



Pharmacological And Phytochemical Evaluation Of *Urtica Dioica* Against *Staphylococcus -Aureus*, *Bacillus Cereus* (Gram Positive And Gram Negative) Curing Dental Problem

Sonika Rani^{1*}, Neelam Painuly²

¹Research Scholar, School of Pharmacy and research, Dev Bhoomi Uttarakhand University, Dehradun.

²Associate Professor, School of Pharmacy and research, Dev Bhoomi Uttarakhand University, Dehradun

Email Id - chaudharysonika04@gmail.com

Citation: Sonika Rani, Neelam Painuly et al ,(2024),. Pharmacological And Photochemical Evaluation Of *Urtica Dioica* Against *Staphylococcus -Aureus*, *Bacillus Cereus* (Gram Positive And Gram Negative) Curing Dental Problem, *Educational Administration: Theory and Practice*, 30(6), 3232-3239, Doi:. 10.53555/kuey.v30i6.5810

ARTICLE INFO

ABSTRACT

Aim: - Pharmacological and phytochemical evaluations of *Urtica dioica* against (gram positive and gram negative) Curing dental problem.

Method: - Shade dried *Urtica dioica* plant and stored leaves in this extraction plant leaves a coarse powder formed then aqueous done from 50 gm to obtain leaves powder using 500ml of ethanol and aqueous using Soxhlet Apparatus.

Result and conclusion: - *Urtica dioica* leaves have shown great anti-bacterial activity toward both bacteria (*staphylococcus Aureus* and *bacillus cereus*). As an extract of *Urtica dioica* of leaves (Ethanollic and Aqueous) were used among be to, ethanollic extract has highly percentage in comparison to aqueous extract. Due to high percentage, ethanollic extract was used for the phytochemical screening and show positive result for percentage of alkaloids, flavonoids, tannins, phenolic compounds and Resins but shown slight negative result for Iodine test.during measurement of zone of inhibition, both the extract (ethanollic and aqueous) has shown grater activity toward *Bacillus cereus* and among the both extract, ethanollic extract was found to be more effective.

Keywords:- U.D. (*Urtica dioica*), zone of inhibition, MIC (Minimum inhibitory concentration) *Staphylococcus aureus*,Dental cavity, Antibacterial.

INTRODUCTION

Dental caries is mostly prevention of disease when it is firstly cause of oral pain and tooth loss, cavities are known as this area is hard surface in our tooth and that is damaged. A cavity when present Hole in tooth that Invent tooth decay cavity found when acid in our Mouth Erode and Enamel (tooth hard outer layer). Proper oral Hygiene and Regular dental cleaning for cure of dental cavity, And Another term of tooth Cavities is dental caries or dental cavities. Dental caries are well known as tooth decay or cavity. Bacterial growth that causes an infection of hard tissue of teeth due to infection of acid by bacterial spitting of waste of food on the tooth that surface. Plaque beans pole our teeth, Acid in plaque that in a short time period erodes tooth Enamel, it's Hard and sure to coat teeth that prevent and cure Again tooth decay, that is tooth Enamel weakness which is Risky for Increase decay, cavity for Rise of Bacterial infection some person like a higher Risky. Carries are usually known as a tooth decay/ dental cavity. [F,Hans-2005]A cavity caused by carries. Our teeth are always responsible for ingestion. Then simply to swallow. Although they look more like bone, teeth are actual Ectodermal organs. Some common diseases that contact our oral health involve cavities (tooth decay, gum disease, oral cancer. More than 40% of adults Report having felt in their mouth within the last year, And more than 80% of people will have had at least one cavity by age 34. *Urtica dioica* known as common nettle, stinging nettle (not all plants of this species sting) are members of Urticaceae. This plant lacks a permanent woody stem, many are flowering garden plants and have some medicinal properties. *Urtica dioica* found a combination of occurring once every year or unchanging herb. [Doughari JH, Talaei A 2006 - 2001] *Urtica dioica* as a produce seed, underground Rhizomes and height 1- 2 meter. *Urtica dioica* found in phenolic compound as a Bioactive Compounds like a p. hydroxybenzoic caffeic Acid, Esculetin, scopoletin, Chrysoberyl, Beta - sitosterol.*Urtica dioica* in Phytochemical mineral are flavonoids (1.88mg), Alkaloids (1.32mg), phenolic (0.09mg), saponin

(1.6mg), tannin (0.8mg). Naturally source used for breath freshener of statistical worthwhile involving managing plaque that has effective antimicrobial properties. In addition to its medicinal uses, *Urtica dioica* has pharmacological significance as well as serves as a habitat and Food source for various insects, including butterflies and bees. The plant also plays a role in soil Improvement and erosion control. *Urtica dioica* or stinging nettle has a wide range of Medicinal uses. Some specific uses include - Allergic relief has been traditionally used to symptoms of allergies, such as a cold will cause a fever and body aches. This study area of Uttarakhand, Almora is situated in ridge Kumaon Himalaya .Its 1,638 meter above sea level is the neighbor's region. Almora covers 3697.2km. The climate of Almora is warm and temperate. The summers here have a good deal of Rainfall, while winters have very little. The Average temperature in Almora is 14.4° about 1575mm. Almira Region consists of very deep well drained soil formed in a loamy mantle and sandy or gravelly outwash sediments. *Urtica dioica* maintained common name stinging nettle extract having therapeutic treat for various oral diseases. Publications have potential for nettle consequently that review attempts for objectively properties. [F, Hans, Dogari, Behnke- 2005, 2006, 2001]

BACTERIA STRAIN

The Bacteria Responsible for dental cavities are *streptococcus* mutans. Its observation of *Staphylococcus aureus* And *Bacillus Cereus* .*Staphylococcus Aureus* is a gram positive bacteria that usually found in Nasal passage ,dental cavity of 14-15% of healthy Human .Bacteria strain are two bacterial strain were tested Including strain were tested including strain *Bacillus cereus* ,*Staphylococcus Aureus*. Those bacteria are affected for dental cavities as a decay, Bacteria infection, sensation.[Behnke, Maleki -2005, 2008]

1 *Staphylococcus aureus* -

Recent reports indicate high methicillin - resistant *Staphylococcus aureus* (MRSA) carriage Rate in oral cavity .Dental cavity should be considered a source of *Staphylococcus Aureus* in term of cross infection and dissemination to other body sites. [Maleki - 2008] The Role of *Staphylococcus Aureus* in the pathogenic of certain oral cavity .*Staphylococcus aureus* is a human commentary and pathogen that causes serious nosocomial and community acquired Infections. [Talei, Behnke - 2001, 2005]

2 *Bacillus Cereus*-

Bacillus cereus known as a family member *Lactobacillus* have been considered as major contributor to human dental caries for over a century .pathogen of human dental caries the ability of biofilm is poor ,although difference exist between difference exist between different major species . *Bacillus* are spore - forming and aerobic, while *Lactobacillus* are non spore - forming, non motile or microaerophilic, *Lactobacillus* are generally beneficial and found in human gut, mouth.[Maleki, BurtSA, Mahboobi - 2008, 2003, 2006]

Materials and Methods:

Plant Materials

Collection and Authentication of plant

Urtica dioica, known as a common nettle, stinging nettle (not all plants of this species sting) is a member of urticaceae. *Urtica dioica* plant is belong to family urticaceae ,common name is stinging nettle and kandali in uttarakhand Region .I have procured and collected this plant from Uttarakhand region local in Dunga in Dehradun , Uttarakhand India and prepare a herbarium and get Authentication of plant from Shaheed Durga Mall Government post Graduate college, Doiwala, Dehradun - 248140 (Uttarakhand) .

Selection Of Solvent

There are selected two solvent extractions of which are used in Research where comparisons to ethanolic and aqueous solvent .Both solvent extraction have been done by soxhlet extraction method. Ethanolic Extraction has high yield comparison to aqueous extraction.

1 Ethanolic as a solvent

2 Aqueous ac a solvent

Extraction Process

Ethanolic Extraction

In this extraction *Urtica dioica* leaves a coarse powder .Then Extraction was done from 50 grams of obtained leaves powder using 500 mL of ethanol for 15-20 hours at 50°temperature using soxhlet Apparatus. After extraction the sample was filtered with a filter paper and measured by measuring cylinder and Extract stored in a Glass jar and glass jar mouth cover by a silver foil. Sterile the china disc in a hot air oven then weigh the Empty chinadisc weighing balance and fill the china disc with extract then again weigh it . Then preheated the wares bath for 30-35 min.at 100°C .put the china dish on a water bath and set the temperature 50°C for 5- 6 hours . Then again weigh china disc with Residue and Residue stored airtight vials. Then Extraction yield of all extract were calculated using the following equation below:

$$\begin{aligned} \text{Formula of percentages yield} &= \text{Actual yield /Theoretical yield} \times 100 \\ &= 2.04/50 \times 100 \\ &= 4.08 \% \end{aligned}$$



Fig. no. 1 extraction of ethanolic solution

Aqueous Extraction

In this extraction *Urtica dioica* leaves a coarse powder .Then Extraction was done from 50 grams of obtained leaves powder using 500 mL of Aqueous for 15-20 hours at 50°temperature using soxhlet Apparatus. After extraction the sample was filtered with a filter paper and measured by measuring cylinder and Extract stored in a Glass jar and glass jar mouth covered by a silver foil. Sterile the china disc in a hot air oven then weigh the Empty china dish weighing balance and fill the china disc with extract then again weigh it . Then preheated the wares bath for 30-35 min.at 100°C .put the china dish on a water bath and set the temperature 100°C for 8-10 hours. Then again weight china dish with Residue and Residue stored airtight vials. Then Extraction yield of all extract were calculated using the following equation below:

$$\begin{aligned} \text{Formula of percentages yield} &= \text{Actual yield} \div \text{Theoretical yield} \times 100 \\ &= 3.52/50 \times 100 \\ &= 7.04 \% \end{aligned}$$



Figure no.2 this picture shows Extraction of Aqueous solution

Phytochemical Analysis

SN. No.	Test	Procedure	Result
1	Alkaloids Test Wagon R test	Take 1ml plant Extract than add 2-3 drop Wagner Reagent	Presence of Brown and Reddish precipitated
2	Dragendorff Test	Take 1ml pant extract than add 2- 3 drop dragendorff Reagent	Presence of Reddish precipitated
3	Picric Acid test	Take 1ml plant extraction than add 2-3 drop picric acid	Presence of orange color

4	Tannic Acid	Acidified Extract + 10% tannic acid solution	Presence of buff colored precipitate
5	Phenolic Test Lead Acetate test	Plant extract is dissolved in 5ml distilled water +3 ml of 10% lead acetate solution	A white precipitated
6	Iodine test	1ml plant Extract + few drop of distilled Iodine solution	A transient Red color
7	Tannins Test Gelatin test	Plant extract is dissolved in 5ml distilled water + 1% gelatin solution +10%NaCl	A white precipitated



Figure no.3 Phytochemical test

Minimum Inhibitory concentrations (MIC)

Minimum inhibitory concentrations, concentrations of an Antibacterial drug that inhibit and bactericidal growth of a microorganisms then one night Incubation. MICs can be determined on plates of solid growth medium diffusion method (In liquid growth media) after a pure culture is isolated. Disk- diffusion testing is most frequently used to antibacterial resistance of isolated. MIC is determined by examining a tube containing the dilution series of antibacterial agents for turbidity. There are three main Reagents necessary to run this assay: the media, antibacterial or antimicrobial agent, the microbes being tested, for the disk- diffusion method [Maleki, BurtSA - 2008, 2004]

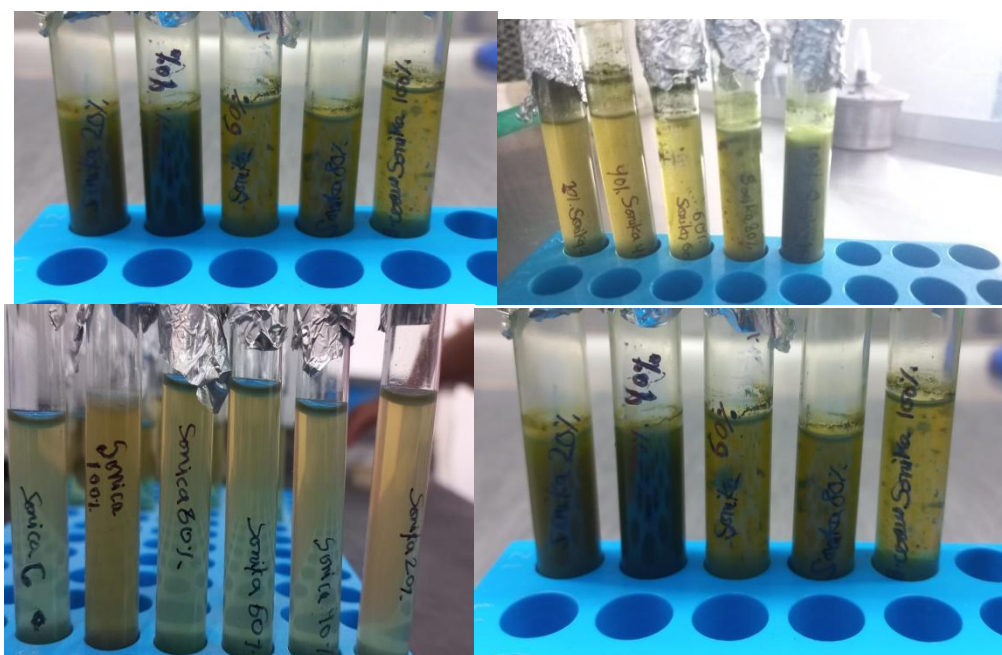


Figure no.3 this picture shows MIC with concentration

Bacteria selection

Selection of Resistant bacteria has been suggested to occur at high Antibiotic concentration Exceeding the Minimal Inhibitory concentrations (MIC) of susceptible Bacteria cells will no longer grow and are therefore outcompeted by Resistance one. These are strains of bacteria that have developed Resistance of many different types of Antibacterial. [Esimone, Tiwari, Zare-zardini -2019, 2016, 2013]

Antibacterial Activity

Anti -bacterial Activity testing for ethanolic and Aqueous Extract of *Urtica dioica* by Disk diffusion method Incubation at 37° C for 22 hours. In the bacterial activity antibacterial Reagent could be divided into bactericidal agents that slow down or bacteria growth. In this activity with disk diffusion method comparison bacteria inhibitory with another solvent [Testai, Cowan- 2012, 2013]

Zone of inhibition:-

Firstly prepare medium culture of (TSA) tryptone soya agar .Dissolved TSA medium into 1 L distilled water shake and heat of dissolved completely .Now autoclave medium at 12° C temperature and 15 pound pressure for 15 min. Bring a pure culture, plate of bacteria, Incubation of cultured plate, sample preparation and placement then incubated and testing bacterial growth inhibit and reading result. [Overereies, Tahri - 2019, 2007]

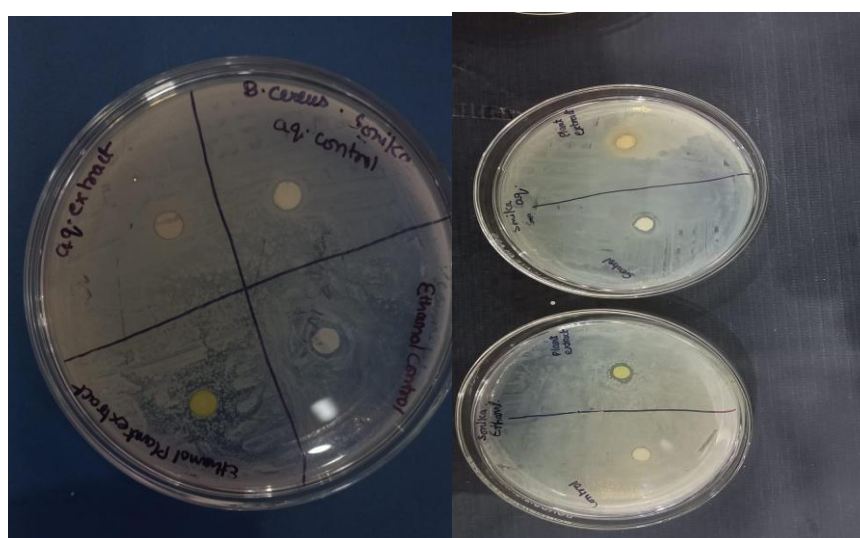


Figure no.4 this picture shows aqueous and ethanolic samples with agar medium culture

Result and discussion :-

Phytochemical testing

S.No.	Test	Procedure	Result
1	Phenolic Test Lead acetate test	Plant extract is dissolved in %mL distilled water + 3mL of 10% lead acetate solution.- A white precipitated	Positive
2	Tannins test Gelatin test	Plant extract is dissolved in 5ml distilled water +1% gelatin solution +10%NaCl - A white precipitated	Positive
3	Braymer test	1 ml filtered + 3 ml distilled water + drop 10% ferric chloride solution - Blue green color	Negative
4	Flavonoid test Alkaline Reagent test	Plant extracts +10% ammonium hydroxide solution - A yellow fluorescence	Positive
5	Ferric chloride test	Extract Aqueous extract solution + few drop 10% ferric chloride solution - A green precipitated	Positive
6	Alkaloids Test Picric acid test	Few mL filtered + 3-4 drop of 2% picric solution - An orange color	Positive
7	Iodine test	3ml extract + 10% tannic acid solution - A buff color precipitated	Negative
8	Resins test Turbidity test	10ml extract + 20 mL 4% HCL -turbidity	Positive

Table No 2. This Table showing the phytochemical test, procedure and Result Zone of inhibition of test Plant extraction

Microorganisms	Concentrations	Ethanollic Extraction	Aqueous Extraction
Staphylococcus Aureus	1 mg/ ml	16 mm± 0.5mm	11mm±0.5mm
Bacillus Cereus	1 mg/ml	9mm±0.5mm	12mm±0.5mm

Table No.3 zone of inhibition of ethanolic and Aqueous Extract against Staphylococcus - aureus and Bacillus cereus.

Minimum inhibitory concentration of test plant extraction

Its lowest concentration (expressed as mg/L of an antimicrobial agent inhibits visible in vitro growth of microorganisms. The MIC test determine the antimicrobial activity of s test agent against a specific bacteria .concentration of 100% visible in test tube .and slowly higher territory with highly concentrations that mean MIC responsible to 100 ,80,60,40,20 highly visible at dose of sample 1 mg/to 100% concentrations high inhibit to bacterial growth.[Ziyat, Esimoni - 2017, 2014]

Conclusion –

Urtica dioica leaves have shown great antibacterial activity toward the both bacteria (staphylococcus Aureus and bacillus cereus). As two extra 5 of *Urtica dioica leaves* (Ethanolic and Aqueous) were used. Among the two, Ethanolic extract has a high yield percentage in comparison to aqueous extract. Due to high yield percentage, ethanolic extract was used for the phytochemical screening and shown Positive result for presence of Alkaloids, flavonoids, tannins, phenolic compound and Resins but shown slight negative result for Iodine test .During the

measurement of zone of inhibition, both the extract (ethanolic and Aqueous) have shown greater activity toward *Bacillus cereus* and among the both extract, ethanolic extract was found to be more effective.

Reference

- Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett Appl Microbiol.* 2003;36(3):162–7. [PubMed] [Google Scholar]
- Bozkurt, M., Fidan, A. F., Kalaycıoğlu, Z., Atalay, E., Kılıç, O., Bilenler, T., ... & Kızıl, M. (2020). Bioactivity potential of *Urtica dioica* L. leaves extract: Health benefits, antioxidant, and antimicrobial properties. *Food Science & Nutrition*, 8(2), 1012-1020.
- Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12(4):564–82. [PMC free article] [PubMed] [Google Scholar]
- Doughari JH. Antimicrobial activity of *Tamarindus indica* Linn. *Trop J Pharm Res.* 2006;5(2):597–603. [Google Scholar]
- Dewanjee S, Maiti A, Majumdar R, Majumdar A, Mandal SC. Evaluation of antimicrobial activity of hydroalcoholic extract *Schima Wallichii* bark. *Pharmacology online.* 2008;1:523–8. [Google Scholar]
- Durak, Y., Aras, S., & Kocaadam, B. (2019). Antioxidant and anti-inflammatory effect of *Urtica dioica* in a model of acute pancreatitis in rats. *European Journal of Medicinal Plants*, 29(1), 1-8.
- Esimone, C. O., Iroha, I. R., Ibezim, E. C. (2014). In vitro antimicrobial activities of extract of *Urtica dioica* Linn. *Afr. J. Microbiol. Res*, 8(16), 1689-1696.
- Fereidouni, E., Asgharian, P., Mahzoni, P., & Vaez, H. (2015). Protective effect of hydroalcoholic extract of *Urtica dioica* L. leaves on cyclophosphamide-induced hemorrhagic cystitis in rats. *Indian Journal of Nephrology*, 25(5), 299.
- Hajiyani, M., Tewari, D., Sobarzo-Sánchez, E., Nabavi, S. F., Farzaei, M. H., & Abdollahi, M. (2018). Natural product-based nanomedicines for wound healing purposes: therapeutic targets and drug delivery systems. *International Journal of Nanomedicine*, 13, 5023.
- Hirano T, Homma M, Oka K. Effects of stinging nettle root extracts and their steroidal components on the Na⁺,K⁽⁺⁾-ATPase of the benign prostatic hyperplasia. *Planta Med.* 1994;60(1):30–3. doi: 10.1055/s-2006-959402. [PubMed] [CrossRef] [Google Scholar]
- Kavalali, G., Tuncel, H., Göksel, S., Hatemi, H. H., Astarci, H. M., Tugcu, V. (2016). Comparative randomized study on the efficacy of herbal dentifrice with fluoride dentifrice on plaque, gingivitis, and salivary *Streptococcus mutans* growth. *Nigerian Journal of Clinical Practice*, 19(5), 641-648
- Krzieski T, Kazon M, Borkowski A, Witeska A, Kuczera J. Combined extracts of *Urtica dioica* and *Pygeum africanum* in the treatment of benign prostatic hyperplasia: double-blind comparison of two doses. *Clin Ther.* 1993;15(6):1011–20. [PubMed] [Google Scholar]
- Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng.* 2005;2:125–34. [Google Scholar]
- Móricz, Á. M., Ott, P. G., & Lemberkovics, É. (2013). Bioactivity guided fractionation of stinging nettle (*Urtica dioica* L.) leaves. *Pharmacognosy Magazine*, 9(33), 219.
- Maleki S, Seyednejad SM, Damabi NM, Motamedi H. Antibacterial activity of the fruits of Iranian *Torilis leptophylla* against some clinical pathogens. *Pak J Biol Sci.* 2008;11(9):1286–9. [PubMed] [Google Scholar]
- Mahboobi M, Shahcheraghi F, Feizabadi MM. Bactericidal effects of essential oils from clove, lavender and geranium on multi-drug resistant isolates of *Pseudomonas aeruginosa*. *Iran J Biotechnol.* 2006;4(2):137–40. [Google Scholar]
- Obertreis B, Giller K, Teuscher T, Behnke B, Schmitz H. [Anti-inflammatory effect of *Urtica dioica* folia extract in comparison to caffeic malic acid]. *Arzneimittelforschung.* 1996;46(1):52–6. [PubMed] [Google Scholar]
- Riehemann K, Behnke B, Schulze-Osthoff K. Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibits the proinflammatory transcription factor NF-kappaB. *FEBS Lett.* 1999;442(1):89–94. [PubMed] [Google Scholar]
- Seyednejad M, Ebrahimzadeh H, Talaei A. Carbohydrate content in olive Zard cv and alternate bearing pattern. *Int Sugar J.* 2001;103(1226):84–7. [Google Scholar]
- Sharifi-Rad, J., Sureda, A., Tenore, G. C., Daglia, M., Sharifi-Rad, M., Valussi, M., ... & Martins, N. (2018). Biological activities of essential oils: From plant chemoeology to traditional healing systems. *Molecules*, 23(12), 2566.
- The National guides to medical herbs and plants. UK: Tiger Books.Int. Plc; 1998. [Google Scholar]
- Testai L, Chericoni S, Calderone V, Nencioni G, Nieri P, Morelli I, et al. Cardiovascular effects of *Urtica dioica* L. (*Urticaceae*) roots extracts: in vitro and in vivo pharmacological studies. *J Ethnopharmacol.* 2002;81(1):105–9. [PubMed] [Google Scholar]
- Tahri A, Yamani S, Legs Ser A, Aziz M, Makhfi H, Bnouham M, et al. Acute diuretic, natriuretic and hypotensive effects of a continuous perfusion of aqueous extract of *Urtica dioica* in the rat. *J Ethnopharmacol.* 2000;73(1-2):95–100. [PubMed] [Google Scholar]

24. Wagner H, Willer F, Kreher B. [Biologically active compounds from the aqueous extract of *Urtica dioica*]. *Planta Med.* 1989;55(5):452–4. doi: 10.1055/s-2006-962062. [PubMed] [CrossRef] [Google Scholar]
25. Ziyat A, Legs Ser A, Makhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. *J Ethnopharmacol.* 1997;58(1):45–54. [PubMed] [Google Scholar]
26. Zare-Zardini, H., Toliat, T., Ahmadian, S., Mollae, M., Salehi, M., & Jafari, M. (2016). *Urtica dioica* (nettle): A review of its chemical, pharmacological, and clinical properties. *Drug Design, Development and Therapy*, 10, 2679.