	3017	-	-	-	-	-	7	-	8	-	8	-	9
Aspergillus flavus	2798	-	8	-	-	-	-	-	-	-	9	-	8
Aspergillus parasiticus	2796	-	9	-	-	-	-	-	8	-	9	-	7

RESULTS AND DISCUSSION

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antimicrobial activity assay. The results of antibacterial, antifungal, nutritional value and phytochemical screening activity, table 1-5 and figure 1-3, reveals that antibacterial, antifungal, nutritional, and phytochemical screening activity of leaf of *Urtica dioica* was evaluated against ten bacterial and three fungal pathogenic strains.

Table 3: Nutritional value of *Urtica dioica* leaf

Nutrients	Value
Moisture (%)	63.4 ± 0.15
Ash (%)	8.5 ± 0.08
Total protein	1.5 ± 0.04
Crude fat (%)	1.72 ± 0.25
Crude fibre (%)	2.9 ± 0.09
Soluble carbohydrates	21.98± 0.11
Organic matter	91.50± 0.16
Mg/100gm nitrogen	0.24 ± 0.07
Ca mg/100gm	0.42 ± 0.10
Mg mg/100gm	0.53± 0.06
K mg/100gm	0.75± 0.08
P mg/100gm	0.086 ± 0.04

Table 4: Qualitative estimation of *Urtica dioica* leaf and stem phytochemical screening

Test	*Urtica dioica* leaf	*Urtica dioica* stem
Carbohydrates/ glycosides		
(1) Molish test	(-)	(-)
(2) Fehling test	(-)	(-)
(3)Benedict test	(-)	(-)
Alkaloid		
(1) Mayer's test	(-)	(+)
(2) Dragondroff test	(-)	(-)
Flavonoid	(+)	(+)
Saponins	(-)	(+)
Tannins		
(1) Pyrogoll & catechol	(-)	(+)
(2) Gallic acid	(-)	(-)
Unsaturated sterol/triterpenes		
(1) Liebermann Burchard test	(-)	(+)
(2)Salkowiskis test	(-)	(+)
Resin	(-)	(-)

Table 5: Qualitative estimation of *Urtica dioica* leaf amino acid screening

Amino acid test	*Urtica dioica* leaf
L- Hydroxy proline	(.)
DL Serine	(-)
DL Iso-leucine	(-)
DL Valine	(-)
DL-2-Aminobutyric acid	(+)
L-Ornithin	(-)
L-Cystein hydroxyl	(-)
DL-Nor-leucine	(-)
DL-Tryptopham	(+)
DL-Alanine	(-)
L-Glutamic acid	(-)
Glycine	(-)
L –Proline	(-)
L- Arginine	(+)
DL – Aspartic acid	(-)
L –Cystein hydroxychloride	(+)
L- Histidine	(-)
L – Leucine	(-)
L –Lysine monochloride	(+)
DL – Methionine	(-)
DL – β-Phenyl alanine	(-)
DL – Threonine	(+)
L – Tyrosine	(-)
3-C-3-4Dihydroxy phenyl	(-)

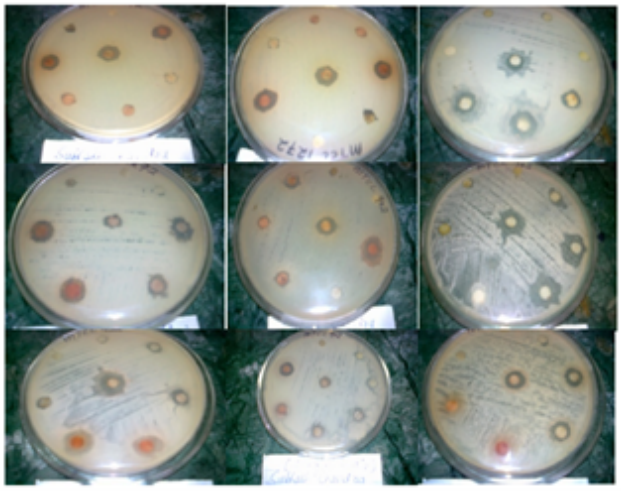

Figure 1: Antibacterial and antifungal activity of ten bacterial strains & three fungal strains against *Urtica dioica* plant stem extract

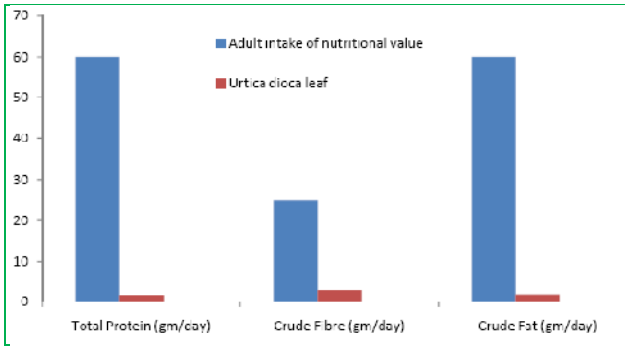

Figure 2: Comparison of per day intake of nutrients by Adults with the nutrients present in the leaf of *Urtica dioica*.

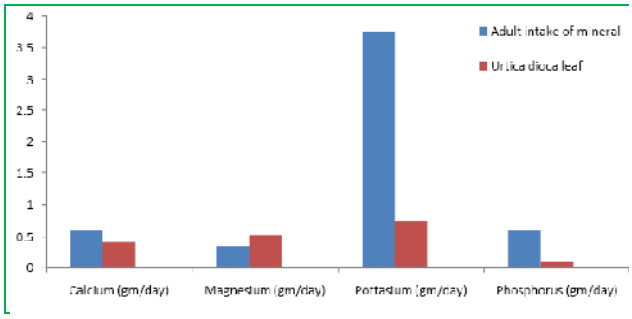

Figure 3: Comparison of per day intake of minerals by Adults with the mineral present in the leaf of *Urtica dioica*.

Antibacterial and antifungal activity

Urtica dioica ethanolic stem extract significant activity 17±1mm, 16±1mm and 14±1mm against *Escherichia coli* (MTCC 729), *Streptococcus pyogenes* and *Salmonella entericatyphim* against food poisoning bacteria, the order of the species based on total antibacterial activity is as follows: *Escherichia coli* > *Streptococcus pyogenes* > *Salmonella entericatyphim*.

Nutritional value

The level of nutrients such as crude protein 1.5%, carbohydrates 21.98%, crude fiber 2.9% and ash content 8.5% and minerals as calcium, magnesium, potassium and

phosphorus 0.42, 0.53, 0.75 and 0.086 mg/100gm) respectively.

Phytochemical screening

The phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins. This analysis revealed that, the leaf contained higher value of fat, protein, fiber and minerals as compared to the cultivated medicinal plant with green vegetable (e.g palak and rayi) and 500 gm leaf contain sufficient amount of nutrients, required per day by a person.

CONCLUSION

The in vitro antimicrobial studies present *Urtica dioica* to have considerable efficacy against various pathogenic bacteria. The study provides a scientific basis for the use of the plant as folk medicine. The leaf and stem of the plant is a good source of essential nutrients including minerals, carbohydrates, proteins and lipids. However, more advanced pharmacological and clinical studies would be required to investigate in vivo mechanism of nutraceuticals effects of this important wild plant.

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