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To Study the Effect of Antioxidants and Photochemical Eavaluation of Plant Urtica Urens

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ABSTRACT
Current review on In situ gelling systems includes polymeric formulations that are in solution forms before entering the body, but change to gel forms under the physiological conditions. Eye is the most sensitive organ of the body. Designing of ocular drug delivery system is the most challenging field for pharmaceutical scientists. Topical administration of ophthalmic drugs is used to alleviate the symptoms and signs caused by ocular surface inflammatory disorders, to treat infections, for glaucoma or intraocular inflammation. The objective of ourwork is to formulate an ocular delivery system of Ciprofloxacin. Due to its elastic properties, in-situ gels resist the ocular drainage of drug leading to longer contact times with ocular surface. The solution-gel transition depends on one or combination of different stimuli, like ph change, temperature modulation, solvent exchange, ultra violet irradiation and the presence of specific ions or molecules. Drug delivery systems having such properties can be widely used for sustained vehicle preparations of the bioactive molecules. Mainly In situ gels are administered by oral, ocular, rectal, vaginal, injectable, and intraperitoneal routes. The In situ gel forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems.

Keywords: Gels, in-situ gel,ophthalmic drugs, glaucoma, bioactive molecules, sustained release, polymers, and drug delivery systems.

I. INTRODUCTION

Herbal medicines are the healthful products that contain plant materials as their pharmacologically active element.[1] The meditative plant is might outlined as any substance with one or additional of its organ containing properties that may be used for therapeutic functions or which might be used as precursors for the synthesis of the varied drug. The plant may be a synthesis laboratory, not just for chemical compounds like sugar, proteins, enzymes, fats and oils, minerals, vitamins, and lipids that are used as food by man, however additionally for a large number of compound like alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, easy synthetic resin glycosides, tannins, saponins, polyphenols.[2] Medicinal qualities of the plants is due to the attribution of these chemicals, that exerts a physiological and therapeutic effect.[3]

Restorative plants are utilized as the dietary assistant and inside the treatment of various illnesses while the best possible learning of their capacity is not known.[4] Traditional medication refers to health practices data and beliefs incorporating plants, animals and mineral primarily based medicines, non-secular therapies, manual techniques, and exercises, applied singularly or together to treat, diagnose and prevent illnesses or maintain well-being.[2] Over the years, medicative plants are found helpful in treatment and management of assorted health issues. The Traditional drug is, without doubt, a reliable different approach to health care delivery within the metropolis as a result of it's low-cost, simply accessible and efficacious. Herbal drugs are invariably single plant extracts of fractions or mixtures of fractions/extracts from completely different plants. Ancient plant medicines may provide a natural key to treat numerous human ailments.[2]

Medicinal plants play an intensive role within the development of modern herbal medicines as several diseases like cancer; liver diseases, and inflammatory disease finds no complete cure in medical care. The bioactive compounds of healthful plants are used as anti diabetic, chemotherapeutic, anti inflammatory, anti arthritic agents wherever no satisfactory cure is present in trendy medicines.[5]

Medicinal plants are necessary for medicine analysis and drug development, not only plant constituents are used directly as therapeutic

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agents, however conjointly as beginning materials for the synthesis of medicine or as models for pharmacologically active compounds. Herbal drugs are of 3 types based on the character of the active metabolites. The drug employed in crude form is the primary class. The dynamic constituents disengaged when the procedure of plant concentrates speak to the second classification of natural medications. These are pure molecules and usually pharmacologically additional active. Herbal medication for that information on acute and chronic toxicity studies in animals is obtainable represent the third class.[1]

The World Health Organization (W.H.O) has listed 21,000 plants that are used for therapeutic purposes far and wide. Among them 2,500 species are in India, out of that 150 species are utilized economically on a genuinely huge scale.6 India is the biggest producer of therapeutic herbs and is called as Botanical garden of the World. It has an extraordinary biodiversity because to its land and climatic conditions. In 2002 W.H.O propelled its first exhaustive customary solution technique to help push to advance moderate, successful and safe utilization of conventional pharmaceutical and complimentary option medicine.[5] Around 25% of the medication recommended overall originated from plants, 121 such dynamic compounds being in current use. Examples of very important drugs obtained from plants are digoxin from Digitalis spp., antimalarial antiarrhythmic drug (quinine and quinidine) from Cinchona spp., vincristine and vinblastine from Catharanthus roseus, atropine from Atropa belladonna and morphine and codeine from Papaver somniferum. It is calculable that 60% of anti-tumour and anti-infectious medicine already in the market or within the clinical test are of natural origin.6 The vast majority of these compound they cannot yet be synthesized economically and these are still achieve from wild or cultivated plants. Natural compounds will be lead compounds, permitting the design and rational planning of novel drugs, biomimetic synthesis development and also the discovery of latest therapeutic properties not yet attributed to known compounds.[7, 8]

Some other important compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters. All obtained from plants are important role used as pharmacological, physiological and biochemical purpose.[9] In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especiall those derived from plants.[10] This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient, abusive and/or incorrect use of synthetic medicine results in side effects in addition to other problems a large proportion of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that natural products are harmless. However the use of these substances is not always authorized by legal authorities dealing with efficacy and safety procedures, and many regulations of phytomedicinal products is the most alternative way.[11] The W.H.O considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries.[7]

Medicinal plants are the basis of huge financial worth in every part of the world. Over three-quarters of the globe population depends principally on plants and plant extracts for health care. More than 30% of the whole plant species, at one time or other, were used for therapeutic purpose.[12] It's been estimated that in developed countries like United States, plant medication constitute the maximum amount as 25% of the total medicine, whereas in fast developing countries like China and India, the contribution is the

maximum amount as 80%. Thus, the financial importance of medicinal plants is much more to countries such as India than to rest of the world. These countries supply two third of the plants used in modern system of medicine and the health care system of rural population depend on native systems of medicine. Ayurveda, Siddha. Unani, and folk medicines are the major systems of native medicines. Among these systems, Ayurveda is mainly developed and extensively practiced in India.[13] About 8,000 herbal remedies have been codified in Ayurveda. The possible use of higher plants as a source of new drugs is still inadequately investigated. Of the estimated 250,000-500,000 plant species, only a small share has been explore phytochemically and even a smaller share has been properly studied in terms of their pharmacological properties; in most case, only pharmacological screening or preliminary studies have been carried out.[14,15] It is estimated that only 5000 species are studied for medicative use. Even the Allopathic system of medicine has adopted a variety of plant-derived drugs that form an essential section of the modern pharmacopoeia. Some essential chemical intermediated required for producing the modern

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drugs are also obtained from plants. Not only, that plant-derived drug offers a stable market worldwide, however additionally plants still be a crucial supply for new medicine.[16]In several of the developing countries the employment of plant drugs is increasing because modern life saving drugs are away from the reach of three quarters of the world's population although several countries expend 40-50% of their total wealth on drugs and health care. As a section of the strategy to cut the economic burden on developing countries, it's obvious that an increased use of plant drugs will be followed in the future.[17] Unfortunately, much of the ancient knowledge and a lot of valuable plants are being lost at an alarming rate. With the rapid exhaustion of forests, impairing the availability of raw drugs.[18] It is logical to presume that a lot of useful drugs will be found in the plant kingdom if the search for these entities is carried out in a rational and systematic manner.

Table 1. Plant species with therapeutic value under different plant groups.[16]

S.No.	Plant groups	Number of species
1	Thalophytes	230
2	Bryophytes	39
3	Pteridophytes	382
4	Gymnospermae	55
5	Angiospermae	
A	Monocotyledons	676
B	Docotyledones	3495
	Total	4877

Table 2.Plant families containing over one hundred species with therapeutic worth.[16]

S.No.	Family	Genera	Species
A	Monocots		
1	Liliaceae	45	165
2	Orchidaceae	45	135
B	Dicots		
1	Compositae	89	331
2	Leguminoseae	91	313
3	Ranuculaceae	31	208
4	Laminaceae	46	189
5	Rosaceae	28	146
6	Umbellliferae	34	123
7	Rubiaceae	35	118
8	Euphorbiaceae	30	104
9	Asclepiadaceae	29	101

Table 3. Major medicinal plant that can be cultivated in India and have established demand for their raw materials.[16]

S.No.	Medicinal plant	S.No.	Medicinal plant
1	Acorus calamus	16	Heracleum candicans
2	Aconitum sp.	17	Hyoscyamus sp. Muticus
3	Adhatodavasica	18	Inula racemosa
4	aloe vera	19	Juglans regia
5	Ammi major	20	Juniperus spp.
6	Atropa acuminata	21	Matricaria chamomilla
7	Berberis aristata	22	Papaver somniferum
8	Carica papaya	23	Plantago ovate
9	Catharanthus roseus	24	Rauvolfia serpentine
10	Cassia senna	25	Rheum emodia
11	Cephalisipecauanha	26	Saussureaiappa
12	Cinchona spp.	27	Swertia chirata

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13	Dioscorea spp.	28	Urginea indica
14	Glycyrrhiza glabara	29	Valeriana wallichii
15	Hedchium spicatum	30	Zingiber officinale

Table 4. Medicinal plants having anti-diabetic activity.[19]

S.No.	Plant name	Family	Part used	Type of extract
1	Alangiumlamarckii	Alangiaceae	Leaves	Alcoholic
2	Albizia odoratissima	Mimosaceae	Bark	Methanol
3	Berberis vulgaris	Berberidaceae	Root	Aqueous
4	Catharanthus roseus	Aapocynaceae	Leaf	Methanol
5	Cocos nucifera	Arecaceae	Leaf	Hydro-methanol
6	Dillenia indica	Dilleniaceae	Leaves	Methanolic
7	Lippa nodiflora	Verbenaceac	Whole plant	Methanol
8	Marrubium vulgare	Lamiaceae	Aerial part	Methanol
9	Ocimum sanctum	Lamiaceae	Aerial part	Hydroalocholic
10	Psidium guajava	Myrtaceae	Fruits	Ethanol
11	Solanum xanthocarpum	Solanaceae	Leaves	Aqueous and methanol
12	Zygophyllum album	Zygophyllaceae	Whole plant	Ethanol

The Urtica urensRoxb is one of the plants that contain many active components that can be used in the treatment of many human diseases. Urtica urensRoxb. Belonging to family Urticaceae is a perennial plant which is commonly known as stinging nettle it is also known as bichughass, bichubuti in Hindi and Punjabi, vrishchhiya – shaaka in Sanskrit. It is widely distributed throughout the temperate and tropical areas around the world.

Diabetes mellitus is understood as a group chronic metabolic diseases characterized by the rise in blood sugar level because of defects in hypoglycemic agent (Insulin) secretion, hypoglycaemic action or both.[20] Insulin is a internal hormonal secretion produced by the beta cells of the pancreas, that is needed to exploit glucose from digested food as an influence supply.[21]The chronic hyperglycemia in diabetes is associated with long-term damage dysfunction and failure of assorted organs chiefly the heart, blood vessels, kidneys, eyes and nerves. A range of pathological changes are liable for the development of diabetes, autoimmune damage of beta cells of the pancreas causes diminish insulin secretion and lead to insulin shortage. The basis of the irregularity in the metabolism of carbohydrate, fat, and protein in diabetes reduce insulin on target tissues known as insulin resistance. Poor insulin action outcome from inadequate insulin discharge and/or diminished tissue responses to hypoglycaemic agent at one or additional points within the advanced pathways of internal secretion action. The symptoms of Hyperglycemia occur in patients because of defects in hypoglycaemic action often coexist in the same patient. Impairment of insulin secretion and hyperglycemia characterized by symptom such as renal disorder, polydipsia, weight loss, generally with polyphagia, and blurred vision.[22, 23, 24.]

Types of diabetes mellitus- Mostly There are two types of diabetes mellitus. The first type 1 or Insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes mellitus and second type 2 or Non-insulin-dependent diabetes mellitus (NIDDM) or type 2 adult onsets. However, as treatment recommendations evolve, actual classification of the type of diabetes mellitus complicates epidemiological analysis and clinical management. The new organization identifies four varieties of diabetes mellitus: type1, type2 as previously mentioned, other specific types and gestational diabetes. Each of the categories of diabetes mellitus known extend across a clinical demand.25

Type 1: Type 1 diabetes, previously called insulin-dependent diabetes mellitus IDDM or juvenile onset diabetes, Could account 5-10% to all diagnosed cases of diabetes. This can be characterized via beta cell destruction caused by an autoimmune process, usually resulting in absolute hypoglycemic agent shortage. The onset is typically acute, developing over a period of a few days to a week. Over 95% of persons with type 1 diabetes mellitus are affected by the disease before the age of 25, with associate degree equal incidence

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in every sex and increased prevalence within the white population. Risk factors are less well defined autoimmune disease, genetic, and environmental factors are involved in the development of this type of diabetes.1 Patients suffering from this are therefore totally dependent on the exogenous source of insulin. [20,21,25,26.]

Type 2: type 2 diabetes was previously called Non-insulin-dependent diabetes mellitus NIDDM or adult-onset diabetes that is characterized by hypoglycemic agent resistance in peripheral tissue and hypoglycemic agent secretary defect of the beta cell. This is the most ordinary type of diabetes mellitus account for about 76-85% of all the diagnosed cases of diabetes. Risk factors of the type 2 diabetes include obesity, older age, previous history of gestational diabetes, impaired glucose tolerance, physical inactivity, and family history of diabetes. [21, 23.25,26.]

Gestational diabetes- this is glucose intolerance being recognized during pregnancy. It can complicate pregnancy leading to prenatal morbidity and mortality, so clinical detection is important. Gestational diabetes is completely treatable but requires watchful medical supervision during the pregnancy. About 20-50% of affected women develop type 2 diabetes later in life.[26,27].

Other specific types of Diabetes

1 Genetic defect of the beta cell- These conditions are linked with monogenetic defects in beta cell function. The onset of hyperglycemia is generally earlier than the age of 25 years. They are stated as maturity- onset diabetes of the young and are characterized by impaired insulin secretion with negligible or no defects in insulin action these defects are inherited in an autosomal dominant pattern. [23, 28, 29.]

2 Genetic defects in insulin action- These are abnormalities linked with mutations of the insulin receptor and may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some persons with these mutations may have acanthuses nigricans. Women may be virilized (development of male sex characteristics in a female) and have enlarged cystic ovaries.[23, 28,29.]

3 Diseases of the exocrine pancreas: any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. Also included in this type are cystic fibrosis and hemochromatosis.[23, 28, 29]

4-Drug –or chemical- induced diabetes: this form of diabetes occurs with drugs or chemical that affect insulin secretion, increase insulin resistance or permanently damage pancreatic beta cells. A commonly encountered example is the patient taking long-term of high-dose steroid therapy for autoimmune diseases or post-organ transplantation, which can result in steroid-induced diabetes. [23, 28, 29.]

Plant Description:

Urtica urensRoxb. (Family-Urticaceae) is a perennial, monoecious herb found in damp and partially shady places of evergreen forest [30, 31]. It is having a place with family Urticaceae. It grows to a height of 1700-2800 m from sea level. It's found in Kingdom of Bhutan, Nepal, Western China, and India, particularly in Himalaya (lower altitude) Kashmir, Uttarakhand, West Bengal, Arunachal Pradesh, Tamilnadu and Sikkim [30, 32, 33]. It's a permanent plant is often referred to as mountain chain Urtica dioica and domestically as Shishoon in kumaun and kaldiya or kandali in Garhwal. The nettle is taken under consideration to be together of nature's best herbs for it consists of proteins, calcium, phosphorus, iron, magnesium, beta-carotene, besides vitamins A, C, D, and B complex. The leaves of the plant have stinging hairs that are answerable for the burning sensation and itching sensation on the contacted skin surface attributed to the presence of aminoalkane (histamine) and 5-hydroxytryptamine.33 Young leaves of the plant are nutritious and are cooked as food in Western mountain chain region. This plant is employed historically to cure numerous disorders. Young leaves of the plant are employed to cure goiter and associated pain. The leaves are also used to cure the allergic disorders like cold and cough. The leaf extract is employed to cure baldness and conjointly employed in hair wash. The fresh leaves and roots of the plants are applied to cure the dislocation of bones. As a result of the many healthful used, it's thought-about as a very important healthful plant. 30, 33

Geographical distribution

Urtica urensRoxb. is mainly found in moist and shady places at height of 1700- 2800 meters from the sea level. It is found in Bhutan, Nepal, Western China and India. In India grows

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naturally in Kashmir, Uttarakhand, West Bengal, Arunachal Pradesh, Tamilnadu and Sikkim.34

Cultivation and collection

Basically the nettle is considered as a weed. They have a perennial root system that spreads quickly and makes it very difficult to eradicate once it is established. The plants may be grown by the seeds or vegetative by divisions. Nettle seeds are small and they are easier to work with if they are mixed with some sand and a number of gardeners suggested that the herb seeds have a cold treatment prior to germinating. If seeds sow indoors, freeze the herb seeds for several weeks before sowing the stinging nettle seeds. Cover the trays or pots with a plastic dome or plastic wrap to keep the moisture high. If initial outdoors sow the stinging nettle seeds directly in the late fall so that the herb seeds can freeze up throughout the winter. Once the seedlings are 3-4 inches in height, transplant them to the herb garden in early spring. Space plants approximately 20 cm apart. Water as needed during dry periods.[35, 36, 37.] Young leaves and shoots are best harvested in spring through to autumn. Wait until the little nettles are 20 cm high. Be sure to wear gloves once harvest to avoid the sting delivered by little hairs on the leaves and stem. [38, 39,]

If the nettle is incredibly young solely harvest the highest bud and initial leaf set. Harvest the terminal bud can stimulate lateral bud growth inflicting the plant to become bushier and permitting harvest regularly from the constant plant. Don't harvest once flowering and avoid harvest old leaves once flowering as these become unpleasant. Collect seeds once they are ripe. Autumn is the time once nettle roots are harvested and employed in the preparation of a variety of herbal remedies. Dry the leaves on well-ventilated screen and place in a dark, warm and dry place.[39, 40, 41.]

Planting Directions

Table 5. Planting Directions

Temperature	65F
Average germ time	12-16 days
Light required	Yes
Depth	Surface sow seed and do not bury
Sowing Rate	7-10 seeds per plant
Moisture	keep seeds moist until germination
Plant spacing	15-18 inches

PHARMACOGNOSTIC PROFILE

Taxonomical categorization. [42, 43]

Table 6. Taxonomical categorization

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Angiospermae
Class	Dicotyledone
Subclass	Archichlamydeae
Order	Urticales
Family	Urticaceae
Genus	Urtica
Species	Urtica urensRoxb.

Common regional and vernacular names [42, 44]

Urtica urensRoxb. Is known by several common regional and vernacular names

Table 7. Common regional and vernacular names

English	Nettle, Sting nettle, Himalayan stinging nettle
Nepalese	Sishnu
Bengali	Paharahbichuti

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Gharwal	Kandli
Kumoun	Shishoon
Hindi	Bichubuti

Macroscopic studies

The Nettle is a perennial, monoecious herb 2-4 feet tall, found in moist and partly shady places of the evergreen forest. The plant generally grows up to 1 m height but may grow up to 2 m depending on place and soil condition. The plant has wide-spreading rhizomes that are long and stoloniferous and are bright in shading as the perennial roots.Leaves are dark green in color and coarsely toothed, with strong edges and a clear venation of the lower leaf surface. Leaves are 5-12 cm in length and 2-8 cm width. Both surfaces of the leaf are armed with stinging hairs. The leaves are borne oppositely on an erect wiry green stem. The stems are strong, hairy, less branched and quadrangular. The stem of the nettle can range between 25-50m in length and is green in young plants and purple/reddish in older ones. The grooved hollow stem of the plant is rigid, wiry and is covered with stinging hairs which release toxins. The whole plant is secured with erect and bristly glandular hairs whose tips come off when touched, transforming the hair into a needle that injects a stinging liquid, that contain acetylcholine, formic acid, 5-hydroxttrotamine, and histamine. The hairs on the leaves are especially excruciating. The plants lose their stinging qualities when they are dried. The plants bloom in mid-year; it has modes greenish or greenish white blossoms that hang down in drooping clusters which range from 4-7 cm in length. The fruiting time of the plant is from June-October Fruits are characteristics broadly ellipsoidal shaped achenes. They are yellow-green in color. They may be slightly compressed and are usually 1mm long. [45, 46, 47, 48]

Microscopy

The leaves of Urtica urensRoxb. have following characteristics: [30]

1. Upper and lower epidermis.
2. Consist of anomocytic stomata in the both epidermises.
3. 5 or 6 layers of collenchymas were visible.
4. Both epidermises covered by the thick cuticle.
5. The xylem and phloem vascular bundles are present.
6. Calcium oxalate prisms.
7. They don't contain palisade in the midrib region.

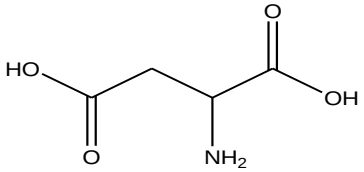
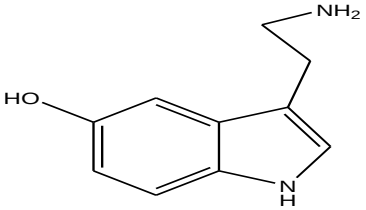
Table 8. Microscopic characters of Urtica urensRoxb. Leaf powder [30]

S.No.	Parameters	Observation
1.	Trichome	Unicellular
2.	Calcium oxalate crystals	Prism
3.	Epidermal cells	Elongated and uniform
4.	Stomata	Anomocytic

Phytochemistry

Urtica. urensRoxb. Contains many chemical constituents like histamine, serotonin (5-hydroxytryptamine) and acetylcholine. The further chemicals found in Urtica. urensRoxb. are malic acid, tryptophan, aspartic acid, serine and tyrosine and others such as alkaloids, flavonoids, phenols, polysaccharides, glycosides, and tannins. 42

Table 9: Phytochemical constituent with their chemical structure [42]

Chemical name	Chemical structure	Chemical name	Chemical structure
Acetylcholine		**Serotonin**	

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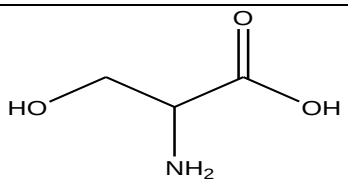
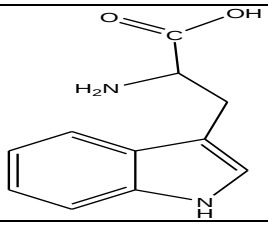
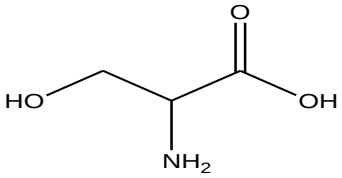
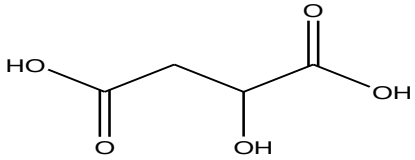
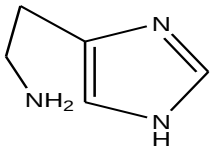
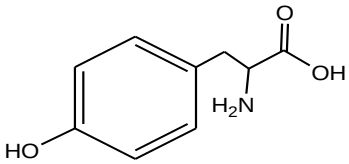
Aspartic acid		**Tryptophan**	
Serine		**Malic acid**	
Histamine		**Tyrosine**	

Chemical constituents

Stinging nettle is a powerhouse of nutrients. It contains on average 22% protein, 4% fats, 37% non-nitrogen extracts, 9-21% fiber, and 19-29% ash. The leaves concerning about 4.8 mg chlorophyl per gram of dry leaves, depending on whether or not the plant was full-grown within the sun or shade. Surprisingly, more chlorophyll and carotenoids are found in plants that have been grown in the shade. The dried leaf of nettle contains 40% protein.[44]

Nettle stems contain the simplest fiber that has been historically used for constant function as linen and is made by an identical setting method. Unlike cotton, nettles grow easily without pesticides

Urtica urensRoxb. contains several chemical constituents' viz. histamine, serotonin (5-hydroxytryptamine) and acetylcholine. The other chemicals found in Urtica. urensRoxb. are malic acid, aspartic acid, serine, tyrosine, and tryptophan. It is also rich in vitamins (Vit. C and α-tocopherol) [31, 38, 45.]

Pharmacological activities

Pharmacological activities-research has been reported on the pharmacologic activities of Urtica urensRoxb.

Hepatoprotective activity- Ethanolic extract of leaves of Urtica urensRoxb. was screened against carbon tetrachloride (CCl_4) elicited hepatotoxicity in rats. The orally administered extract of Urtica urensRoxb. was capable of decreasing elevated levels of aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total bilirubin, and humor macromolecule. The histopathology of the liver of the rats furthermore confirmed the useful effects.[39,]

Wound healing activity – Methanolic extract of the leaves of Urtica urens was investigated for wound healing property within the rats exploitation the excision, incision, and dead area wound models by administering the methanolic extracts of the plant at the dose of 300mg kg^{-1} day^{-1} and by applying alcoholic extracts (5%w/w) developed as an ointment prepared by Indian pharmacopoeia method. Healing was evaluated by the rate of wound contraction, time till complete epithelialization, granulation tissue weight, breaking strength, assessment of hydroxyproline and histopathological parameters. The investigated drug showed vital to wound healing activities compared to the control. It considerably cured wounds at a dose of 300mg/kg/day. [31]

Anti-oxidant activity- The inhibitor action of the hydromethanolic extract of Urtica urensRoxb. Was examined by completely different in-vitro ways specifically nitric oxide scavenging, DPPH scavenging, and reducing power assay. The antioxidant activity of the hydromethanolic extract of Urtica urensRoxb. Was compared with ascorbic acid as standard. The hydromethanolic extract of Urtica urensRoxb. The leaves were capable of guarding the cells against injuries caused by reactive oxygen species.[33]

Cardioprotective activity- Hydroethanolic extract of Urtica urensRoxb. leaf material was investigated

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for the cardioprotective property against doxorubicin-induced cardiotoxicity in rats. The hydroethanolic extract of Urtica urensRoxb. Guard the heart muscle by decreasing the elevated level of malondialdehyde (MDA), elevating the diminished levels of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and high density lipoprotein (HDL), with a concomitant decrease in the elevated levels of low-density lipoprotein(LDL), and Triglyceride(TG). Hydroethanolic extract of Urtica urensRoxb. Conjointly considerably reduced the raised activities of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). It disclosed that Hydroethanolic extract of Urtica urensRoxb. Exhibited vital cardioprotective action against cardiotoxicity elicited by doxorubicin in Wistar rats.[40,]

Nephroprotective activity- An extract of Urtica urensRoxb. was capable of exerting the nephroprotective impact in PCM-induced nephrotoxicity within the rat model. This action was evident by decrease in blood urea nitrogen (BUN) macromolecules and creatinine levels. [45]

Ethnobotanical uses

- Young leaves of the plant are used to cure goiter and associated pain. Young twigs with stinging hairs are applied to cure goiter or pain. The vegetable is eaten to cure cold and cough.[30,48]
- The leaves are also used to cure the allergic disorders such as cold and cough.[30]
- The fresh juice of the leaves is used to cure fracture, dislocation of bone; and boils. [30, 31]
- The plant decoction has been reported as febrifuge. [31]
- Plant decoction used in the treatment of fevers, root juice applied in case of throat pain, also taken for gonorrhea, roots decoction given in dog bite.[40]
- Branches with leaves applied externally on sprains and swelling for their counter-irritant properties.[40]
- Young leafy shoots taken as vegetables to get relief from rheumatic pain.[40]
- Veterinary medicine, poultice form the root applied to alleviate inflammation of the fractured or injured parts of domestic animals.[40]
- The stem fiber is of high quality and used to make cloth, fishing nets, and ropes and for some industrial materials.[49]
- It also used in Fever and illnesses to women following to child birth. [50]
- The leaves are used in dysentery, joint pain and liver disorders. [33]

II. REVIEW OF LITRATURE

S.No.	Title	Year	Author
1	Anti-pyretic, anti-inflammatory and analgesic activities of aqueous leaf extract of Urtica dioica L. In albino mice	2016	**Safari V.Z. et al** [51]
2	Antihyperglycemic evaluations of herbal drug combinations by in vitro methods	2016	Singh Y., Upadhayay A., et al [52]
3	Medicinal plants: Future source of new drugs	2016	Shakya A.K. et al [53]
4	Genetic Diversity analysis of Urtica urens in Uttarakhand Himalayas by rapid marker	2015	Sharma S.,et al [54]
5	Highlights on nutritional and therapeutic value of stinging nettle	2015	Said A.A. et al [48]
6	Phytochemical analysis of Stinging nettle leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry	2015	Al-tameme H.J. et al [55]

7	Evaluation of a root extract gel from Stinging nettle as analgesic and and-inflammatory therapy in rheumatoid arthritis in mice	2015	Jia-cheng L. et al [56]
8	A review on diabetes mellitus	2015	Harikummar K. et al [26]
9	Diabetes mellitus: a review	2015	Deshmukh C D., et al [57]
10	Models to study in vitro antidiabetic activity of plants: a review	2015	Dsouza D., et al [58]
11	Antioxidant potantial and total phenolic content of Stinging nettle (whole plant)	2015	Joshi B.C. et al [59]
12	Study of nettle leaves mineral composition and antioxidant capacity of their extracts obtained by Maceration and supercritical fluid extraction.	2015	Sidaoui F. et al [60]
13	Determination of bioactive molecules and antioxidant activity in stinging nettle	2015	Hijazi A. et al [61]
14	Assessment of antifungal activity of extracts from nettle (Urtica dioica L.) Against alternariasolani.	2015	Malgorzata N., et al [62]
15	Phytochemical and pharmacological accounts of some reviewed plants with andtidiabetic potential	2014	Kharjul M., et al [63]
16	Pharmacognostical standardization of Urtica urensRoxb. Leaves	2014	Kumar S., et al [30]
17	Pharmacognostical and pharmacological studies on Urtica urensRoxb: a review	2014	Kumar S., et al [42]
18	Stinging nettle: a review of its chemical, pharmacological, toxicological and ethnomedical properties	2014	Mamta S. Preeti K., et al [64]
19	Current status of some tropical plants with anti- diabetic potential- a review	2014	Majekodunmi S. O. et al [25]
20	A review on: potential antidiabetic herbal medicines	2014	Talaviya P.A., et al [65]
21	The effect of hydro-alcoholic extrct of Urtica dioica root on testes in adult rats	2014	Mohammad P., et al [66]
22	Pharmacognostical review of Urtica dioica L.	2014	Bhuwan C. J. et al [67]
23	Extraction, phytochemical screening, chemical quantification and identification of bioactive compounds from lebaneseUrtica	2014	Akram H. et al [68]

	dioica		
24	Determination of bioactive compounds including gc-ms investigation of hexane and dicloromethane extracts and trace metal composition of Urtica dioica	2014	Akram H., et al [69]
25	Molecular and chemical therapeutic features of Urtica species	2013	Alshuwayeb M.H., et al [70]
26	Herbal medicine- A rational approach in health care system	2013	Pathak K., et al [71]
27	Cardioprotective effect of ethanolic extract of Urtica urensRoxb. Against isoproterenol induced myocardial infarction in rats	2013	Barman N.R., et al [40]
28	Evaluation of in vitro anti diabetic activity of selected plant extracts	2013	Nair S.S., et al [72]
29	Current status of herbal drugs in the development of newer therapeutics agents	2013	Wahab S., et al [17]
30	A review on: potential anti-diabetic herbal medicines	2013	Arumugam G., et al [23]
31	Phytochemical and cytotoxic studies of polyphenolic flavonoids contents of Urtica dioica.	2013	Nohad A. Al. O., et al [73]
32	Leaves of common nettle As a source of ascorbic acid (vitamin c)	2013	Tatyana A. S., et al [74]
33	Determination of protein and mineral contents in stinging nettle	2013	Vesna R., et al [75]
34	Mineral properties and dietary value of raw and processed stinging nettle	2013	Laban k., et al [76]
35	Techniques for the extraction of bioactive Compounds from Stinging nettle.	2013	Hanan B., et al [77]
36	Study of nutritional quality, phytochemical constituents and Antioxidant activities by different solvents of nettle (Urtica urens).	2013	Kumar M., et al [78]
37	Antidepressant-like effect of extracts from Urtica dioica in mice model of depression.	2012	Amir M.C.,et al [79]
38	Screening antimicrobial activity of various extracts of Urtica dioica.	2012	Amir M.C., et al [80]
39	Potent Indian herbs used for the cure and management of diabetes	2012	Murgan T., et al [1]
40	Diabetes management: a literature	2012	Bregu A., et al [81]

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	review		
41	An overview on anti diabetic medicinal plants having insulin mimetic property	2012	Patel D.K., et al [82]
42	Investigating the chemical composition of different parts extracts of Urtica dioica	2012	Saeideh H. L. et al [83]
43	Characterization of antioxidant and antimicrobial activities of nettle leaves	2012	Zoran Z.,.K., et al [84]
44	Molecular characterization of the nettle plant Urtica urens based on RAPD marker	2011	Goel C., et al [85]
45	Pharmacological evaluation of herbal extracts for their in-vitro hypoglycemic activity	2011	Rao A.P., et al [86]
46	Plants having potential antidiabetic activity: a review	2010	Chauhan A., et al [87]
47	An antioxidant potential of hydro-methanolic extract of Urtica urensRoxb	2010	Pandey S., et al [33]
48	Antidiabetic and hypolipidaemic effects of few common plants extract in type 2 diabetic patients at Bengal	2010	Kumar B.D., et al 88
49	Evaluation of wound- healing activity of leaves of Urtica urensRoxb. And Callicarpa arboreaRoxb. In rats	2009	Kumar P., et al [31]
50	Hepatoprotective effect of the ethanolic extract of Urtica urensRoxb. In CCl4 treated rats	2007	Nath L., et al

III. MATERIAL AND METHODS

Preparation of plant material

Collections of plant: The whole plant of Urtica urensRoxb.was collected in the month of October-November from road side of Selaqui, Dehradun.

Washing: The collected plants were washed in tap water so that the dust can be removed and after this the plant were soaked in 1% saline water for 5min. to remove microbes.

Drying: Shade dry is recommended for the drying process for protection the rich vitamins and other photosensitive constituents. The plant material was spread on the sterile clean green net in a well ventilated room.

Grinding: Dried plant material was crushed in small pieces and then grinded by electric grinding machine for coarse powder and the powdered was stored in an airtight container which is protected from light and humidity.

Chemical requirement and their source: Required enzymes for the ant diabetic provided by the guide Mr. Ajay Singh Bisht. And other chemical and reagents used for standardization of drug taken from our college. HIPR Dehradun.

Table 10.List of chemicals used in our study

S.No.	Chemical name
1	Distilled water
2	n-hexane
3	Petroleum ether
4	Chloroform
5	Ethyl acetate
6	Methanol

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7	Phenol
8	Sulphuric acid
9	Hydrochloric acid
10	Soluble starch
11	Perchloric acid
12	Folin &ciocalteu's reagent
13	Sodium carbonate
14	Gallic acid
15	Aluminum chloride
16	Sodium nitrite
17	Sodium hydroxide
18	Rutine
19	Sodium acetate
20	Vanillin methanol solution
21	Cathechin
22	Alpha amulase enzyme
23	Benedict reagents
24	Fehling solution
25	Tannic acid
26	Hager's solution
27	Wegner reagents
28	Mayer reagents
29	Dragon doffs reagents

Physiochemical standardization:-

Determination of moisture content (loss on drying)

Procedure: for determining the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, was appropriately used. About 10 g of drug after precisely weighing (exactly weighed to within 0.01 g) it in a tarred evaporating dish. For example, for ungrounded or unpowderd drug. Prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness. Avoid the employment of high-speed mills in getting ready the samples, and exercise care that no considerable quantity of wet is lost throughout preparation which the portion taken is representative of the official sample. After placing the above said amount of the drug in the tarred evaporating dish, dry at **105** °c for 5 hours, and weigh. Continue the drying and consideration at one hour interval till the distinction between 2 sequent considerations corresponds to not more than 0.25 %. Constant weight is reached once 2 consecutive considerations when drying for a half- hour and cooling for a half hour in the desiccators, shows no more than 0.001 distinctions.

Determination of extractive values:-

Extractive value is a measure of the content of the drug extracted by solvents. Extractive value can be water soluble, alcohol soluble and hexane soluble.

Water extractive:

Procedure: Air dried 2 gram accurately weighted powdered drug placed in 250 ml of conical flask, macerated 24 hr, with 100 ml of water as a solvent in duplicate with proper marking and put on shaker for 6 hr for extraction allow to stand for 18 hr, after extraction process filter out and be precocious for solvent loss and arrange 2-2 petri plate for each conical flask with proper coding. These plates should be pre-weighted previously, pipette out 10 ml extract for each petri plate from the respective conical flask and dried till the solvent loss.

Formula used for calculation:

10 ml of extract solution contain = X gram extract
100 ml of extract solution contain = X100/10 =10X gram extract
2 gram powdered drug contain = 10X gram extract
100 gram powdered drug contain = 10X × 100/2 gram extract = 500X %

X = difference in pre weight and final weigh

Alcohol extractive:

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Procedure: Air dried 2 gram accurately weighted powdered drug placed in 250 ml of conical flask, macerated 24 hr, with 100 ml of alcohol as a solvent in duplicate with proper marking and put on shaker for 6 hr for extraction allow to stand for 18 hr, after extraction process filter out and be precocious for solvent loss and arrange 2-2 petri plate for each conical flask with proper coding. These plates should be pre-weighted previously, pipette out 10 ml extract for each petri plate from the respective conical flask and dried till the solvent loss.

Formula used for calculation:
10 ml of extract solution contain = X gram extract
100 ml of extract solution contain = X 100/10 =10X gram extract
2 gram powdered drug contain = 10X gram extract
100 gram powdered drug contain = 10X × 100/2 gram extract = 500X %

X = difference in pre weight and final weigh

Hexane extractive
Procedure: Air dried 2 gram accurately weighted powdered drug placed in 250 ml of conical flask, macerated 24 hr, with 100 ml of hexane as a solvent in duplicate with proper marking and put on shaker for 6 hr for extraction allow to stand for 18 hr, after extraction process filter out and be precocious for solvent loss and arrange 2-2 petri plate for each conical flask with proper coding. These plates should be pre-weighted previously, pipette out 10 ml extract for each petri plate from the respective conical flask and dried till the solvent loss.
10 ml of extract solution contain = X gram extract
100 ml of extract solution contain = X 100/10 =10X gram extract
2 gram powdered drug contain = 10X gram extract
100 gram powdered drug contain = 10X × 100/2 gram extract = 500X %

X = difference in pre weight and final weigh

Determination of total ash value:

Ash value is used to determine quality and purity of a curde drug and to establish the identity of it. The residue remain later than incineration is the ash contented of the drug, which basically correspond to inorganic redicals like phosphate, Carbonates, and silicates of sodium, potassium, magnesium, calcium etc. naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Many a time, the crude drugs are admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents. For determination of total ash, the powdered drug is incinerated so as to burn out all organic matter. Hence, quantitative determination in terms of various ash values helps in their standardization.

Procedure: 2 gram of air dried powdered drug was accurately weighted and put in pre weighted completely dried crucible and place in the muffle furnace at 550 °C after incineration powdered drug converted in to white ash, post weight was taken, difference in weight shows the total ash content.

% Ash value calculated by the formula:
2 gram powdered drug contain = X gram of ash
100 gram powdered drug contain = 100X/2 =50X

X = difference in the weight

Determination of total acid insoluble ash value:

Acid-insoluble ash which is a part total ash insoluble in diluted hydrochloric acid is also recommended for natural drugs. Adhering dirt and sand may be determined by acid-insoluble ash contain.

Procedure: Ash obtain after incineration was dissolved in the 10% 25 ml of the hydrochloric acid, heat for 5 minutes than solution was filtered with the ash less filter paper,after filtration filter paper made to neutralized by washing with the hot water. Took pre-weight of crucible and placed filter paper in the crucible incinerate at 550 °C till white ash obtain.

Formula used for calculation:
2 gram powdered drug contain = X gram acid insoluble ash

100 gram of powdered drug contain = 100X/2 = 50X

X = difference in the weight

Determination of total sugars:-
Reagent used:

A. 80% ethanol, 80% phenol, concentrated sulphuric acid.
B. Dextrose standard solution: (0.1 mg/ml) dissolve in 100 ml of 80% ethanol.

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Procedure: 0.5 gm homogenate of the plant tissue in 80% ethanol. Centrifuge at 2000 rpm for 15 min. The supernatant obtained is made up to known volume (generakky up tp 10 ml or depending on the expected concentration of sugar). Take 0.2 ml aliquot; add 0.1 ml of 80% phenol and 5ml conc. sulphuric acid, then make up the volume up to 10 ml with 80% ethanol, cool in ice bath. Total sugar was calculated as sucrose (mg/ml) using y= 0.003x +0.003, R^2 = 0.998 at 490nmusing UV-1 Double beam spectrophotometer, where y was the absorbance and x the dextrose equivalent (mg/ml).

Determination of total starch:

Reagent used

A. 80%ethanol, 80% perchloric acid, 80% phenol, concentrated sulfuric acid

B. Starch(soluble)standard solution: (0.1 mg/ml) in 100 ml of distilled water

Procedure: 0.5 gm homogenate of the plant tissue in 80% ethanol was centrifuge at 2000 rpm for 15 min. to the residue therefore obtained, put in 4 ml of distilled water, heat on a water bath for 15 min. and macerate by the help of glass rod. To each of the samples, add 3 ml of 52% perchloric acid and centfifuge at 2000 rpm for 15 min. The supernatant thus obtained is made up to known volume (generally up to 10 ml or depending on the expected concentration of starch). Take 0.1 ml aliquot, add 0.1 ml of 80% phenol and 5 ml conc. sulphuric acid, make up the volume up to 10 ml. cool and calculate total starch as starch soluble (mg/ml) using y= 0.004x + 0.001, R2 = 0.998, at 490nmusing UV-1 Double beam spectrophotometer,where y was the absorbance and x the starch equivalent (mg/ml).

Determination of total tannins

Regents used:

A. saturated Sodium carbonate solution: It was prepared by adding 35 g anhydrous sodium carbonate to each 100 ml distilled water, dissolved it at 70-80^{o}c and get cool overnight, filtered through glass wool.

B. Tannic acid standard solution: (0.1 mg/ml) dissolve 10 mg tannic acid in 100 ml of distilled water.

C. Folin &Ciocalteu's phenol reagent.

Preparation of standard curve: Standard curve was prepared using tannic acid as standard (10 mg tannic acid in 100 ml of distilled water).

Procedure: Extracted 2 g powdered plant material with 100 ml distilled water by boiling on water bath for 6-8 hrs., filtered and made up the volume to 100 ml volumetric flask. Took 1 ml aliquot of it, added 5 ml Folin&Ciocalteu's reagent, 10 ml saturated sodium carbonate and make up the volume up to 100 ml in volumetric flask. The instrument was calibrated through blank and took the corresponding absorbance of different samples, total tannin content calculated by using y = 0.003x + 0.009, R2 = 0.990, at 760 nm, using UV-1 Double beam spectrophotometer, where y was the absorbance and x the tannic acid equivalent (mg/ml).

Determination of total phenolics

Estimation of Total Phenolic content in the plant was carried out according to modified colorimetric Folin-Ciocalteu method.

Extract preparation: 1 gram air dried powdered drug percolated with pure methanol, three-time filter the extract and lyophilized to dry and was weighted.

Regents used:

A. 7% Sodium carbonate saturated solution: It was prepared by adding 7 g anhydrous sodium carbonate dissolved in 100 ml distilled water, and get cool.

B. Gallic acid standard solution: (1mg/ml) dissolve 10 mg gallic acid in 10 ml of deionized water.

C. Folin &Ciocalteu's phenol reagent

Procedure: A volume of 0.5 ml of deionized water and 0.125 ml of a known dilution of the extract were added to a test tube, Folin-Ciocalteu's reagent (0.125 ml) was added to the solution and allowed to react for 6 min. Then, 1.25 ml of 7% sodium carbonate solution was liquated into the test tubes, and the mixture was diluted to the 3 ml with deionized water. The color developed for 90 min, and the absorbance was read at 760nm, 0.002x + 0.051, R2 = 0.998, using UV-1Double beam spectrophotometer. The measurement was then compared to the standard curve of prepared Gallic acid solution and expressed as milligrams of Gallic acid equivalents per 100g of the sample extract.

Determination of total flavonoids:

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Total flavonoid contents of the extract solution based on the formation of a complex flavonoid-aluminium.

Extract preparation: 5 gram of dried powdered leaf and rhizomes cold percolated with known volume of methanol.
Reagent used:
2% Aluminum chloride
Rutin standard: 0.1mg/ml solution
Methanol

Procedure: A volume of 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ in methanolic solution was added. After one hour at room temperature, the absorbance was measured at 420 nm, using UV-1 Double beam spectrophotometer. Extract samples were evaluated at a final concentration of 0.01 mg/ml. All the determinations were done in triplicate. Total flavonoid content was calculated as Rutin (mg/ml) using the following equation based on the calibration curve: $y = 0.006x + 0.057$, $R^2 = 0.998$, where y was the absorbance and x was the Rutin equivalent (mg/ml).

Determination of total flavonols:
Extract preparation: 5 gram of dried powdered leaf and rhizomes percolated with known volume of methanol.
Reagent used:
2% Aluminum chloride solution.
5% Sodium acetate solution
Rutin standard: 0.1mg/ml solution
Methanol
Procedure: To 1.0 ml of sample, 2 ml of 2% $AlCl_3$ in methanol and 3 ml 5% sodium acetate solution were added. The absorbance at 440 nm was read after 2.5 hour at 20°C using UV-1 Double beam spectrophotometer. Extract sample were evaluated at a final concentration of 0.05 mg/ml. Total flavonol content was calculated as Rutin (mg/ml) using the following equation based on the calibration curve: $y = 0.002x + 0.058$, $R^2 = 0.999$, where y was the absorbance and x the Rutin equivalent (mg/ml).

Determination of total Proanthocyanidins:
Extract preparation: 5 gram of dried powdered leaf and rhizomes percolated with methanol for 48 hrs, and concentrated under vacuum using rota-vapour.

Reagent used:
A. 4% vanillin-methanol solution.
B. Concentrated hydrochloric acid
C. Catechin standard: 0.1mg/ml solution
D. Methanol

Procedure: To 0.4 ml of sample with 3 ml of 4% vanillin-methanol solution and 1.5 ml concentrated hydrochloric acid were added and shake well. The absorbance at 500 nm was read after 15 min at room temperature using UV-1 Double beam spectrophotometer. Extract sample were evaluated at a final concentration of 0.01 mg/ml. Total proanthocyanidin content was calculated as Catechin (mg/ml) equivalent using the following equation based on the calibration curve: $y = 0.004x + 0.053$, $R^2 = 0.997$, where y was the absorbance and x the rutin equivalent (mg/ml).

Successive extraction: In successive extraction process the powdered plant material extracted with non polar to polar solvent i.e. Hexane, chloroform, Ethyl acetate, methanol, Water so on the bases of the polarity of contain in the plant material will be extracted out in particular solvent like non polar in hexane and chloroform, intermediate polar in ethyl acetate or high polar in methanol and water. Soxhlet apparatus for (Hot percolation method) was used for successive extraction. Here continous extraction of a drug or any other substance which is recommended in monograph is done. The process consists of percolating it with suitable solvents at a temperature approximately that of the boiling point of the solvent. Any apparatus that permits the uniform percolation of the drug and the continuous flow of the vapor of the solvent around the percolator may be used.

Procedure: Assembly was arranged and thimble was prepared and place 5 gram of air dried powdered drug and was extracted with Hexane 3 days, than extract solution collected and concentrate under vacuum using Rota-vapour. Then the plant material was again collected and air dried. When completely dried placed back in thimble again. Similarly was done for chloroform, ethyl acetate, methanol, than water. Finally the dried extracts were collected in pre-weighted glass vials and post-weight for each vial was taken. calculated % yield.

Formula used:
5 gram air dried powder contain = X gram of extract
100 gram air dried powder contain = 100X/5 = 20X

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X = difference in weight of vial

Phyto-chemical screening:

Phyto-chemical screening comprise of the chemical evaluation of the plant successive extract, these are qualitative test which shows the presence or absence of different type phyto-constituent in plant from successive fraction i.e. Hexane, Chloroform, Ethyl acetate, Methanol, Water by using Soxhlet apparatus(Hot percolation method), subjected to qualitative tests for the identification of various active constituents including, Carbohydrate, Glycoside, Alkaloid, Amino acids, Flavanoids, Fixed oil, Tannins, Gum and Mucilage, Phytosterols.

Chemical requirement:

α-Naphtol, Benedict reagent, Fehling's A and B, conc Sulfuric acid, Ferric chloride, Vanillin hydrochloride reagent, Sodium hydroxide,Copper sulphate, Millon's reagent, Wagner's reagent, Hager's reagent, Ninhydrin, Dragandroff's reagent etc.

Procedure:

Test for carbohydrate:

- Molish's Test: Treat the extract with a small number of drop of alcoholic alpha-naphthol, put in 0.2 ml of sulfuric acid gradually through side surface of test tube, purple to violet color band appears at the junction.
- Benedict"s reagent: Treat the extract with few drop of benedicts reagent (alkaline solution containing cupric citrate complex) and boil on water bath, reddish brown ppt. forms if reducing sugar is present.
- Fehling's test: the same volume of Fehling A (copper sulfate in distilled water) and Fehling B (potassium tartarate and sodium hydroxide in distilled water) reagent are mixed along amount of extract, boil on water bath, brick red ppt of cuprous oxide form, if reducing sugar is present.
- Caramelisation: Carbohydrate when treated with strong sulfuric acid, they undergo charring with the dehydration along with burning sugar smell.

Test for tannin:

- Ferric chloride test : Extract give blue-green color with 5% ferric chloride solution.
- Vanillin hydrochloride test: Extract when treated with few drops of vanillin-hydrochloride reagent give purple red color.
- Alkaline reagent test: Extract treated with 5% sodium hydroxide solution give yellow to red ppt within short time.

Test for protein and amino acid:

- Test: Extract with few ml of 5% sodium hydroxide solution and 1% copper sulphate produce pink to purple color.
- Millon's test: Extract with 2 ml of millon'sreagent(mercuric nitrate in nitric acid containing traces of nitrous acid) white ppt appears which turns red upon gentle heating.

Test for alkaloids:

- Dragendroff's test (potassium bismuth iodide solution): Alkaloids give reddish brown ppt with dragendroff's reagent.
- Wagner's test (solution of iodine in potassium iodide): Alkaloids give reddish brown ppt with wagner"s reagent.
- Hager's test (saturated solution of picric acid): Alkaloid give yellow color ppt with hager's reagent.
- Mayer's test(potassium mercuric iodide): Alkaloids give yellow color ppt. with Mayer reagent.

Test for Flavonoids:

- Zinc hydrochloride reduction test: To extract add a mixture of zink dust and conc. Hydrochloric acid, it gives red color after few minutes.
- Alkaline reagent test: To the extract put in a small number of drops of sodium hydroxide solution, development of an deep yellow color which turns to colorless on adding of few drops of dilute acetic acid indicate the presence of flavonoid.

Test for saponins:

Test for resins:

- Extract dissolve in acetone and this solution was added to distilled water turbidity indicate the presence of resins.

Test for glycoside:

- The alcoholic, chloroform and water extractive were treated with acetic acid, ferric chloride and 2-4 drops of concentrated sulfuric acid,

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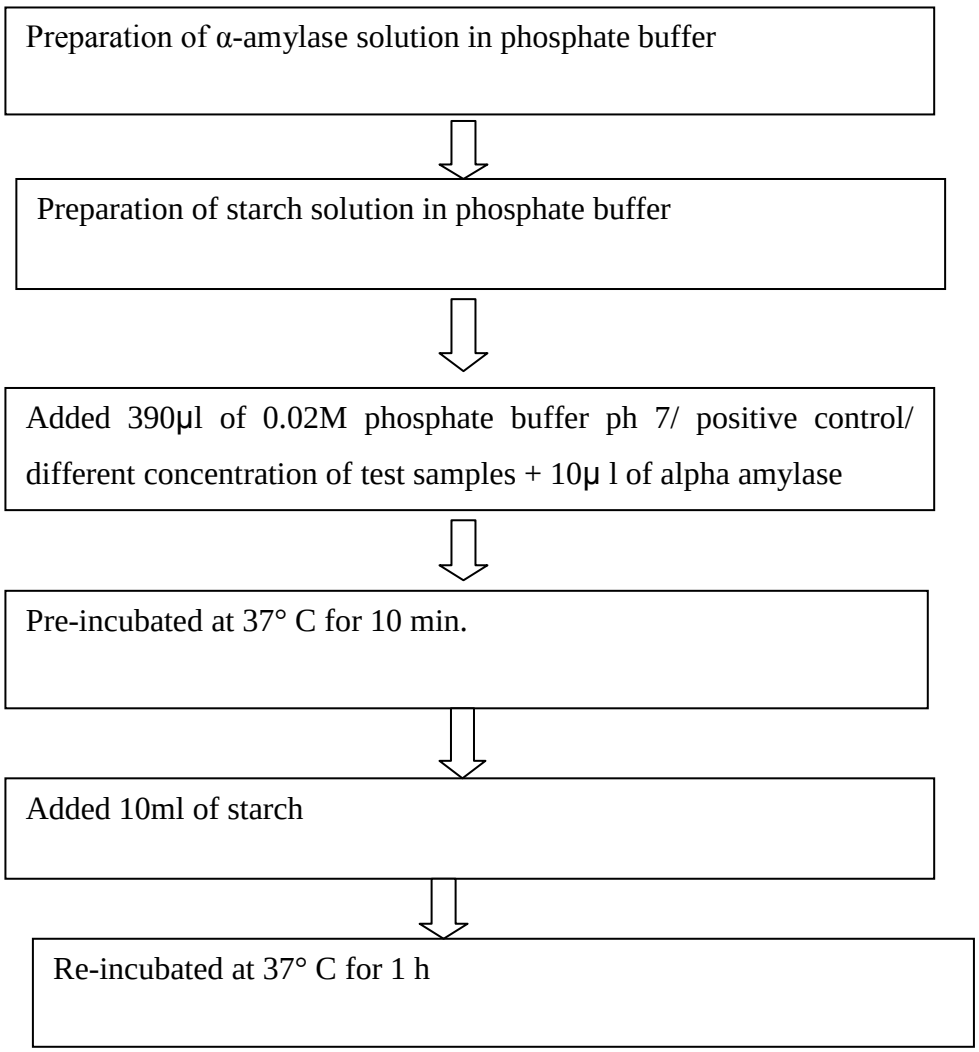
formation of blue color indicate the presence of glycoside.

IN VITRO ANIDIABETIC ACTIVITY

Alpha-amylase inhibition method. Alpha-amylase activity can be measured in-vitro by hydrolysis of starch in presence of alpha-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance/extract possesses alpha-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α -
amylase inhibitory activity.

Procedure:

Alpha-amylase activity was carried out by starch-iodine method. 10 µL of α-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µL of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and C= absorbance of control (without starch).



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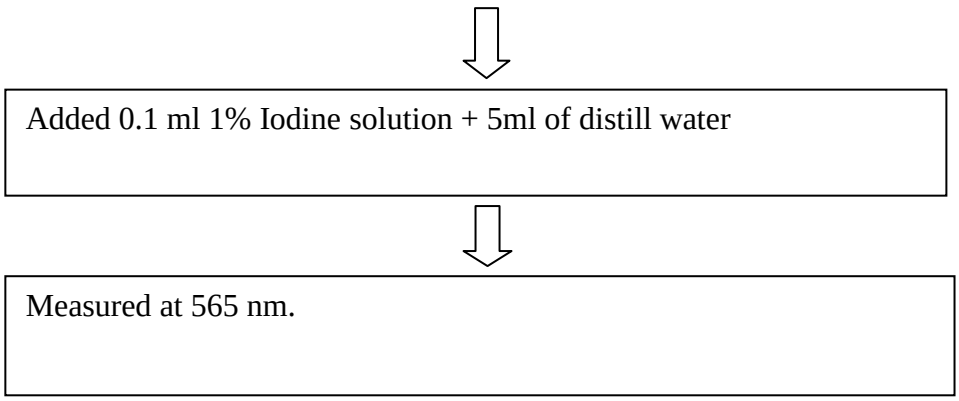

Figure 1: Chart Schematic flow chart of alpha-amylase enzyme inhibition assay procedure.

IV. RESULTS AND DISCUSSION

Total moisture percent: Result obtain from the study is tabulated in table

Table 11. Moisture content

Parameters	Moisture content %
Loss on Drying at 105°c(LOD)	11.43%

Loss on Drying of powdered of Urtica urensRoxb. was found to 11.43% and it is an important parameter to minimize the decomposition of drug either due to chemical change or microbial contamination.

Total Ash value and acid insoluble ash: Result obtained from the current study is recorded in table.

Table 12. Percent total Ash value and acid insoluble ash

Sample	% Total Ash value	% acid insoluble ash
Whole plant	10.6	4.7

Flouroscence Analysis:-Flouroscence analysis it was found that when the powdered of Urtica urensRoxb. was treated with different reagents on different U.V. wavelength i.e. 254nm and 366nm. It showed different colours which are characteristics of drugs. Which is recorded in table

Table 13. Observation of Flouroscence Analysis of powdered of Urtica urensRoxb.

Sn.	Experiments	Daylight		UV-light	
				254nm	365nm
1	Powder + water	Amber green		Light green	Amber green
2	Powder + aqueous NaOH	Green		Brown	Dark green
3	Powder +alcoholic NaOH	Light green		Brick red	Green
4	Powder + conc. H2SO4	Black		Black	Black
5	Powder + conc. Hcl	Dark brown		Black	Black
6	Powder + Nitric acid	Yellow		Yellow	Yellowish green
7	Powder + 5% ferric chloride	Brown		Brown	Amber green

Total extractive value:-

Total hexane, chloroform, ethyl acetate, alcohol, and water extractive value reported in table

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Table14. Total extractive value of hexane, chloroform, ethyl acetate, methanol, and water extractvie.

Sample	% hexane extractive	% chloroform extractive	% ethyl acetate extractvie	% methanol extractive	% water extractive
Plant	1.41%	2.42%	3.6%	7.67%	8.28%

Determination of total sugar contents:-

Table 15. Calibration curves of dextrose (standard).

S.No.	**Concentration(µg/ml)**	Absorbance
1	10	0.0412
2	20	0.0721
3	30	0.1032
4	40	0.1356
5	50	0.1721

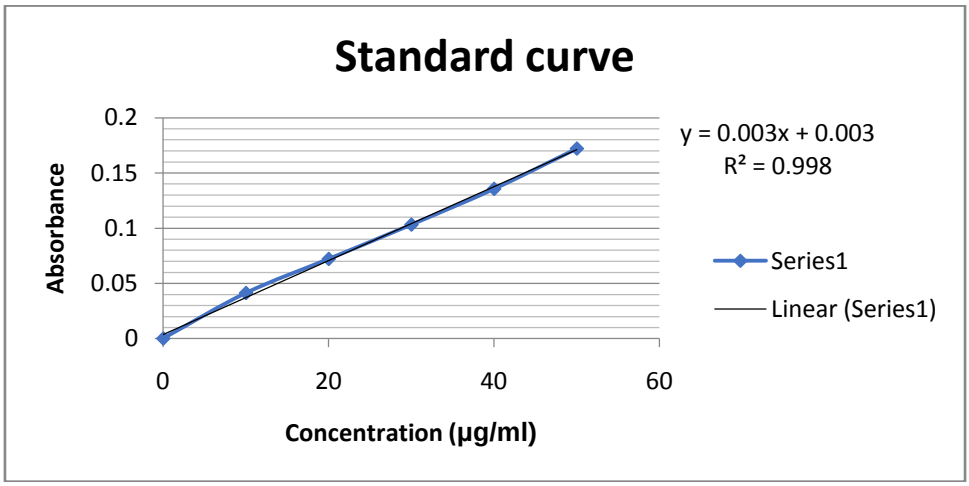

Figure 2: Standard curve of Dextrose.

Table 16. Observation of sugar content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	26.16	0.0815

Determination of total starch contents:

Table 17. Calibration curve of starch soluble (standard)

S.No.	**Concentration(µg/ml)**	**Absorbance**
1	10	0.0431
2	20	0.0912
3	30	0.1285
4	40	0.1671
5	50	0.2154

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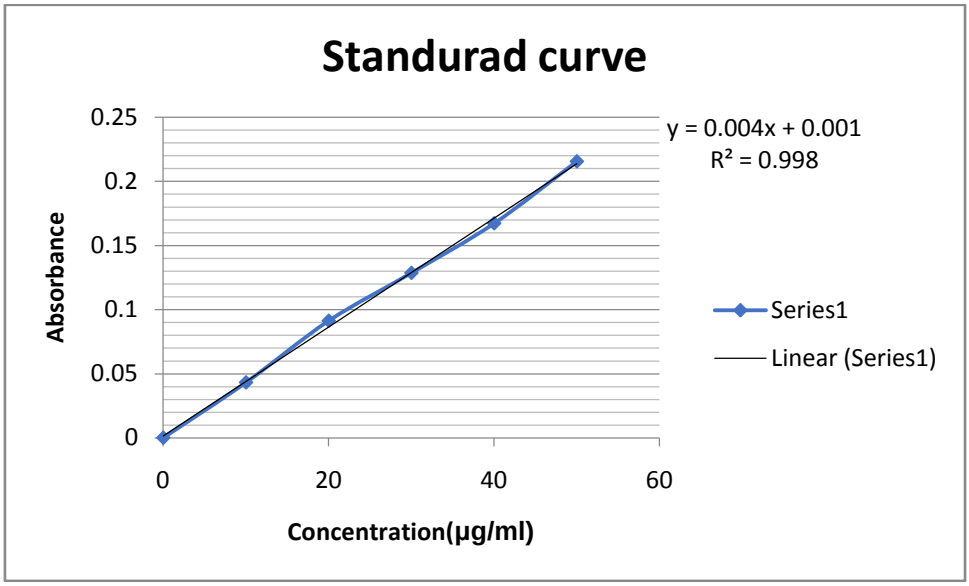

Figure 3: Standard curve of starch soluble.

Table 18. Observation of starch content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	22.17	0.0897

Total tannin contents:

Table.19 Calibration curve of tannic acid (standard)

S.No.	Concentration(µg/ml)	Absorbance
1	10	0.0498
2	20	0.0894
3	30	0.1256
4	40	0.1542
5	50	0.1864

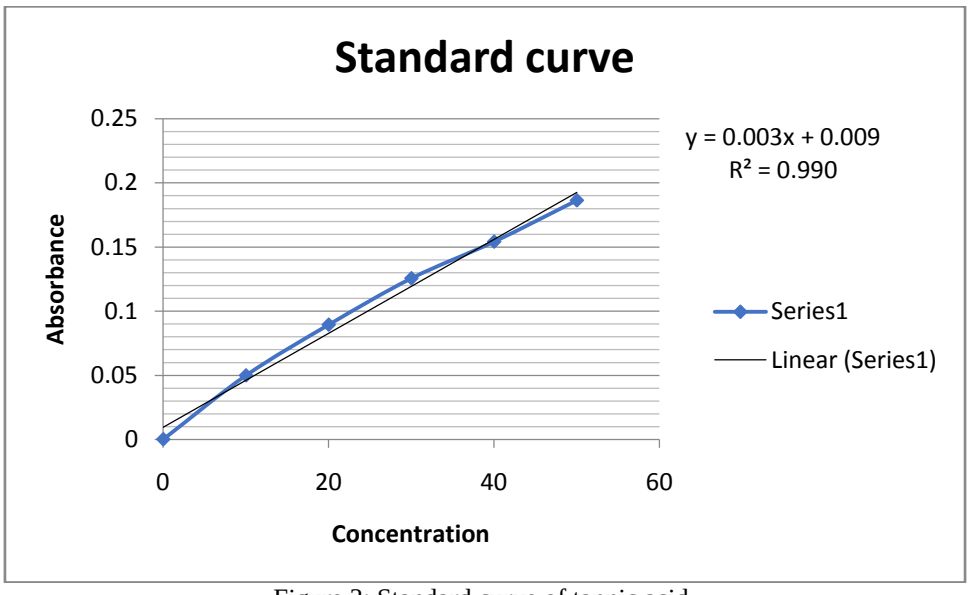

Figure 3: Standard curve of tannic acid.

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Table 20. Observation of tannin content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	23.4	0.0792

Determination of total phenolic content:

Table 21. Calibration curve of galic acid (standard)

S.No.	Concentration(µg/ml)	Absorbance
1	10	0.0821
2	20	0.1032
3	30	0.1254
4	40	0.1531
5	50	0.1876

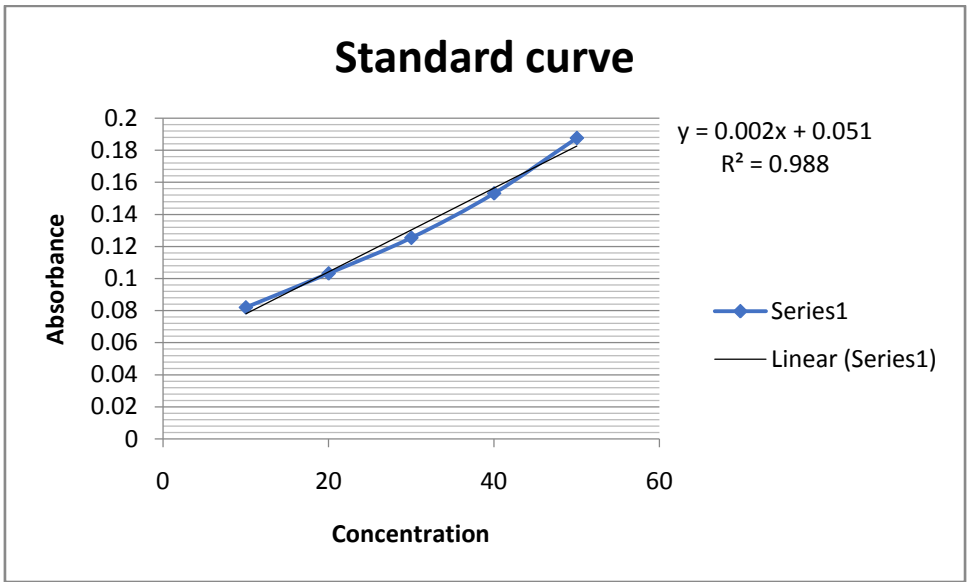

Figure 4: Standard curve of gallic acid.

Table 22. Observation of phenolic content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	31.05	.1131

Determination of total flavonoids content:

Table 23. Calibration curve of Rutin (standard)

S.No.	Concentration(µg/ml)	Absorbance
1	10	0.1251
2	20	0.1843
3	30	0.2432
4	40	0.3153
5	50	0.3789

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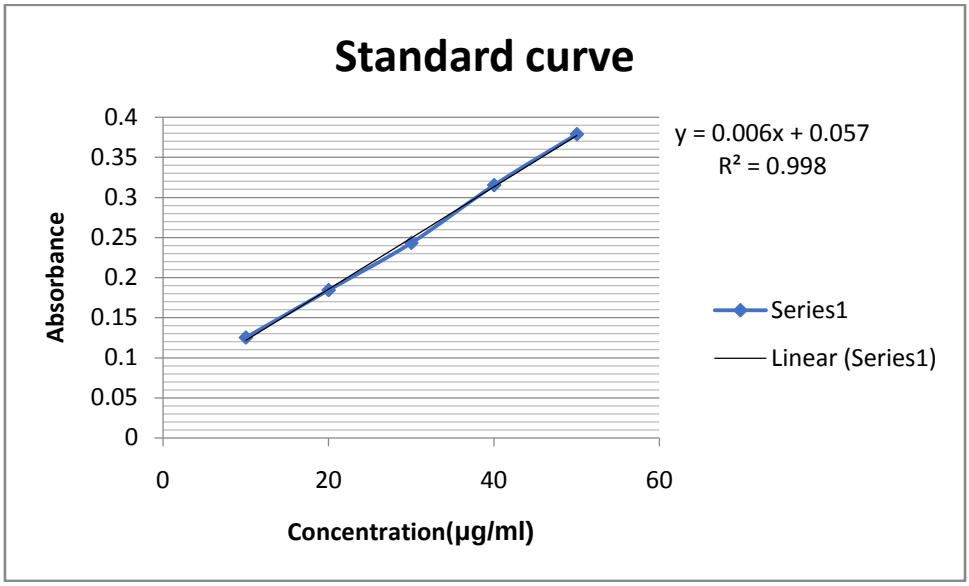

Figure 5: Standard curve of Rutin

Table 24. Observation of flavonoids content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	24.01	0.2011

Determination of total flavonols content:

Table 25 Calibration curve of Rutin (standard)

S.No.	**Concentration(µg/ml)**	**Absorbance**
1	10	0.0812
2	20	0.1034
3	30	0.1254
4	40	0.1487
5	50	0.1703

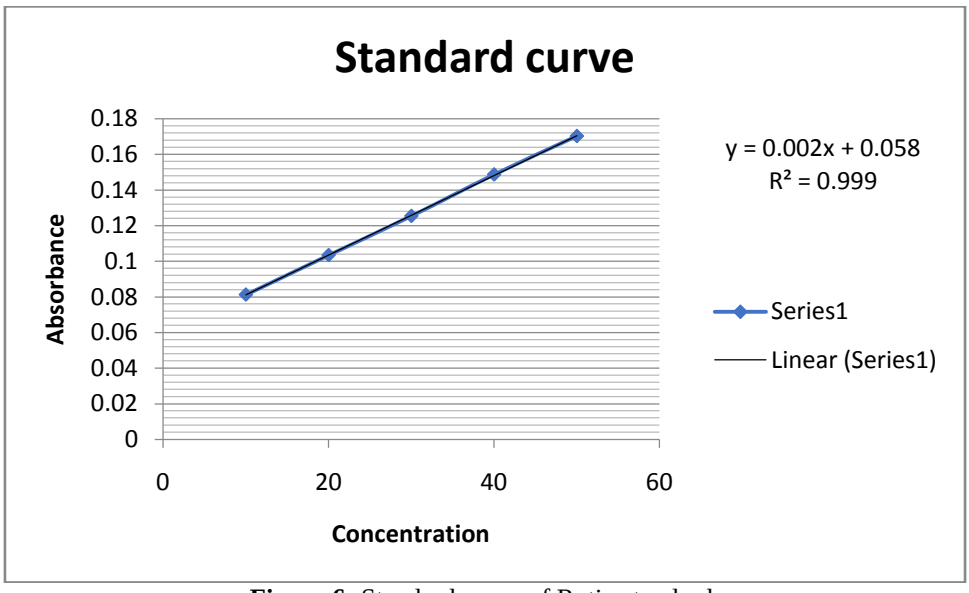

Figure 6: Standard curve of Rutin standard.

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Tablet 26. Observation of flavonols content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	23.1	0.1042

Determination of total proanthocyanidins content:

Table 27 Calibration curve of Catechin (standard)

S.No.	Concentration(µg/ml)	Absorbance
1	10	0.0943
2	20	0.1343
3	30	0.1676
4	40	0.2098
5	50	0.2543

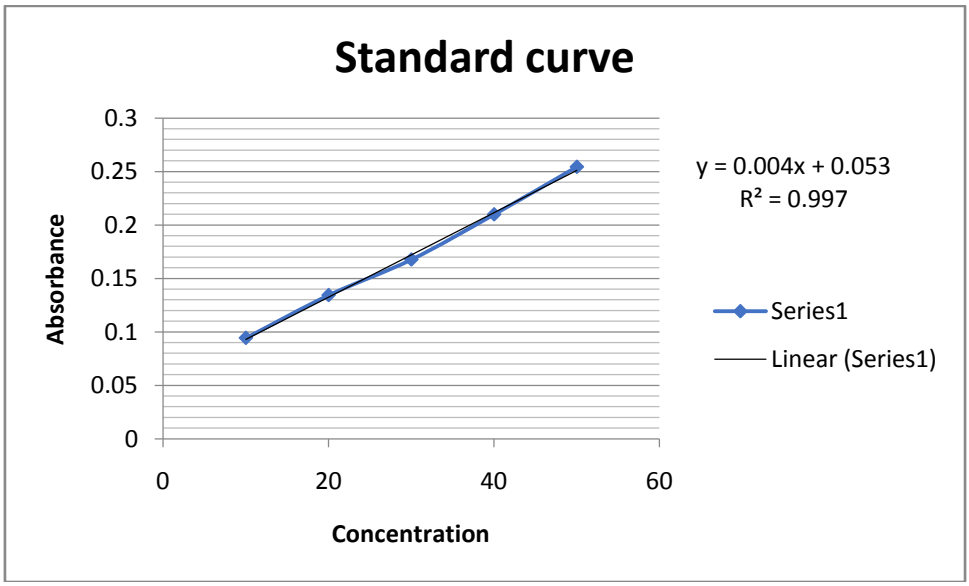

Figure 7: Standard curve of Catechin.

Table 28. Observation of proanthocyanidins content in test solution of plant Urtica urensRoxb.

S.No.	Concentration(µg/ml)	Absorbance
1	21.85	0.1404

Preliminary Phyto-chemical screening:

Table 29. Phytochemical screening of successive fraction from soxhlet, (+) shows presence, (-) and show absence of content of extract.

S.No.	Compound	Test	n-hexan extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Water extract
1	Carbohydrates	Molish's	-	-	+	+	+
		Fehling's	-	+	+	+	+
		Benedict's	-	+	+	-	-
		Caramelisatin	+	+	+	+	+
2	Alkaloids	Dragendroff's	+	+	-	+	+
		Mayer's	+	-	+	-	+
		Wagner's	-	+	+	-	+
		Hager's	+	+	+	+	+
3	Tannin	Ferric chloride	+	+	+	-	-
		Vanillin Hcl	-	-	-	-	-

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		test					
		Alkalin reagent	+	+	+	-	+
4	Test for protein and amino acid	Biuret	+	+	-	+	-
		Millon's	+	+	+	-	-
		Ninhydrin	+	+	-	+	-
5	Flavonoids	Alkaline reagent	-	+	-	+	+
		Zinc hydrochloride	+	-	+	+	+
6	Glycosides	General test	+	+	+	-	-
		Froth test	-	+	+	-	-

IN VITRO ALPHA-AMYLASE INHIBITION METHOD

Table 30: Show % inhibition of alpha-amylase enzyme

S.No.	Concentration of sample (ml)	% Inhibition
1	0.2	34.14%
2	0.4	48.14%
3	0.6	65.7%
4	0.8	75.4%
5	1.0	78.4%

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