Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 4325-4331
Received: 26-03-2019
Accepted: 28-04-2019

Maneesha Singh
Assistant Professor, Shri Guru Ram Rai School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India

Garima Kali
Research Scholar, Shri Guru Ram Rai School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India

Correspondence
Maneesha Singh
Assistant Professor, Shri Guru Ram Rai School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India

Study on morpho-anatomical and histo-chemical charaterisation of stinging nettle, *Urtica dioica* L in Uttarakhand, India

Maneesha Singh and Garima Kali

Abstract
Stinging nettle (*Urtica dioica* L., Urticaceae) is a ubiquitous herb grown in large part of the world mainly in temperate and tropical wasteland areas used as medicinal herb, fabric dye, textiles, food. The present study aims for investigation of *Urtica dioica* (nettle leaf) on the basis of morpho- anatomical and histochemical characterization on samples collected from Uttarakhand. The collected plant samples showed marked variation in morphological characters. The ridges and furrows were more prominent in sample A as compare to sample B and C. Medullary rays in sample B and C were more distinct and consist of about 23-30 rows of cells as compare with sample A. Raphid shaped calcium oxalate crystals were observed in all the samples. These studies revealed that present studies were significant for the characterization and identification of plants and altitudinal effects on distribution of phyto-constituents on various plant parts.

Keywords: *Urtica dioica* L., Urticaceae, phyto-constituents, histochemical analysis

Introduction
Biodiversity refers to the variety and variability of all types of microbes, plants and animals on the earth. It includes not only the many species that exist, but also the diversity of population that makes up a species, the genetic diversity among individual's life form and the many different habitats and ecosystems around the globe. India has a total geographical area of about 329 million hectares with a coastline of over 7500 km. The ecological or ecosystem diversity of the country is enormous, ranging from sea level to the highest mountainous ranges in the world; hot and arid conditions in the northwest to cold arid conditions in the trans-Himalayan region; tropical wet evergreen forests in Northeast India and the Western Ghats; mangroves of Sunder ban and fresh water aquatic to marine ecosystems [1].

Uttarakhand one of the newly formed state of India, is endowed with a unique and diverse range of biodiversity. From the snowbound peaks of the Himalayas to the moist Alpine scrub, sub Alpine forests, dry - temperate and moist- temperate forests to moist deciduous forests, the state possesses a wide biodiversity that in return nurtures a large multiplicity of floral and faunal forms. The state is home to nearly 4048 species of Angiosperms and Gymnosperms belonging to 1198 genera under 192 families. Of these nearly 116 species are specific to Uttarakhand i.e. their geographical distribution is limited to the boundaries of the state. In Uttarakhand, 161 species of flora found are recognized as rare or threatened under the categorization of the International Union for Conservation of Nature (IUCN).

Stinging nettle (*Urtica dioica* L., Urticaceae) is a ubiquitous herb which is available in large part of the world mainly in temperate and tropical wasteland areas all over the world. In India, *Urtica dioica* L found growing in temperate and sub- tropical Himalayas, from Kashmir to Sikkim between 1200 to 3500m above sea level. It grows wild as undergrowth particularly in all districts of Uttarakhand. *Urtica dioica* L. occurs on almost all soil types, although it prefers moist or damp soils and is absent from permanently waterlogged soils and weakly acid or weakly basic conditions [2]. This perennial typically grows to between 1 to 3m tall with dark green leaves in an opposite pattern that are oval to heart shaped, simple and saw toothed and are sparsely covered with stinging hairs with greenish and brownish flowers are mostly unisexual with male and female flowers on the same or different plants [3]. The plant is common in herbal medicine, and young leaves can be cooked and eaten as a nutritious potherb. *Urtica dioica* L. can be used in osteoarthritis, benign prostatic hypertrophy, allergic rhinitis and asthma, bleeding, diabetes [4], gout, excessive menstrual bleeding, acne treatment, etc. Additionally, stinging nettle has been used as a source of bast fibers for textiles and is sometimes used in cosmetics [5].

Most of the studies on *Urtica dioica* were concentrated on its general study that whether it is beneficial or harmful [4], its Pharmacognostical and phytochemical study [2, 6, 7, 8]. Historically, the plant was cultivated for food and other industries in European countries such as Scotland, Denmark and Norway for food and other industries.

Therefore, the present study deals with the comparative morphological and anatomical characterization along with the histological analysis of different samples of *Urtica dioica* L. collected from different locations of Uttarakhand to evaluate the effect of climatic conditions.

Material and methods

Location of the experiment and climatic condition

The experiment was carried out at Botany laboratory in the Department of Botany of College of Basic and Applied Sciences, Sri Guru Ram Rai University, Patel Nagar, Dehradun Uttarakhand. Uttarakhand considered as land of gods and is located amongst Shivalik range on the foothills of the Himalayas. The Uttrakhand is a tropical region and hence rich in biodiversity. There are thirteen districts in Uttarakhand and the plant material was collected from three different districts i.e. Dehradun, Pauri and Rudraprayag. Uttarakhand is located between the latitude 29 ^{0} 55"north and longitude 77 ^{0}35"east covering the area of 2002.4sq km with the elevation of 2000m above the sea level and Pauri is the district of Garhwal mandal and is located between the latitude at 30.15 ^{0} north and longitude 78.78^0 east covering the area of 5,951 feet with the elevation of 1,814m above the sea level and the last third one is the Rudraprayg district and is located between the latitude at 30.28 ^{0} north and longitude 78.98 ^{0}east covering the area of 2,936 feet with the elevation of 895m above the sea level.

Material and Methodology

The material for the comparative study comprises of aerial parts of *Urtica dioica* L. which belongs to the Urticaceae and it is found in different districts of Uttarakhand.

Collection of plant materials

Fresh and disease free aerial parts of the plant were collected from three districts Dehradun district (Sahastradhara area), Pauri and Rudraprayag district of Uttrakhand. It was very necessary to separate the dust, contaminants and some other solids from the leaves to get best results.

Morphological study of *Urtica dioca* L.

Collected plant sample was subjected for morphological studies which includes plant height, length of petiole, leaves arrangements, sample of the apex marginal internodes etc.

Anatomical study of *Urtica dioica* L.

The fresh plant parts were preserved in alcoholic acetic solutions which was prepared by the mixture of 50% of alcohol, 40% of formalin, 2% of acetic acid. Then the plant was preserved for further microscopic studies of stem, petiole and leaves.

The transverse section was dehydrated in 30%, 50%, 70% and 95% solution of ethanol. For double staining safranin and fast green was used. Double staining was done so that the living tissues can be differentiated from the dead one and at last this double stained section was fixed in the slide by the use of Canada balsam and kept for further studies.

Histo-chemical Analysis

Leaves, petiole and stems were examined to study morphological and organoleptic characters. Samples for microscopy were prepared by putting it in a preserving solution. Then the transverse section was taken. The section should be as thin as possible and then see under microscope at 10 X after staining with –

1. Safranine- for vascular bundles
2. 20% H_2SO_4- Stele and calcium oxalate
3. Grams's iodine- for starch in cortical medullary ray cells
4. Phloro glucinol- for stellar tissue

Result and discussion

Urtica dioica L. (Stinging nettle) is a perennial herbaceous plant of the Urticaceae family [9] (Ahmed and Parasuraman, 2014). The plant has a long history of use as a source for traditional medicine, food and tea. It is found in orchards, neglected yards, waste places, roadsides, flood plains, stream banks and ditches. In the present study the plant was subjected to morphological, anatomical and histo-chemical characterization collected from different locations of Uttarakhand. The findings of the present studies were as follows.

Morphological studies of *Urtica dioica* L

Urtica dioica L. is a perennial herb; the height of the plant varies from about 2 to 4 feet. The three samples varied in their height as maximum height of sample C (40-50cm) and the minimum height of sample A (30-40cm). In all the studied samples, the leaves were simple (i.e., lobed or unlobed but not separated into leaflets). The leaves were dark green in colour and leaves face each other in opposite direction and superimposed. The leaves were about 3 to 15cm long and 2 to 4 cm wide the leaves. The petiole was moderately long and arises from a leaf axil with two linear stipules at the base. The leaves were ovate to lanceolate in shape with a shallowly chordate base and acuminate tips. The margins were prominently dentate with the terminal tooth larger than the other marginal teeth as also reported by Testai *et al*., (2002) [3].

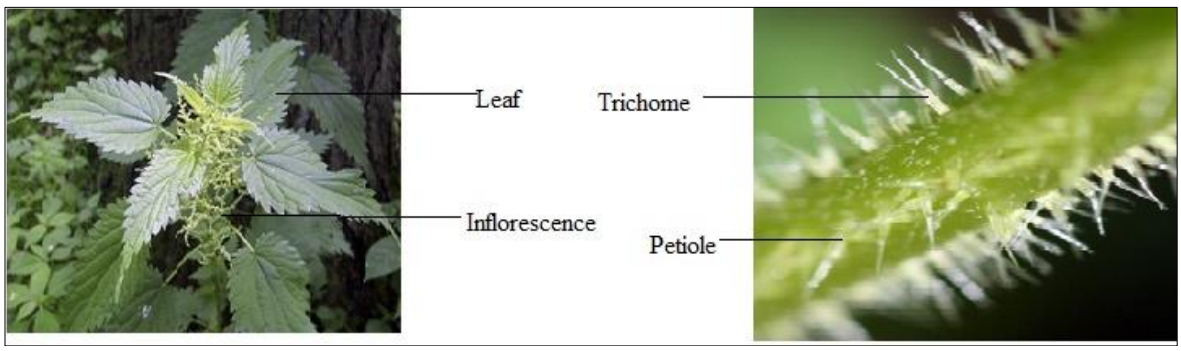

Fig 1: Aerial view of *Urtica dioica* L

Fig 2: Stem of *Urtica dioica* L showing arrangement of stings

Table 1: Vegetative characters of three aerial plant part samples of *Urtica dioica* L.

S.N.	Characters	Sample A (Dehradun)	Sample B (Rudraprayag)	Sample C (Pauri)
1.	Height of the plant	30-40 cm	40-50 cm	40-50 cm
2.	Leaves length	1-20 cm	1-23cm	1-26 cm
3.	Width	2-3 cm	2-3 cm	3-4 cm
4.	Petiole	1-9 cm	1-11cm	1-11cm
5.	Internodes	3-6cm	3-6cm	3-8cm

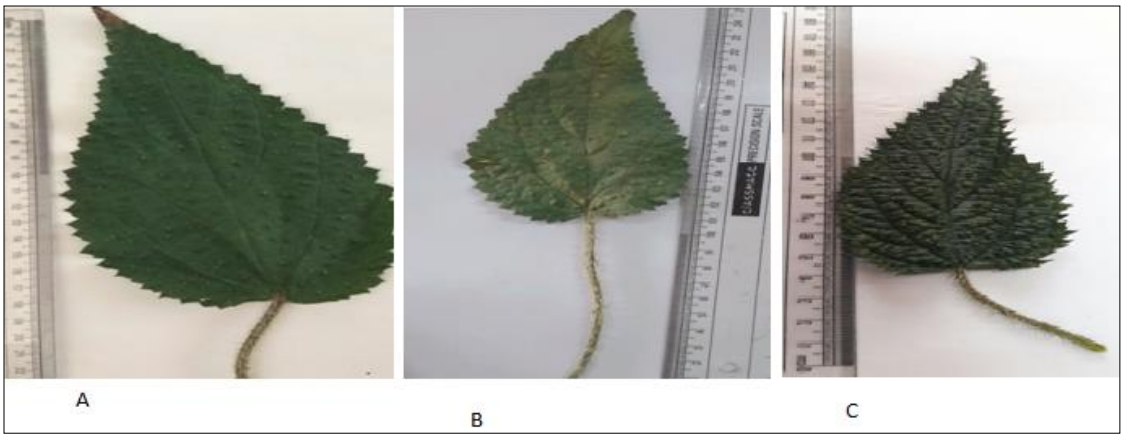

Fig 3 (A, B, C): Adaxial surface of leaf showing arrangement of veins and strings on different samples of *Urtica dioica*

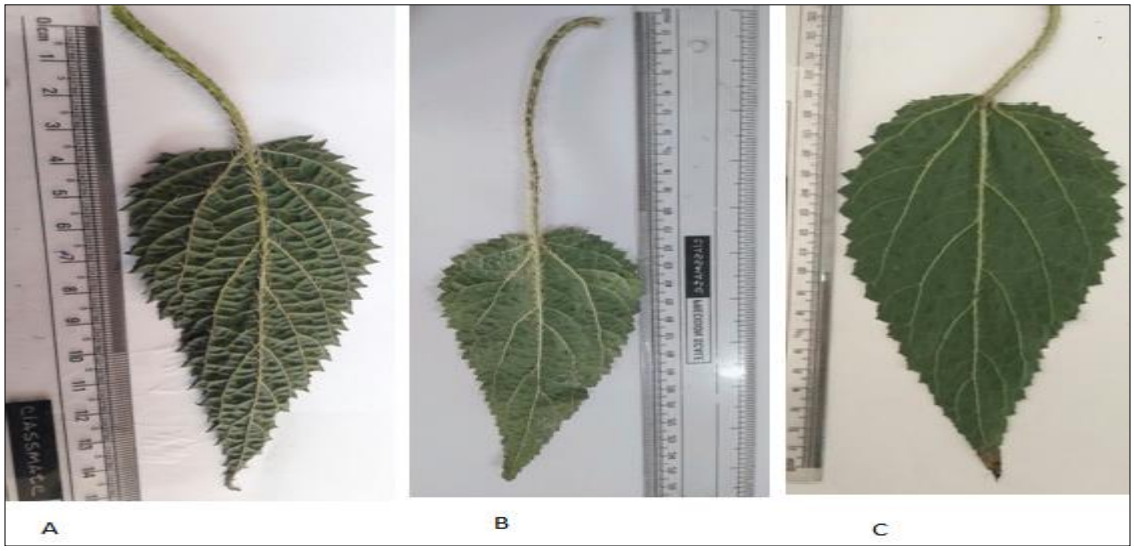

Fig 4: Different samples A(Dehradun), B(Rudraprayag), C (Pauri) showing lower surface of *Urtica dioica* L.

Fig 5: Different samples A(Dehradun), B(Rudraprayag), C (Pauri) showing nodes and internodes of *Urtica dioica* L.

Flowering and fruiting time was from June to October in all the studied plant samples. Inflorescence was normally chyme arising from the axil of leaf or, terminal in position. Flowers were monoecious, incomplete, regular, unisexual- pistillate or, Staminate. The number of petals and sepals were four. Petals and sepals both were separate and not fused. Stamens were four in number and the fruit was dry but did not split open when ripe, and fruit length was 1 – 1.4mm.

Anatomical characterization

In general, the outline of the stem were wavy due to the presence of ridges and furrows. The cortical region were differentiated from outer side into single layer epidermis with cellulosic wall and stinging hairs followed by 3to 4 layered collenchymatous hypodermal region and towards inner side parenchymatous cortex was present. The stele were present below the endodermis where 12-16 vascular bundles were arranged in a ring and showed endarch, conjoint, collateral and open condition. Vascular bundles encirclde parenchymatous pith in the centre. Similar types of studies have been conducted by Corsi and Masini, (1997) [10] that in the transverse section of stem, fibro-vascular bundles ranges from 12-20 in number. In the present study of the transverse section of stem, the ridges and furrows were more prominent in sample A as compare to sample B and C. Medullary rays in sample B and C were more distinct and consist of about 23-30 rows of cells as compare with sample A where rays have less number of rows of cells. Vascular bundles were present in the ridges only and in the furrow medullary rays were arranged.

Transverse section of petiole collected from different locations showed the organization of different types of tissues. Both the adaxial and abaxial side of the petiole was covered by a layer of epidermal cell. The upper side of the petiole has two swelling. The protective layers of cells such as collenchyma was abundant. Below the collenchymatous hypodermis, the ground tissue is made up of parenchymatous tissues. The vascular bundles were arranged in horseshoe manner. In the centre, vascular bundle were larger in size and size of vascular bundles decreases from the centre towards the notch. In the transverse section of petiole, more prominent notch was present in sample A as compared to other samples.

In general anatomical studies of leaf, under the upper epidermis is hypodermis, the cells with cystolith or crystals could be seen in all samples. The mesophyll has been with compact palisade parenchyma just under the upper epidermis. Spongy parenchyma cells under the palisade cells formed the pith. A thickened vascular bundle was present in the main midrib, and lower the midrib was collenchymas cells. Large stinging hairs (trichomes) were present on lower surface of leaf. According to Zohreh and Maryam in 2012 [11], the occurrence of laticiferous elements, glandular, non glandular and stinging hair types, hypostomata presence of hydathodes, fluid loaded epidermis, silicified and calcified cell walls which occurs as cystoliths of different forms in members of Urticaceae.

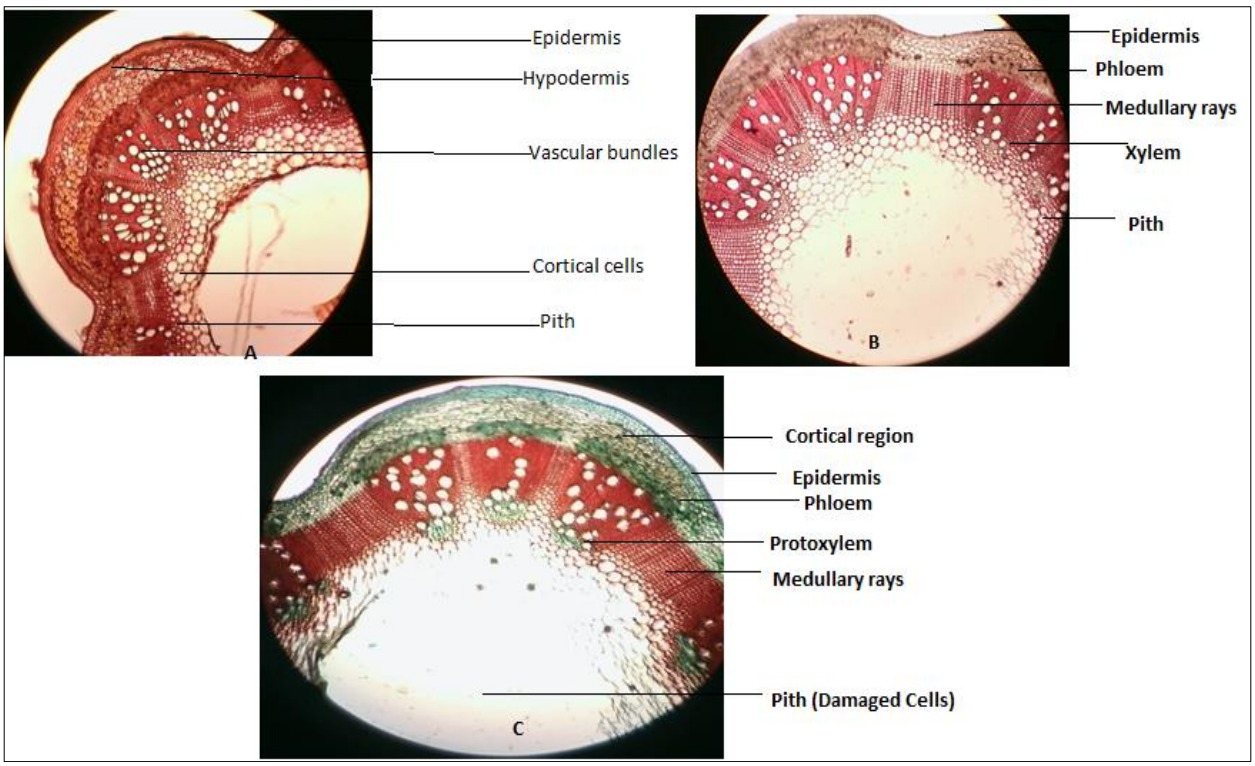

Fig 6: (A, B, C) Transverse section of stem showing vascular bundles in different samples of *Urtica dioica* L with double staining

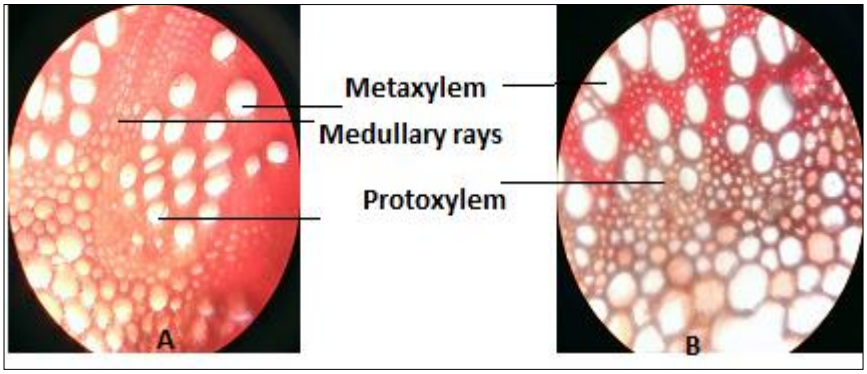

Fig 7: (A, B) Transverse section of stem showing Meta xylem and proto xylem vascular bundles in different samples of *Urtica dioica* L

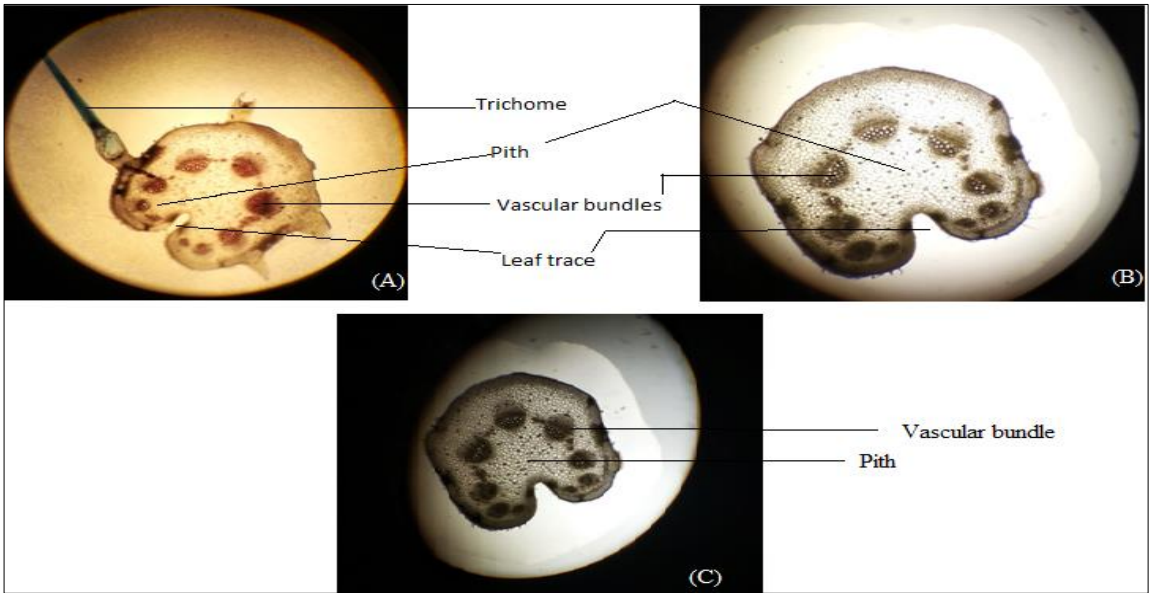

Fig 8: (A, B, C) Transverse section of petiole showing organization of tissues in different samples of *Urtica dioica.*

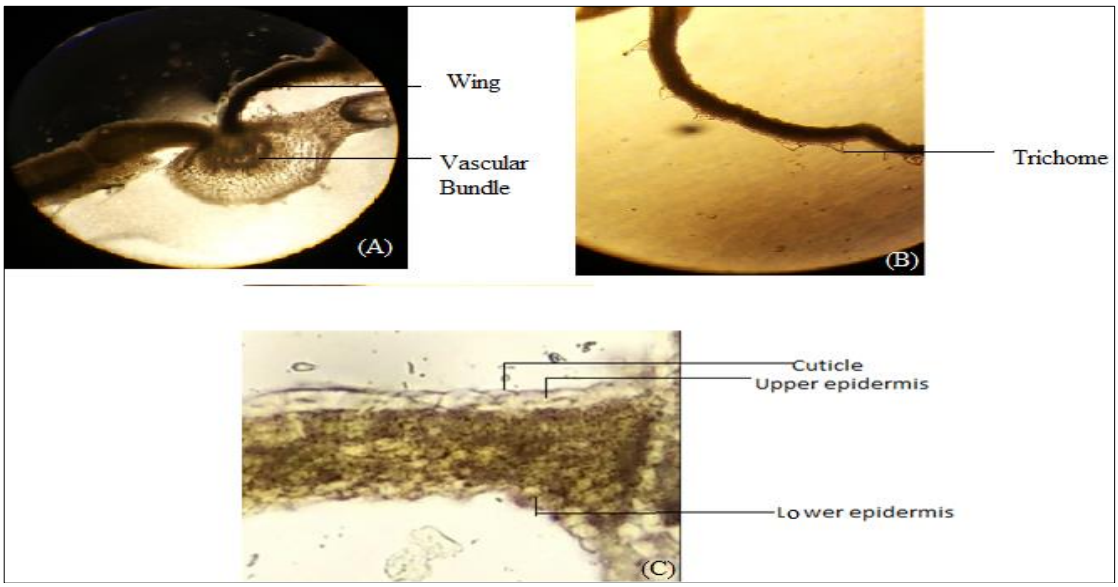

Fig 9: (A, B, C) Transverse section of leaf showing organization of tissues in different samples of *Urtica dioica* L.

Histo-chemical analysis

Histochemical analysis is one of the valuable standardization processes of quality control of crude drugs to locate the presence of ergastic cell contents such as vascular bundles, calcium oxalate crystals, cortical and medullary ray and stellar tissue in the histological zone of the plant [12, 13]. The biosystematic importance and implication of histochemical features of various compounds such as ergastics, calcium oxalate crystals helpful for the characterization and identification of inter and intra specific variations [14]. In the current study, Hand section of the stem and petiole were mounted in suitable chemical reagents to determine the presence of various chemical substances and their zone of distribution. The presences of calcium oxalate crystals, its size and shape in various plant families helpful for the inter and intra specific differences that may be used for taxonomic references [15]. Raphid shaped calcium oxalate crystals were observed in all the samples. The number of crystals were more in sample A and B. Staining with Iodine solution for cortical and medullary ray cell showed the presence of starch granules mainly in the cortical and medullary region of stem and petiole consisting of parenchyma cells. Starch is mainly stored within xylem parenchyma ray tissue of underground organs. This type of storage tissue can be considered to be expensive in terms of resource allocation as ray parenchyma cells of wood are living and non-photosynthetic and require a high metabolic demand to be both created and maintained [14, 16]. The staining with phloroglucinol for stellar tissue showed the presence of vascular bundles in a ring in all the studied samples.

Table 2: Observations of histo-chemical analysis of T.S. of Stem and petiole obtained by treatment of chemicals

S. N.	Reagent	Histo-chemical zone	Sample A (D.dun)	Sample B (RPG)	Sample C (Pauri)
1	Safranine	Vascular bundles	+	+	+
2	20% H_2SO_4	Stele and calcium oxalate	+	+	+
3	Iodine solution	Starch in cortical medullary ray cell	+	+	+
4	Phloroglucinol	Stelar tissue	+	+	+

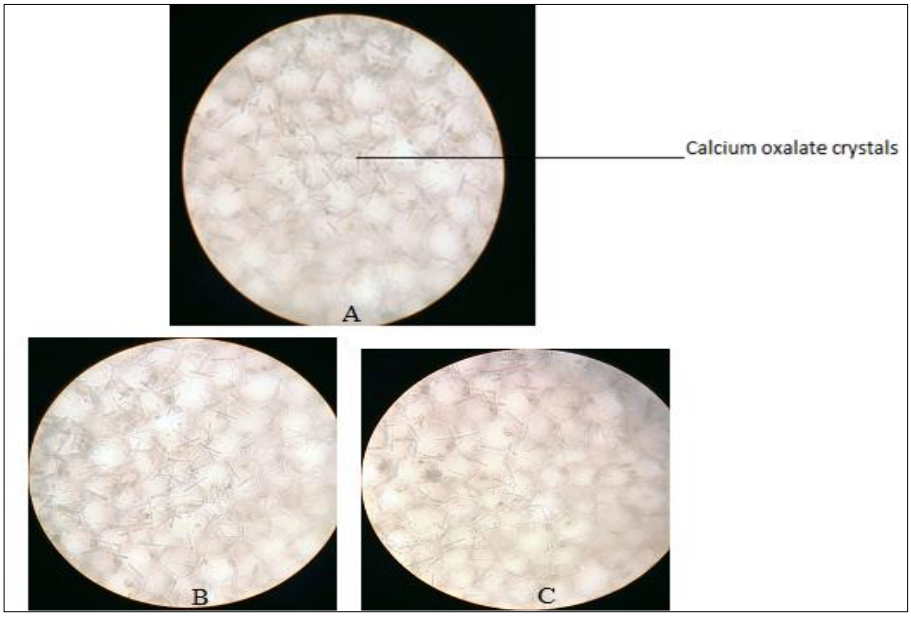

Fig 10: (A, B, C) Transverse section of stem showing calcium oxalate crystals in cortical cells of *Urtica dioica*.

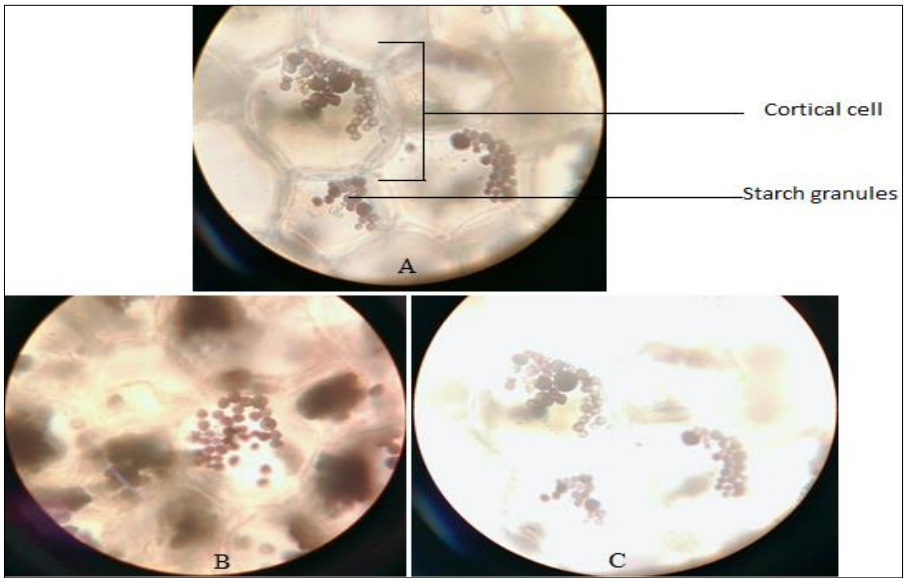

Fig 11: (A, B, C) transverse section of stem showing cortical cells and starch granules in different samples of *Urtica dioica.*

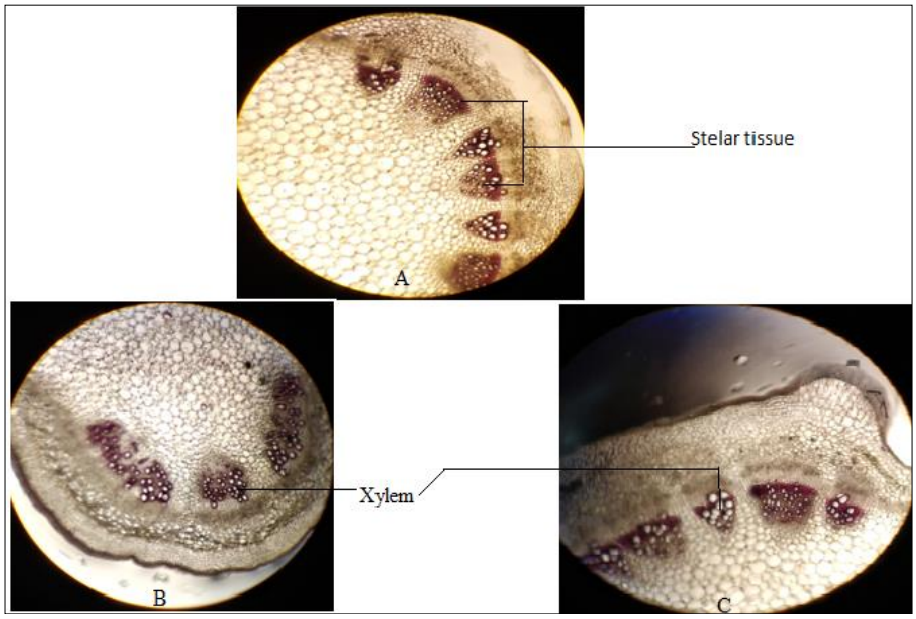

Fig 12 (A, B, C): Transverse section of stem showing stellar tissue in different samples of *Urtica dioica* L.

Conclusion

The present studies on the basis of the morphological, anatomical and histo-chemical basis was found to be significant for the characterization and identification of plants belonging to the same and different localities as there may be significant variations of altitude on the morphological and anatomical features. Histo-chemical studies are useful for the location of different phytochemical compounds in different parts of the plant cells on the basis of distinct color reactions. Therefore, these studies would be beneficial for evaluation and characterization of plants on the basis of effects of altitudinal effects on its morphological and anatomical characters.

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