ISOLATION AND PHYTOCHEMICAL SCREENING OF STINGING NETTLE

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DEPARTMENT OF BIOTECHNOLOGY, BIOINFORMATICS AND PHARMACY

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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

CERTIFICATE

This is to certify that the work titled " ISOLATION AND PHYTOCHEMICAL SCREENING OF STINGING NETTLE" submitted by "ADITI VERMA" (081754) AND "PRIYANKA SINGH" (081761) in partial fulfillment for the award of degree of bachelor of pharmacy of jaypee university of information technology, Waknaghat has been carried out under my supervision . This work has not been the submitted partially or wholly to any other university or institute for the award of this or another degree or diploma.

Signature of Supervisor :

Name of Supervisor : Dr. Udayabanu. M

Designation : Lecturer

Date : 28/5/12

ACKNOWLEDGEMENT

Foremost, I would like to express my sincere gratitude to my Project Supervisor for the Dr. Udayabanu. M continuous support of my project work and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to thank the rest of my project panel : Dr. Pradeep K. Naik, Mr Rajkumar Tiwari, Dr. Rahul Shrivastava for their encouragement, insightful comments, and hard questions. I would also like to thank Mr. Sita Sharan Patel for his support and guidance throughout our project work.

Last but not the least I would like to thank my parents for being the constant guiding light in my life and being the pillars of support always.

NAME OF THE STUDENTS : ADITI VERMA (081754)

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DATE : 28|5|2012

SUMMARY

Our project " Isolation and characterization of active constituents from the stinging nettle" includes different extraction method such as conventional extraction method, soxlet apparatus column chromatography and TLC as well as the various phytochemical screening of the extract obtained .

The extract were futher studied on rat colon for their pharmacological action i.e, the muscarinic agonist effect on mice.

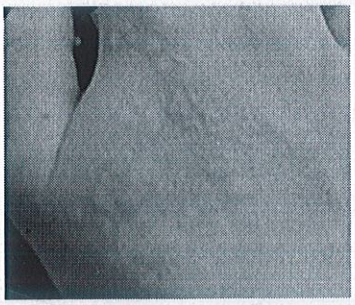
Subsequent doses of ***Urtica dioica*** (ml) : 0.1, 0.2, 0.4, 0.8 was given. Extract showed muscarinic agonist effect on rat colon.

Chapter 1

INTRODUCTION

Stinging nettle or **common nettle**, ***Urtica dioica***, is a herbaceous perennial flowering plant, native to Europe, Asia, northern Africa, and North America, and is the best-known member of the nettle genus *Urtica*.

The plant has many hollow stinging hairs called trichomes on its leaves and stems, which act like hypodermic needles that inject histamine and other chemicals that produce a stinging sensation when contacted by humans and other animals.



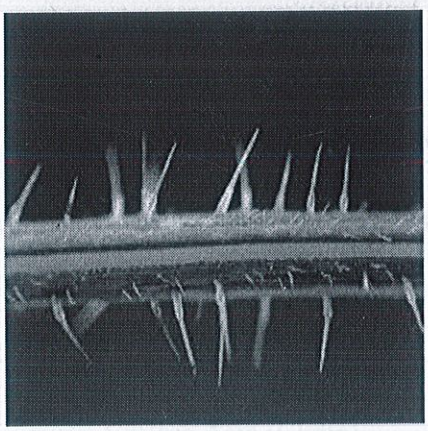
1.1 DISTRIBUTION

- Stinging nettles are abundant in northern Europe and much of Asia.
- In North America it is widely distributed in Canada and the United States, also can be found in northernmost Mexico. It grows in abundance in the Pacific Northwest, especially in places where annual rainfall is high.

Human and animal waste may be responsible for elevated levels of phosphate and nitrogen in the soil, providing an ideal environment for stinging nettle.

Chapter 2

Review of literature



2.1 SCIENTIFIC CLASSIFICATION

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Rosales
- Family: Urticaceae
- Genus: *Urtica*
- Species: ***U. dioica***
- Binomial Name : *Urtica dioica*

2.2 TAXONOMY

There are at least five clear subspecies :

- *U. dioica* subsp. *dioica* (European stinging nettle). Europe, Asia, northern Africa.
- *U. dioica* subsp. *galeopsifolia* (fen nettle or stingless nettle). Europe. (also known as *Urtica galeopsifolia*)
- *U. dioica* subsp. *afghanica*. Southwestern and central Asia. (Gazaneh in Iran)
- *U. dioica* subsp. *gansuensis*. Eastern Asia (China).
- *U. dioica* subsp. *gracilis* (Ait.) Selander (American stinging nettle). North America.
- *U. dioica* subsp. *holosericea* (Nutt.) Thorne (hairy nettle). North America.

2.3 MORPHOLOGY

- Stinging nettle is a dioecious herbaceous perennial, 1 to 2 m (3 to 7 ft) tall in the summer and dying down to the ground in winter.
- It has widely spreading rhizomes and stolons, which are bright yellow as are the roots.
- The soft green leaves are 3 to 15 cm (1 to 6 in) long and are borne oppositely on an erect wiry green stem.
- The leaves have a strongly serrated margin, a cordate base and an acuminate tip with a terminal leaf tooth longer than adjacent laterals.
- It bears small greenish or brownish numerous flowers in dense axillary inflorescences.
- The leaves and stems are very hairy with non-stinging hairs and also bear many stinging hairs (trichomes), whose tips come off when touched, transforming the hair into a needle that will inject several :-

CHEMICALS: acetylcholine, histamine, 5-HT or serotonin, moroidin, leukotrienes, and possibly formic acid.

- This mixture of chemical compounds cause a painful sting or paresthesia from which the species derives its common name, as well as the colloquial names burn nettle, burn weed, burn hazel.

2.4 CONSTITUENTS

- It contains on average 22% protein, 4% fats, 37% non- nitrogen extracts, 9-21% fiber, and 19-29% ash.
- The leaves contain about 4.8 mg chlorophyll per gram of dry leaves, depending on whether the plant was grown in 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. They are one of the highest known sources of protein in a leafy green, and of superior quality than many other green leafy vegetables [1]
- The leaves are also noted for their particularly high content of the metals selenium, zinc, iron, and magnesium.
- They contain boron, sodium, iodine, chromium, copper, and sulfur. They also contain tannic and gallic acids, gum, and wax. Sixteen free amino acids have been found in the leaves, as well as high silicon levels in the leaves, stems and roots. [4]
- The fresh leaves contain vitamins A, C, D, E, F, K, P, and b-complexes as well as thiamin, riboflavin, niacin, and vitamin B-6, all of which were found in high levels, and act as antioxidants.
- Formic acid, silicon, potassium, tannins, glucoquinones, acerylocholine, serotonin, carbonic acid, mucilage, many minerals. [2]
- **Phytochemicals:** histamine, acetylcholine, serotonin, flavonol glycosides, sitosterol, lectin, coumarins, hydroxysitosterol, scopoletin, tannins, lignans.

2.5 STINGING NETTLE AS FOOD

Stinging nettle has a flavour similar to spinach when cooked and is rich in vitamins A, C, iron, potassium, manganese, and calcium.

- Soaking nettles in water or cooking will remove the stinging chemicals from the plant, which allows them to be handled and eaten without incidence of stinging. [5]

2.6 USES

- The roots and leaves, are used for the treatment of asthma, wheezing and short breath by opening up the passage of lungs.[14]
- As a gargle it helps the swelling of the mouth and throat.
- A decoction of the leaves provokes the courses and urine and expels gravel and stone.
- It kills worms in children, eases pain in the sides and dissolves wind in the spleen [15]

- The seed taken as a drink is remedy against the bites of dogs, cleansing the body of all accumulated toxins and in the rapid removal of metabolic waste.
- Act as blood purifier.
- Juices of the leaves are helpful or a decoration of the root is used for fistulas and gangrenes and corrorind scabs or itch.
- The leaves are helpful in the treating blood pressure, cystitis and anemia.
- The roots are also used in treating diarrhoea, dysentery and inflammation and ulcerations affecting the digestive tract. [14]
- It is claimed to stimulate the growth of hair in persons who are suffering from hair loss and gives natural colour to hair.
- Nettle helps in curing rheumatism, arthritis, gout and skin infections.[10]
- Tonics are manufactured for the treatment of physical weakness.
- Bleeding in any area of the body is treatable by the strong astringent action of the nettle.
- It is also used in treating burns and scaled injuries.
- The leaves help in anemia and improve breast-milk production[6]
- The root is now used to treat enlarged prostate. Abscess, Addictions and Herpes.
- The warm tea is used for asthma, hay fever, allergies, colds, fever, grippe, flu, mucous condition of the lungs, pleurisy, leprosy, diarrhea, cholecystitis, dysentery, hemorrhoids, various hemorrhages, scorbutic affections, and mucous in the colon in adults.[8]

2.7 APPLICATIONS

AERIAL

INFUSION – This herbal form of the nettle remedy can be used to stimulate the circulatory system in people suffering from impairment in the flow of blood and it can also be used as a detoxification agent to cleanse the system of toxins in individuals afflicted by disorders such as arthritis, it can be used to treat rheumatism, to treat symptoms of gout, and to treat symptoms of eczema. The herbal infusion made from the nettle also helps in increasing the flow of milk in nursing mothers with lactation issues. A revitalizing spring tonic can be produced from the fresh shoots of the nettle.

TINCTURE --The herbal tincture form of the nettle is utilized in combination with other beneficial herbs in the treatment of various disorders such as arthritic conditions, to treat various skin problems, and in the treatment of heavy uterine bleeding in women suffering from menstrual diseases.

WASH – The herbal remedies made from the nettle can also be used as a healing salve and herbal wash and applied to burns, to insect bites, and to wounds.

JUICE – The herbal nettle remedy can also be used in the form of a nettle juice and this can be prepared by liquefying the whole fresh plant to make a good herbal tonic for the treatment of debilitating conditions and cases of anemia, this same tonic can be used to soothe the stings of the nettle hairs.

The nettle based tonics are also often prescribed for the treatment of cardiac insufficiency coming along with disorders such as edema.

POWDER – Herbal remedies made from the powdered leaves of the nettle can be inhaled as a snuff for the treatment of nosebleeds.

ROOT

HAIR RINSE – The nettle roots can also be used to make an herbal decoction, which can be used as a rinse for the treatment of dandruff, to stem the causes of falling hair, and as a general conditioner for a healthy scalp.

OINTMENT – As an herbal nettle ointment, the nettle is used to topically treat cases of hemorrhoids, the ointment is directly applied to the affected region of the body.

COMPRESS – The herbal remedies made from the nettle can be used to make a herbal compress by soaking a pad in the herbal tincture of the nettle.

This compress can be applied to the areas affected by painful arthritic joints, this compress can also be used in the treatment of gout, to treat cases of neuralgia, in the treatment of various kinds of sprains and cramps, in the treatment of tendinitis, and to treat sciatica in the lower limbs.

2.8 DOSAGE

Infusion: Steep 2 to 3 tbsp. leaves or plant in 1 cup water for 10 minutes.
Juice: Mix with an equal amount of water and take 1 tsp. at a time.

Solid : Take 1 to 2 capsules daily. Upto 4 times per day for hayfever (not to exceed 8 capsules per day).

2.9 DRYING PROCEDURE

The drying process includes several operations or treatments depending on the source of the crude drugs (animal or plant) and its chemical nature. Drying consists of removal of sufficient

moisture content of crude drug, so as to improve its quality and make it resistant to the growth of microorganisms. Drying inhibits partially enzymatic reactions. Drying also facilitates pulverizing or grinding of a crude drug. In certain drugs, some special methods are required to be followed to attain specefic standards. The slicing and cutting into smaller pieces is done to enhance drying.

There are two type of drying process depending upon the type of chemical constituents present.

1) **NATURAL**

sun drying : in case of natural drying, it may be either direct or sundrying in the shed.if the contents of the drug are stable to the temperature and sunlight the drug can be dried directly in sunshine.shed drying is done for the compounds containing volatile components.

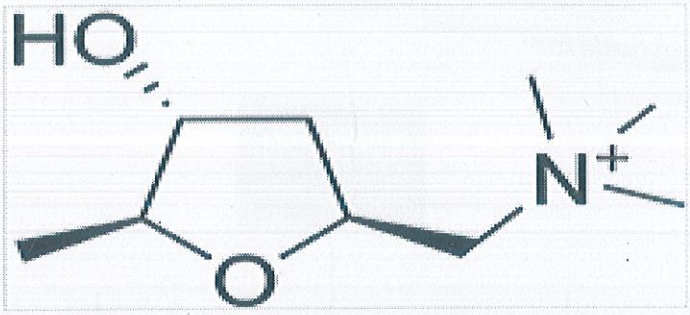
2) **ARTIFICIAL DRIERS**

a) **vaccum driers**: For the drugs that are sensitive to higher temperature.

b) **Spray driers**: For the drugs that are highly sensitive to atmospheric conditions and also temperature or vaccum drying .

c) **Tray driers**: For the drugs which do not contain volatile oil.

And are quite stable to heat or which need deactivation of enzymes. In this method hot air of desired temperature is circulated through the driers that facilitates the removal of water content of the drug.



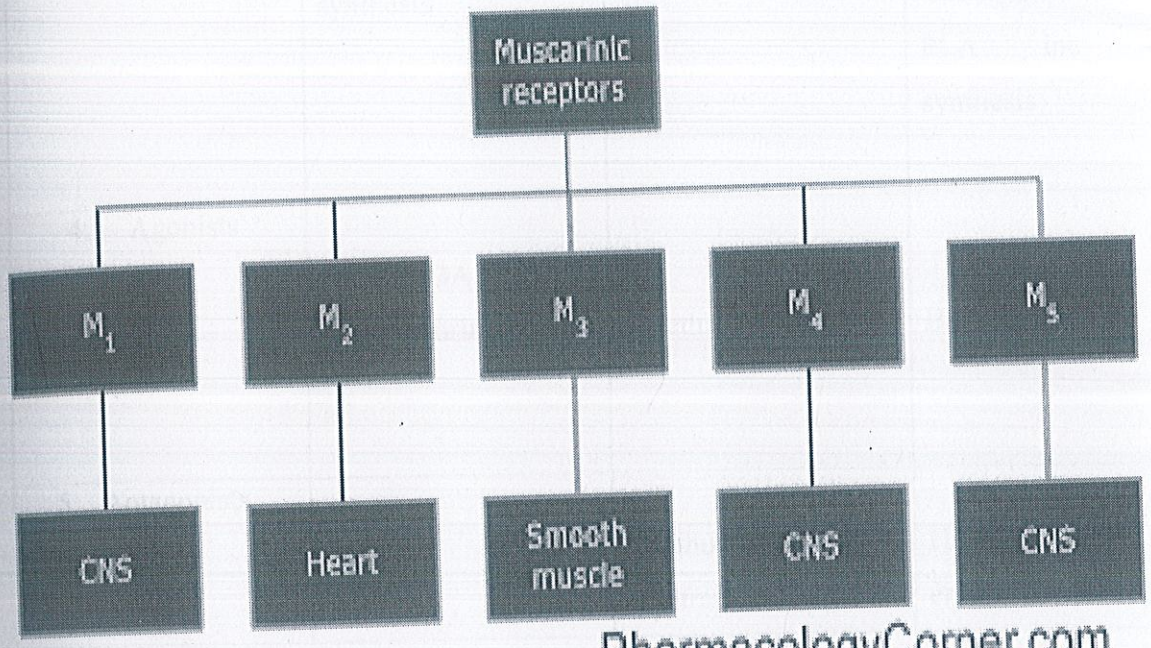
MOLECULAR STRUCTURE OF MUSCARINE

2.10 **What are muscarinic receptors ?**

- These are acetylcholine receptors selectively stimulated by muscarinic and blocked by atropine.
- These are located primarily on autonomic effector cells in heart, blood vessels, smooth muscles, eye and glands of gastrointestinal, respiratory and urinary tract, sweat glands etc and in CNS.

Subtypes of muscarnic receptor :

- They are divide into 5 subtypes : M1 M2 M3 M4 M5
- The first five the major subtypes present on effector cells and on prejunctional nerve ending as well in the CNS. The M4 and M5 are present on the nerve ending on the certain areas of brain and regulate the release of other neuro transmitters.
- Functionally M1, M3 and M5 fall in one class and M2 and M4 in another class.



4. transducer mechanism	IP3 /DAG-inc cytosolic Ca2* PLA inc. -PG synthesis	K* channel opening, Decreased CAMP	IP3/DAG-inc. cytosolic Ca2* PLA inc -PG synthesis
4. Agonists	MCN-343A, Oxotremorine	Methacholine	Bethanechol
5. Antagonists		Methoctramine, tripitramine	Hexahydrosiladif enidol, darifenacin

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Chapter 3

OBJECTIVES

3.1 WHAT MAKES STINGING NETTLE SO IMPORTANT ?

Source

Stinging nettle is a perennial member of the nettle family, native to both Europe and the United States. The root and the leaves are used.

- CurrentStatus

  Recent studies suggest that the leaf tea aids coagulation and formation of hemoglobin in red blood cells. A freeze-dried nettle leaf product has shown slight activity in the treatment of allergies.

- Because of its highly effective pharmacological effects and therapeutic uses, it has been considered as an important medicinal plant. And is being currently studied and research is being done to explore more effective uses and applications.

- Our project is basically related to isolation and characterisation of chemical constituents from stinging nettle.

3.2 Why *Urtica dioica* chosen for the study ?

In the present scenario, where the world is completely dependant on drugs for the various treatment of diseases worldwide, chances are also there of the side-effects caused by these curing chemically synthesized drugs which may be even sometimes fatal and have a lethal effect on the a patient .Therefore, there was a paradigm shift in the preference of the synthetically modified natural products or semi-synthetic drugs over the already existing synthetic drugs. Natural drug from the plants are gaining popularity because of several advantages such as few side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other system of medicine.

The objective of the present work is extraction of phytoconstituents from dried leaves of ***Urtica dioica*** .Phytochemical screening and evaluation of its muscarinic agonist activity on rat colon.

Chapter 4

METHODOLOGY

4.1 COLLECTION AND DRYING

COLLECTION

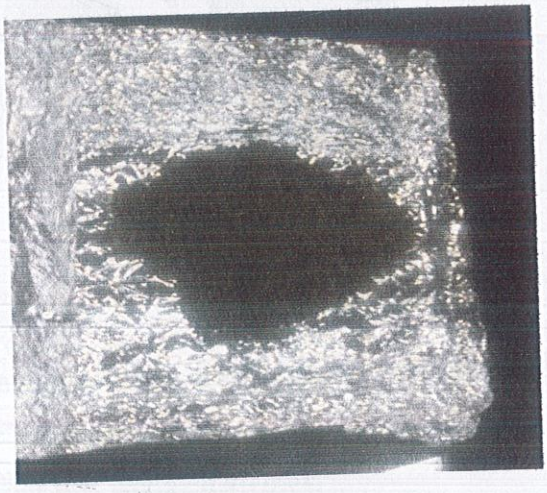
The whole plant was uprooted and washed with water and then the different parts of the plant such as roots, stems , leaves etc were separated.

DRYING PROCEDURE

Leaves were separated from the plant, was washed with water and air dried .

Afterwards, the dried leaves were crushed into powder form manually i.e, by hands.

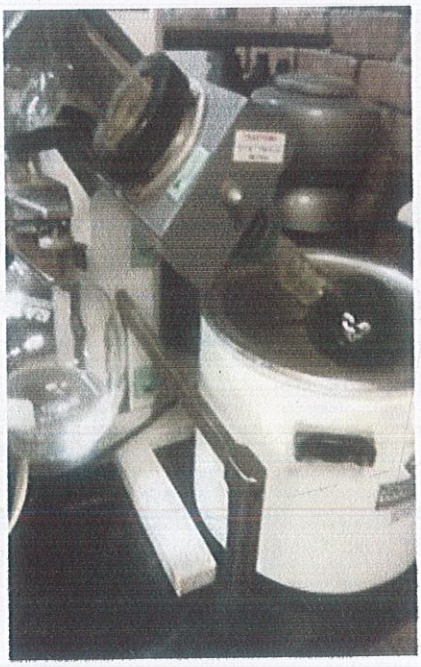
Leaves kept for drying



POWDERED LEAVES

4.2 CONVENTIONAL EXTRACTION METHOD

- *Urtica diocia* leaves were ground into powder .
- The powdered material was defatted with hexane by immersing 150 gms of leaves in 300 ml hexane and was kept for 1 to 2 hrs .
- The leaves were taken out, air dried and dipped in 650 ml of methanol and kept overnight
- The pure methanol obtained, was evaporated using rota evaporation . The concentrated extract was run on TLC (thin layer chromatography).

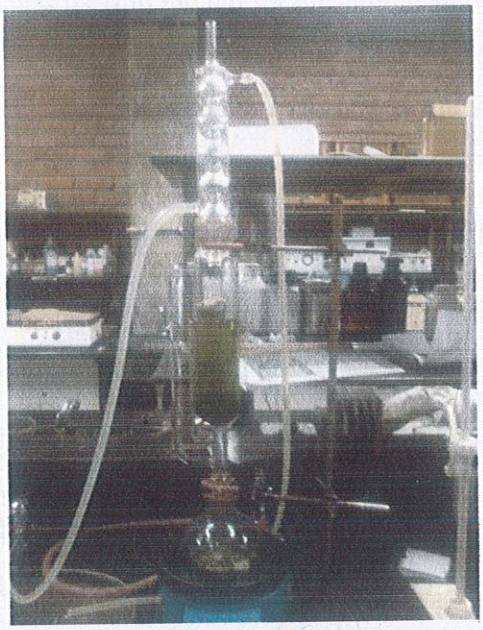


Rota evaporator

4.3 SOXLET APPARATUS

- The leaves of the stinging nettle i.e, ***Urtica dioica*** were collected and washed with water.
- Dried in sunlight for 2 to 3 days. The leaves after fully dried crushed into powder form.
- Weight of dried powder was weighed 46 gms.

- Futher an experiment was setup for soxlet apparatus. The given 46 gms of crushed leaves were immersed in hexane in the soxlet apparatus to remove chlorophyll and waxy substances till clear solution is seen in tube of the apparatus .
- When the color of the extract is clear in the tubes the extraction setup for hexane is removed and the leaves were left for drying.
- Further 200 ml of distilled water and 200 ml of methanol mixture were put in the RBF.
- The assembly was set up again for the soxlet apparatus to run for 72 hrs till the color of leaves turned brown.



Soxlet apparatus

- The methanolic extract was concentrated using rota evaporator.
- The concentrated methanolic extract was evaporated in open in the lab and redissolved in 50% aqueous methanol and separated in chloroform three times with sepratory funnel.
- Three layers were formed, the chloroform being separated at the bottom most layer.
- TLC was run using chloroform and ethyl acetate as solvent system (9:1 ratio).

Distance travelled by the spot : 5.9 cm

Distance travelled by the solvent : 5.9 cm

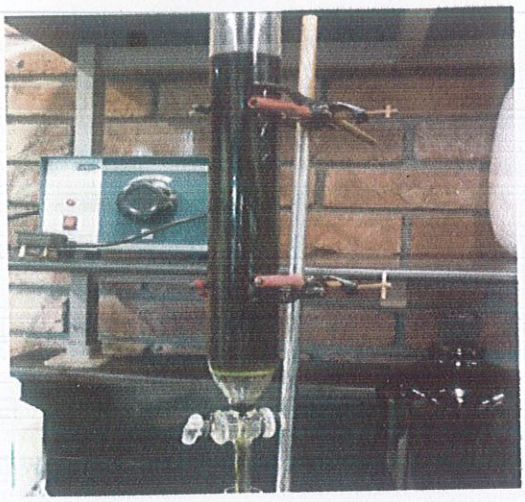
Distance travelled by the solute : 3.7 cm

RF Value : 0.62cm

- Anhydrous sodium sulphate was added to the chloroform layer separated so as to remove water and further filtered, evaporated and crystals were formed in three different petri plates.
- One petri plate was used in the lab for the animal study
- The second petri plate was preserved for further project studies in the lab.
- In the third petri plate 25% methanol was added and left for crystallization.

4.4 COLUMN CHROMATOGRAPHY

- The powdered material was defatted with hexane by immersing 150 gms of leaves in 300 ml hexane and was kept for 1 to 2 hrs .
- The leaves were taken out air dried and dipped in 650 ml of methanol and kept overnight .
- The methanol extract was evaporated in rota evaporation. The concentrated extract was run on TLC (thin layer chromatography).
- Column chromatography of the methanolic layer was performed using silica gel.
- The solution was checked for the presence of flourosence in 12-13 TLC plates and the solution which showed flouroscence was seperarted from the one which did not flouroscence.
- The flouroscene emmiting layer was concentrated in the rota evaporator and the extract was redissolved in methanol (25ml) and kept overnight for crystallization.



Column chromatograph

4.5 TLC SOLVENT SYSTEM

Chloroform (9ml) : Ethyl Acetate (1ml)

Flouroscence tested for the presence of active constituents in the U.V range using the U.V light instrument. The same TLC slides kept in iodine chamber.

Further, separation performed three times in the separatory funnel using chloroform (75ml) each time . During the separation 3 layers observed i.e, the middle layer, upper layer and the lower layer . TLC of the upper layer and the chloroform layer was made to run in TLC . TLC showed more flouroscence in the methanolic layer and less floroscenes in the chloroform layer. Therefore solvent system changed due to chances of presence of glycosides ie, sugar moeity.

TLC made to run again using the solvent system of

- Acetic acid (1ml): n- butanol (4ml): water (2ml)
- TLC made to run again and results came positive. The chloroform layer ran less due to presence of active constituent and methanolic layer ran more.
- The chloroform layer again concentrated in the rota evaporator .
- This rota extract was made to concentrate over water bath to obtain solid residue which was redissolved with methnol(25 ml) and left for crystallisation over night.

4.6 PHYTOCHEMICAL SCREENING

TEST FOR CARBOHYDRATES:

- MOLISH TEST: To 2-3 ml aqueous extract, add few drops of alpha-napthol solution in alcohol , shake and add conc.sulphuric acid from sides of the test tube.

TEST FOR REDUCING SUGARS

- BENEDICT'S TEST: Mix equal volume of benedict's reagent and extract in test tube. Heat in boiling water bat for 5 min.

TEST FOR HEXOSE SUGAR

- SELWINOFF'S TEST: Heat 3ml of selwinoff reagent and 1 ml extract in water bath for 1-2 min.

TEST FOR PROTIENS

- MILLION'S TEST: Mix 3ml extract with 5 ml million's reagent.

TEST FOR CARDIAC GLYCOSIDES

- LEGAL'S TEST : To aqueous extract add 1ml sodium nitroprusside.

TEST FOR FLAVONOIDS

- Addition of increasing amount of sodium hydroxide to the extract.

TEST FOR ALKALOIDS

- MAYER'S REAGENT : To 2-3 ml extract, add few drops of mayer's reagent.

TEST FOR TANNINS AND PHENOLIC COMPOUNDS

- To 2-3 ml alcoholic extract, add few drops of acetic acid solution .

TEST FOR IRON

- To 5ml extract add few drops of potassium ferrocyanide.

TEST FOR AMINO ACIDS

- Heat 3ml extract and drops ninhydrin solution in boiling water bath 10 min.

TEST FOR IODINE

- Mix 3ml extract and few drops of dilute iodine solution.

4.7 BIOLOGICAL EVALUATION

MUSCARINIC AGONISTACTIVITY ASSAY

- Rat was sacrified after cerebral dislocation.
- Colon was immediately removed and was aerated in krebs henseleit buffer solution.

Components in krebs henseleit buffer solution (g/L) :

D-Glucose 2.0

Magnesium Sulfate [Anhydrous] 0.141

Potassium Phosphate Monobasic 0.16

Potassium Chloride 0.35

Sodium Chloride 6.9

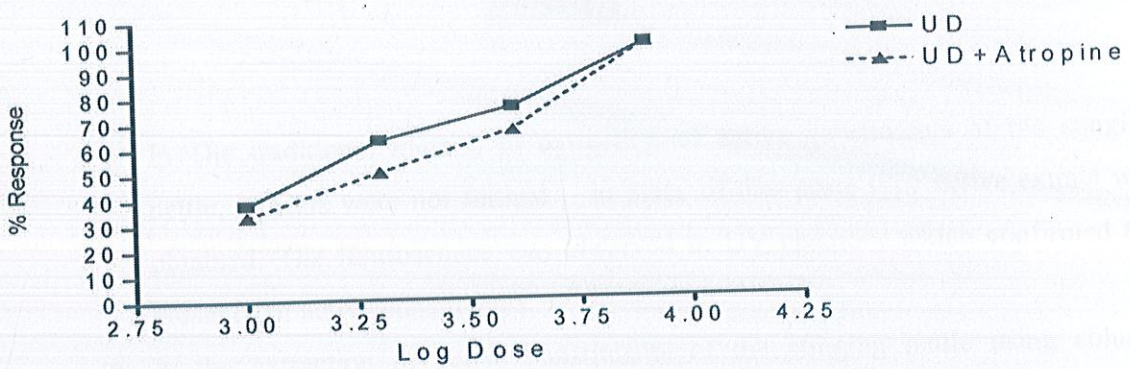
- Tied the thread to the top and bottom of muscle preparation before deataching the muscle from the body of the rat.
- Preparation mounted in up right position in the organ bath containing rat ringer solution under a tension of 1 g.
- Organ bath was bubbled with air.
- Tissue relaxed for 45 min , during which tissue was washed with ringer for atleast 4 times.
- Contraction due to ***Urtica dioica*** extract was recorded using frontal writing lever.
- Concentration – response curve of ***Urtica dioica*** extract was recorded using four doses.
- The concentration response curve of ***urtica dioica*** extract was repeated using atropine
- Labelled and fixed the sstracing.
- A plot was graphed and concentration response curve of ***urtica dioica*** in presence and absence of atropine was compared.
- Inhibition in response of ***Urtica dioica*** recorded.

EC 50 VALUES

Dose Ratio = EC50 in presence of ***Urtica dioica*** / EC50 in the absence of atropine

S.NO	Dose (ml)	Dose (ug)	Dose 10g	Response in mm	%age Response (Extract)	%age Response (Extract+atropine)	Height (mm)
1	0.1	1000	3.0	3	37.5%	33%	2
2	0.2	2000	3.30	5	62.5%	50%	3
3	0.4	4000	3.60	6	75%	66%	4
4	0.8	8000	3.90	8	100%	100%	6

Dose response curve of Stinging Nettle extract in rat colon



4.8 TEST FOR SCOPOLETIN

- Pinch of crushed dried leaves were taken in test tube and was moistened with water.
- The filter paper soaked with 1% NaOH was kept on the opening of the test tube.
- Test tubes were kept in water bath for 5-7 minutes. The same filter paper was checked for flourescence under UV chamber.

Chapter 5

RESULT

- In The traditional method of extraction of active constituents of the stinging nettle, crystals were not formed , in place of that paste type active extract was obtained. The flouroscence was observed in UV chamber which confirmed the presence of active constituents.
- In the extraction of active constituents of the stinging nettle using column chromatography, crystals were not formed, in place of that paste type active extract was obtained. The flouroscence was observed in UV chamber which confirmed the presence of active constituents.
- In the extraction of active constituents of the stinging nettle using soxlet apparatus, crystals were obtained. The crystals were further studied on rat (khymograph) which showed the muscarinic agonist effect of the extract on colon of rat.

The various phytochemical screening of the extract showed the presence of following constituents in our plant:	REAGENTS	RESULT
TEST FOR CARBOHYDRATES	MOLISH TEST	VIOLET RING FORMED AT JUNCTION OF TWO LIQUIDS
FOR REDUCING SUGARS	BENEDICT'S TEST	GREEN COLOR
FOR HEXOSE SUGAR	SEWINOFF'S TEST	RED COLOR
FOR PROTIENS	MILLION'S TEST	BRICK RED PPT
FOR CARDIAC GLYCOSIDES	LEGAL'S TEST	PINK COLOR
FOR ALKALOIDS	MAYER'S TEST	PPT. NOT FORMED
TANNINS AND PHENOLIC	ACETIC ACID	RED COLOR
FOR IRON	POTASSIUM FERROCYNAIDE	DARK BLUE COLORATION
FOR FLAVONOIDS	SODIUM HYDROXIDE	YELLOW COLORATION, DISCOLORISES WITH ACID
FOR AMINO ACIDS	NINHYDRIN SOLUTION	PURPLE COLOR APPEARS
FOR IODINE	DIL IODINE SOLUTION	BLUE COLOR APPEARS WHICH DISAPPEARS ON BOILING

Chapter 6

DISCUSSION

- The different methodologies were carried out in project lab such conventional extraction method, soxlet apparatus, column chromatography and TLC.
- Drying and crushing of leaves the leaves were washed in water and expose to sunlight for 2-3 days so as to avoid fungal growth which could decompose the leaves. During the crushing it was noted that the stem portion not included. Gloves were worn as an essential precaution so as to avoid stings and skin allergy. In case of any kind of inflammation on contact , leaves of the plant ***Spinacia oleraceae*** were rubbed against the skin. In the conventional extraction method, leaves were immersed and the extract was filtered and separated using a separatory funnel .
- In the column chromatography, afte setting up the experiment, the separated layers TLC was run and tested for the presence of flouroscence.
- In the soxlate apparatus method, number of cycles were run so as to have a clear solution of the extract running in the tubes of the soxlate. Care was taken, the solvent did not evaporate as the instrument was covered with aluminium foil when not in use. During the separation precipitates were formed therefore 50% methanol was added.
- During the phytochemical screening it was made sure the reagents being used were fresh and the dropper was cleaned by methanol or ethanol when used each time for screening with a different reagent. The extract is very dark in color which can cause varied inference. This problem was overcome by diluting the extract with methanol, so the color change during the phytochemical screening is cleary visible.
- During the usage of the hot plate, it was made sure that the thermometer being used to check the temperatures and its cut off is known before.

Chapter 7

CONCLUSION

The different extraction methodologies were performed in the lab . However all three of show different results . Using the soxlet apparatus gave the most conviencing results in lab . It give a clear picture of characterisation and isolation of the chemical constituent of the stinging nettle.

The different extraction methods helped in understanding the basic principles involved behind them and also the efficiency of each of the methodology performed in lab during the project period.

The extract has muscarinic agonist effect on rat colon .

The phytochemical screening performed in lab also showed the presence and absence of various organic chemical constituents.

TESTS	CONCLUSION
FOR CARBOHYDRATES	PRESENT
FOR REDUCING SUGARS	PRESENT
FOR HEXOSE SUGARS	ABSENT
FOR PROTIENS	PRESENT
FOR CARDIAC GLYCOSIDES	PRESENT
FOR ALKALOIDS	ABSENT
FOR TANNINS AND PHENOLS	PRESENT
FOR IRON	PRESENT
FOR FLAVONOIDS	PRESENT
FOR IODINE	PRESENT
FOR AMINO ACIDS	PRESENT

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