International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 223-234 www.ijprajournal.com ISSN: 2249-7781

Evaluation of Antibacterial Properties of N. Nucifera.

Rutuja Lolage*, Dr.Vivek M Satpute2, Mr Santosh A Waghmare3, Dr Hemant V Kambale4.

1M Pharm Student ,Loknete Shri Dadapatil Pharate College Of Pharmacy , A/p-Mandavgan Pharata, Tal-Shirur, Dist-Pune

2,3 Assistant Professor, Loknete Shri Dadapatil Pharate College Of Pharmacy , A/p-Mandavgan Pharata, Tal-Shirur, Dist-Pune

4Principle, Loknete Shri Dadapatil Pharate College Of Pharmacy , A/p-Mandavgan Pharata, Tal-Shirur, Dist-Pune

Submitted: 09-03-2023 Accepted: 18-03-2023

ABSTRACT –

The ethanol extract of the plant was shown to have the maximum antimicrobial activity, with the diameter of inhibition zones ranging from 17.2 to 17.8 mm. The antibacterial activity of several solvent extracts from lotus leaves.The ethanol extract of the lotus leaves was then further separated using an MCI-gel column, and the macrodilution broth method was used to test the antibacterial activity. Moreover, first phytochemical tests were also performed. The most potent antibacterial activity was reported in fraction 3, which was eluted from MCI-gel, with minimum inhibitory concentration and minimum bactericide concentration values in the ranges of 0.0313–0.125 g mL-1 and 0.0626–0.25 g mL-1, respectively.The ethanol extract of lotus leaves displayed only weak inhibitory action against all fungi, according to an examination of its antifungal activity. The extract contained phenolic chemicals, flavones, and alkaloids, which may be the source of its antibacterial activity, according to the results of the phytochemical study. Also, it was shown that lotus leaf extract has superior antibacterial properties to sodium benzoate in apple juice (preservative used in food). Lotus leaves can therefore be used as a botanical natural food preservative against infections that cause food poisoning.

Staphylococcus aureus and Streptococcus pyogenes, two gram-positive bacteria, and three gram-negative bacteria, including Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, which were isolated from wounds by the disc diffusion method, were tested for antimicrobial activity against ethanol extracts of Nymphaea lotus leaves (DDM). The extract contained bioactive substances such tannins, flavonoids, alkaloids, anthraquinones, saponins, cardiac glycosides, and phenolics, according to the results of the phytochemical study. The findings also demonstrated that N. lotus was very susceptible to the isolates of S. aureus, S. pyogenes, and E. coli, with zones of inhibition ranging from 8 to 25 mm, while K. pneumoniae and P. aureginosa were only moderately susceptible, with zones of inhibition ranging from 8 to 15 mm.

Key Words –Nymphaea lotus, susceptibility, wound, phytochemical, Lotus Leaves, Antibacterial Natural Product, Food Preservative, Phytochemicals

I. INTRODUCTION–

Herbal medicine is the use of plants to treat disease and enhance general health and wellbeing. Herbs should be used with caution because they may interact with other pharmaceutical drugs. Please consult your primary care physician (GP) about any health issues, and let them know if you are taking or considering taking any herbal medications. Never quit using prescription drugs in favors of herbal remedies without first speaking to your doctor. While buying herbal medications online, exercise caution. Herbal remedies that aren't regulated, like some traditional folk medicines, might not be produced to the same quality and standards as those that are. The use of herbal medicine dates back to early civilizations. It entails the use of plants as medicines to cure illness and improve people's overall health and wellness.

Active substances are found in herbal medications. Several herbal preparations' active components are yet unknown. Several pharmaceutical drugs have just one active component that comes from a botanical source. Herbalists hold that if an active element is utilized separately from the rest of the plant, it may lose its effectiveness or become less safe. For instance, the

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 223-234 www.ijprajournal.com ISSN: 2249-7781

plant meadowsweet contains salicylic acid, which is used to manufacture aspirin. Aspirin can make the stomach lining bleed, however meadowsweet naturally includes other substances that shield the skin from salicylic acid irritants. Practitioners of herbal medicine think that the complete plant has a bigger impact than the sum of its parts. Opponents assert that herbal medication is challenging to administer because of its nature.

Lotusasaher balingredient–

The lotus is a lovely representation of healing. For millennia, Traditional Eastern medicine and gastronomy have made use of the potent lotus plant. India's national flower, the lotus, is revered in Buddhist and Hindu cultures as a representation of purity. For centuries, people have used this herb as medicine. The perennial lotus has a bowl-like form and is frequently mistaken for a water lily. Almost 100 different species of lotus exist. The white single-flower lotus, which has about 20 petals, is perhaps the one you know best. The double-flowered lotus, which can have over 100 petals, as well as pink and red variations with Blue Lotus are further varieties of Lotuses. The usage of lotus for bleeding, cough, fever, liver and stomach issues, among other diseases, is widespread, although there is no scientific backing for it. Several components of the lotus plant are frequently used in Asia for baking, cooking, and flavoring beverages.

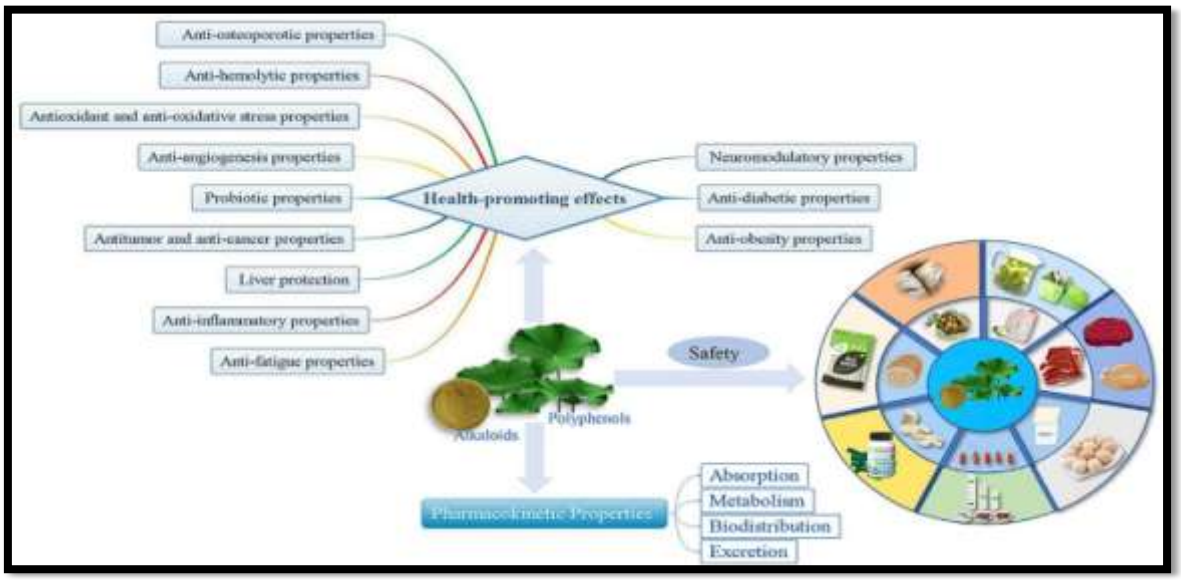
❖

Usesoflotus–

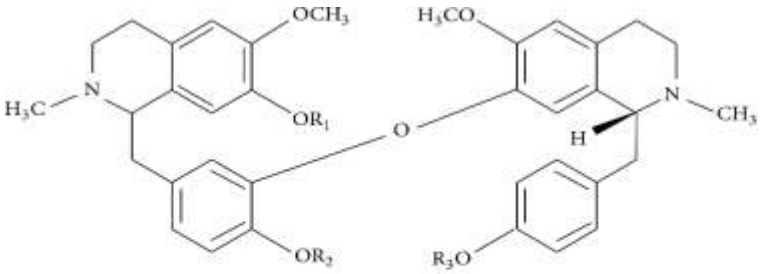
- ✓ StressbusterandAntidepressant.
- ✓ Helpsprevent cardiovasculardisease
- ✓ Regulatesbloodpressure
- ✓ Improvebloodcirculation
- ✓ antdiabetic
- ✓ Richvitamin‘C’source
- ✓ Improve yourmental function
- ✓ Promotesbetterdigestion
- ✓ Detoxify.
- ✓ GItractandstomachacids
- ✓ Fertility
- ✓ Skinhealth
- ✓ Antinflamatoryeffect
- ✓ Antifungal,Antibacterial andAntioxidants

Phytochemicals and chemical constituents present in lotus leaves

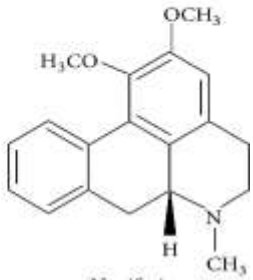
- ❖ Liensinine
- ❖ Dauricin
- ❖ Nuciferin
- ❖ Queracitin



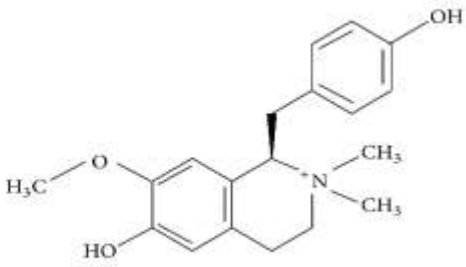
International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 223-234 www.ijprajournal.com ISSN: 2249-7781



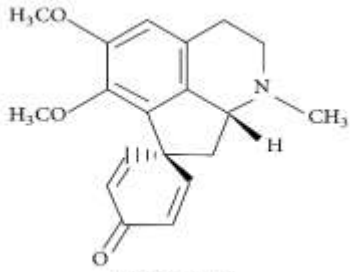
	R_1	R_2	R_3
Liensinine	CH_3	H	H
Isoliensinine	H	H	CH_3
Neferine	CH_3	CH_3	H



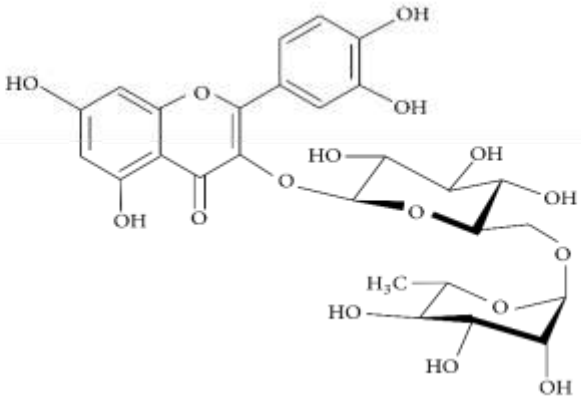
Nuciferine



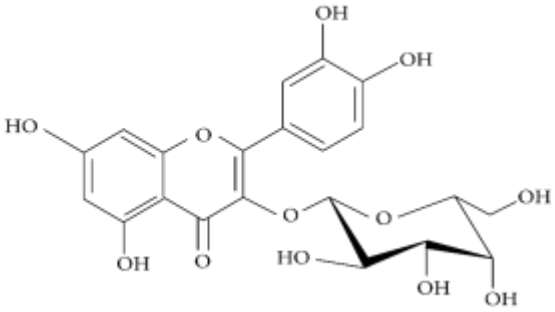
Lotusine



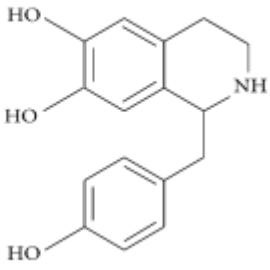
Pronuciferine



Rutin

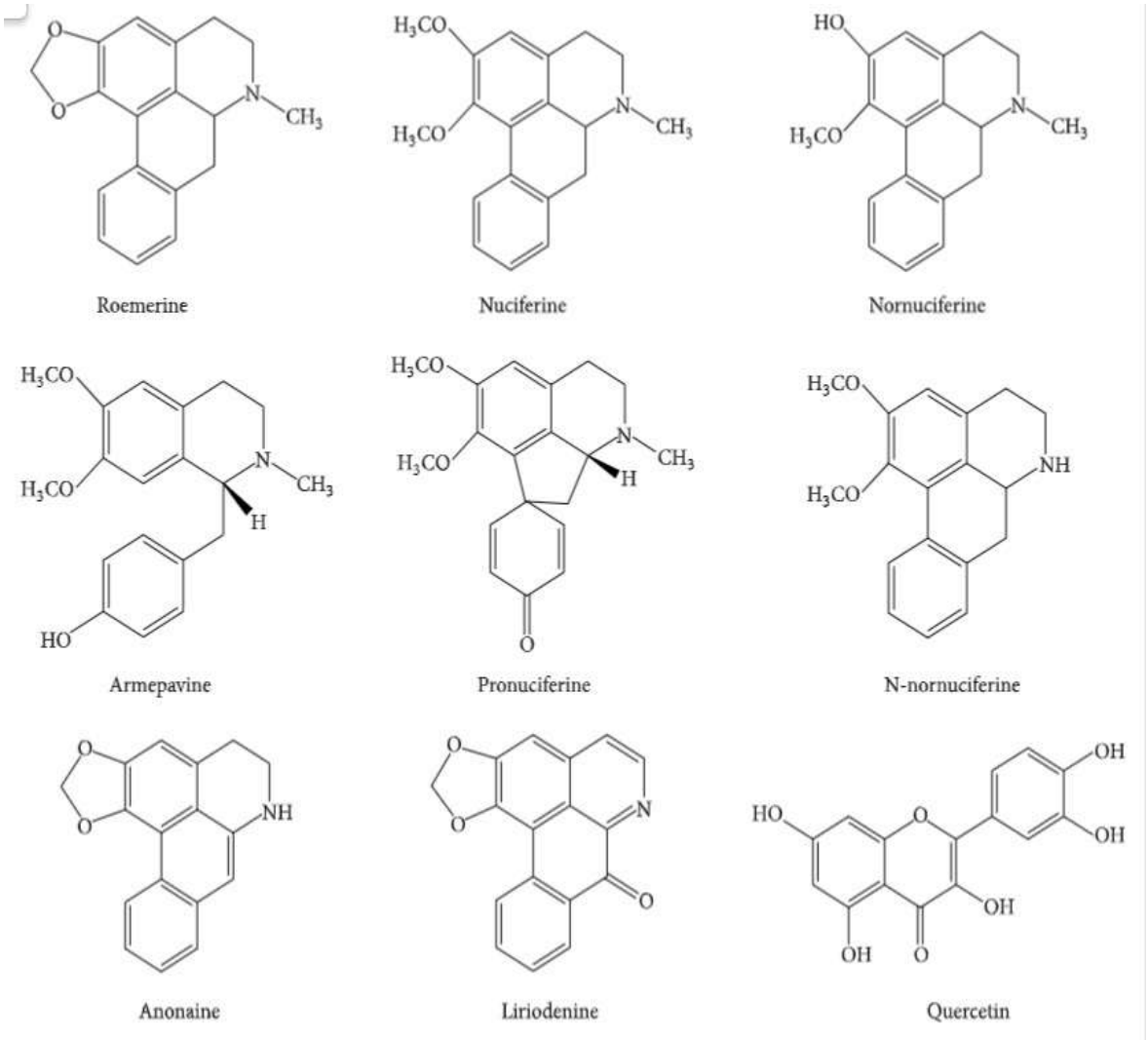


Hyperin



Demethylcoclaurine

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781



Extraction–

The first and most crucial stage in creating herbal medicine formulations is extraction. By isolating the active components from the crude medication using various extraction procedures and adding the right solvent, it acts as an alternative method to identify the lead compound. The choice of solvent is the most crucial phase because it determines the success rate. In order to find the lead molecule, extraction is crucial in the drug discovery process. The many extraction techniques utilized to obtain the herbal extracts have been reviewed in this review along with their advantages and downsides have also been mentioned, along with a brief discussion of solvent choice and the activities of a few phytochemicals.

The initial stage in separating the desired natural products from the base materials is extraction. According to the extraction principle, there are several extraction procedures, including solvent extraction, distillation, pressing, and sublimation. The technique with the highest usage is solvent extraction.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

Method	Solvent	Temperature	Pressure	Time	Volume of organic solvent consumed	Polarity of natural products extracted
Maceration	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extracting solvent
Percolation	Water, aqueous and non-aqueous solvents	Room temperature, occasionally under heat	Atmospheric	Long	Large	Dependent on extracting solvent
Decoction	Water	Under heat	Atmospheric	Moderate	None	Polar compounds
Reflux extraction	Aqueous and non-aqueous solvents	Under heat	Atmospheric	Moderate	Moderate	Dependent on extracting solvent
Soxhlet extraction	Organic solvents	Under heat	Atmospheric	Long	Moderate	Dependent on extracting solvent
Pressurized liquid	Water, aqueous and non-	Under heat	High	Short	Small	Dependent on extracting

TableNo. 1– Methodsof Extraction.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

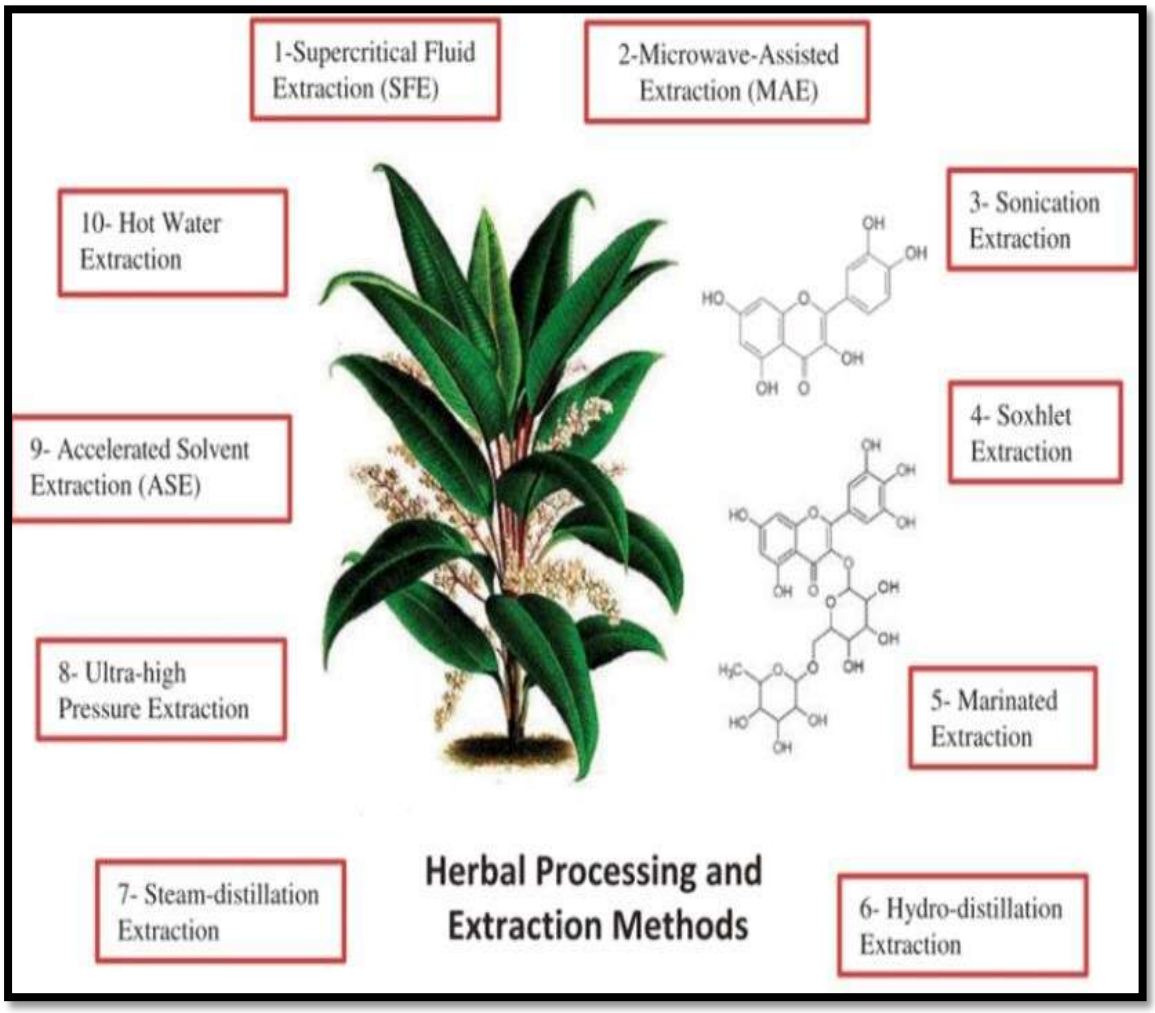


Fig3 –Herbal processingandExtraction Method

Choiceof solvents

The type of solvent employed in the extraction method is a key factor in the successful evaluation of biologically active chemicals from plant parts. Low toxicity, ease of evaporation at low temperatures, quick physiologic absorption of the extract, preservation activity, and inability to enable the extract to form complexes or dissociate are characteristics of an ideal solvent in plant extractions. The amount of phytochemicals to be extracted, the rate of extraction, the variety of different compounds extracted, the variety of inhibitory compounds extracted, the ease of handling the extracts after extraction, the toxicity of the solvent in the bioassay method, the ability health hazard of the extractants residual solvent, the solvent must be nonpoisonous, and the solvent need not interfere with the bioassay are the factors affecting the choice of solvent.

Water:

The most common solvent for extracting plant materials with antibacterial properties is water. Although traditional healers typically employ water, it has been discovered that plant extracts from organic solvents have more consistent antibacterial properties than water extract. Moreover, water soluble phenolics and flavonoids (often known as anthocyanins) are only significant as antioxidant compounds.

Acetone

Acetone is a particularly effective extractant, especially for antimicrobial research where more phenolic compounds need to be extracted because it dissolves various hydrophilic

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

and lipophilic additions from the flora utilized, is miscible with water, is volatile, and has a low toxicity to the bioassay employed. According to a study, aqueous acetone extracts tannins and other phenolic compounds better than aqueous methanol. It was revealed that saponins, which have antibacterial properties, may be extracted using both acetone and methanol.

Alcohol

The ethanolic extracts' improved potency over the aqueous extract is most likely due to higher levels of polyphenols. in comparison to aqueous extracts. This indicates that they are better at dissolving nonpolar materials, such as cell walls and seeds, which causes the release of polyphenols from cells. A more beneficial explanation for the decline in activity of aqueous extract could be the enzyme polyphenol oxidase, which destroys polyphenols in water extracts while remaining inactive in methanol and ethanol. Moreover, water is a better medium for the growth of microbes than ethanol. It has been discovered that 70% ethanol contains more bioactive flavonoid molecules than pure ethanol because it has a greater polarity. To manufacture the pure ethanol up to 30% water and 70% ethanol, the polarity of the solvent was increased. Also, it was found that by entering the cell membrane, ethanol could more readily extract the intracellular components from the plant material. Chloroform

Terpenoid lactones were discovered after dried barks were serially extracted with hexane, chloroform, and methanol, with activity concentrating in the chloroform fraction. The aqueous phase can infrequently contain tannins and terpenoids.

II. MATERIAL&METHODS

Material –

Figno 4–image of lotusleaves

Lotus –

Biological Name:Nelumbo nucifera.Common names: Lotus, Indian lotus , Kamal.Family:Nelumbonaceae.
Clade : Angiosperms.Class :Dicotyledons.Order:Proteales.
Genus:Nelumbo

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

OtherBenefitsof theLotusLeaf

- Helpingpeople whosuffer fromdiabetes
- Healingfattyliver
- Relievingstressandhelpingyousleepbetter
- Reducingcramps
- Loweringstomachacidandhelpingwithgastriculcers
- Improvingfertilityforboth menand women
- Coolinginternalorgans
- Moistening yourskin

SOXHLETEXTRACTION–

Figno-5soxhletextractionapparatus

ChemicalsusedforDifferentTests

Sr. No	ChemicalUsed
1.	Ethanol
2.	Ether
3.	Chloroform
4.	PotassiumMercuricIodideSolution
5.	Potassium Iodidesolution
6.	PicricAcidSolution
7.	Sudan IIIReagent
8.	LeadAcetate

Methods-

❖ Extraction–

Using a mill, dry lotus leaves were ground into 2 mm-sized particles. The ethanol extraction was concentrated to dryness, dried, and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

circumfluence extracted twice with 40% ethanol, once by filtration. 40% ethanol extracts were made from the remaining material, which was then dried and kept for later analysis employing assays for antibacterial activity.

Plant matter can be either fresh (like a leaf of a plant) or dry. To create more surface area, it needs to be crushed using a pestle and mortar. The plant matter should be enough to completely fill the cellulose's pores. Students should be able to construct all of the offered equipment. As opposed to testing an antimicrobial drug out of a purchased container, letting students build the extraction apparatus may help them better understand the extraction process. In order to support the extraction apparatus, the students need start by constructing a rig out of stands and clamps. The solvent (100 ml of ethanol) is then added to a flask with a circular bottom that is connected to a Soxhlet extractor and condenser on an Isomantle. The thimble, which is put into the Soxhlet extractor, is filled with the crushed plant material. Glass wool is trailed along the side arm. The Isomantle is used to heat the solvent, which then starts to evaporate as it travels through the device to the condenser. Following that, the condensate drips into the reservoir holding the thimble. The cycle restarts when the solvent level reaches the syphon and flows back into the flask. A total of 16 hours should pass during the procedure.

EVAPORATION–

After extracting the lotus leaf material from the leaves using the Soxhlet method, the ethanol must be evaporated in order to obtain the pure extract.

A Rotary evaporator was employed to evaporate the ethanol.

ROTARYEVAPOREATOR–

Figno 6– Rotaryevaporator

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

By dispersing a thin film of a solvent over a vessel's interior at a high temperature and low pressure, rotary evaporation reduces the volume of the solvent. As a result, more solvent can be quickly removed from less volatile samples. Heat bath, rotor, condenser, and solvent trap make up the majority of rotary evaporators. Together with a bump trap and round bottom flask holding the sample to be concentrated, an aspirator or vacuum pump also needs to be connected.

Evaluation Parameters for Extract –Test foralkaloids

Test	observation	Inference
Mayerstest– Tosmallamoutofmayersreagent (potassium mercuriciodiedsolution)	Givescream colouror ppt	Presenceofalkaloids
Dragendroffstest– To the small amount of crudedrug add dragendroffs reagent(potassiumbismuthiodidesolution)	Givesreddishbrowncolourorppt	Presenceofalkaloids
Wagenerstests– Tosmallamountofcrudedrug Hagners reagent (iodinepotassiumiodidesolution)	Givesbrownorreddishbrowncolourorppt	Presenceofalkaloids
Hagerstest– Tosmallamountofcrudedrug,hagersreagent(saturatedsolutionofpicricacid)	Gives yellowppt	Presenceofalkaloids

Tableno2–test foralkaloids

Test for volatileoils

Test	**Observation**	**Inference**
To the section of the drug addalcoholicsolutionofSudanIII	Red colour obtain byglobules	Presenceofvolatileoils
Tothethinsectionofthedrug , add a drop of tincturealakaline	Redcolourisobtain	Presenceofvolatileoils

Tableno3 –testforvolatileoils

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 164-175 www.ijprajournal.com ISSN: 2249-7781

Testforflavonoids

Test	Observation	Inference
Shinodatest– Todry powderextractadd5mlof90%ethanol,fewdrops of pf conc HCL adddd0.5gogmagneshium tunings	Pinkcolourisobserved	Presenceofflavonoids
Tosmallamountofresidueaddlead acetatesolution	Yellowcoloredpptisformed	Presenceofflavonoids
AddincreasingamountofNaOHto theresidue	It shows yellow coloration ,whichdecolorizesafteradditionof acids	Presenceofflavonoids

Tableno 4 –Testfor flavonoids

III. DISCUSSION

Nelumbo nucifera, the Lotus plant, is a common plant in the Lakes Area. It is asserted to have a variety of qualities according to Chinese traditional medicine (CTM) theory. The majority of lotus leaves exhibit numerous medicinal qualities that are vital to our bodies. Lotus leaves are used as a herbal remedy in several nations utilizing different sorts of Lotus leaves are also used in the food sector and as a source of nutritional fiber.

Various tests, such as those for alkaloids, flavonoids, volatile oils, etc., were conducted to determine the conformation of the various chemical constituents found in it. After the test, it was discovered that the lotus extract contains anthraquinones alkaloids, proving that quercetin was also present. The antibacterial, antifungal, and many other effects of quercetin are evident. After the lotus extract's antibacterial characteristics have been confirmed, it is possible to formulate the extract to demonstrate those properties and use it in antibacterial creams. The cream has a number of organic substances that have an antimicrobial impact on our skin. The ingredients were effective against several kinds of bacteria that come into contact with our skin on a daily basis.

IV. CONCLUSION–

Lotusleavesisoneofthepopulartraditionalmedicinalherbs.IthasbeenwidelyusedtotreatmanydiseaseslikeSkinhealth,Antinflamatoryeffect,Antifungalandantibacterial ,Antioxidantsproperties

Pharmacological studies revealed that its major constituents are alkaloids, such as liensinine,Isoliensinine, Neferine, Nuciferine, N-Nornuciferineand Quercetin. The alkaloid compoundshavebeenreportedtoshowremarkableeffectsasantibacterial,protecttheskin,Soaneffectivemethod extraction is to increase the content of alkaloids from lotus leaves and to decrease theimpurities content. The best extract method needs considering not only the yield of alkaloid,contentofimpuritiesbutalsopossibilityscaleupatanindustrialorcommerciallevel.Becausethosareeimportant aspect of standardization ofherbal.

REFERENCES

[1]. Rauha, J. P., Remes, S., and Heinonen, M., Antimicrobial effects of Finnish plantextracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol.,56,3-1 (2000).

[2]. Tian, N., Liu, Z., Huang, J., Luo, G., Liu, S., and Liu, X., Isolation and preparation offlavonoids from the leaves of Nelumbo nucifera Gaertn by preparative reversed-phasehigh-performanceliquidchromatography.Sepu., 25, 88-92(2007).

[3]. Xu,Z.andLi,M.,ComparisonofInhibitionstoOralBacteriaamong10ChineseHerbs.ChineseJournal of Modern AppliedPharmacy, 17, 282-284 (2000).

[4]. Burdass,D.,J.Grainger,andJ.Hurst(ed).2006.Basicpracticalmicrobiology.SocietyforGeneral Microbiology,Reading,UnitedKingdom.
[5]. Cavanagh, H. M. A., and J. M. Wilkinson. 2002. Biological activities of lavenderessentialoil. PhytotherapyRes. 16(4):301–308.
[6]. Haitham, Q. 2009. Antibacterial activity in vitro of Thymus capitatus from Jordan.Pak.J. Pharm. Sci. 22(3):247–251.
[7]. Hammer, K. A., C. F. Carson, and T. V. Riley. 1999. Antimicrobial activity ofessentialoils and otherplant extracts. J. Appl. Microbiol. 86(6):985–990.
[8]. Manisha Yogesh Sonalkar, SachinAnnasahebNitave. Formulation and evaluation ofpolyherbalcosmeticcream. WorldJPharm PharmSci 2016;5:772-9.
[9]. T Reynolds, AC Dweck. Aloe vera leaf gel: a review update. J EthnoPharmacol1999;68:3-37.
[10]. Priyanka Sharma, Amit C Kharkwal, Harsha Kharkwal, MZ Abdin, Ajit Varma. Areview on the pharmacological properties of Aloe Vera. Int J Pharm Sci Rev Res2014;29:31-7.
[11]. Sharma Pankaj, Tomar Lokeshwar, Bachwani Mukesh, Bansal Vishnu. Review onneem(Azadirachtaindica):thousandproblemsonesolution.IntResJPharm2011;2:97-102.
[12]. KPSampathKumar,DebjitBhowmik,Biswajit,Chiranjib,Pankaj,KKTripathiMargret Chandira. Traditional Indian herbal plants Tulsi and its medical importance: areview.Res Rev: JPharmacognPhytochem2010;2:103-8.
[13]. Chopra,A.andDoiphode,V.V.(2002)'Ayurvedicmedicine.Coreconcept,therapeuticprinciples, and current relevance, Medical Clinics of North America, Vol. 86, pp. 75-89.
[14]. Mothana, R. and Lindequist, U. (2005). Antimicrobial activitu of some medicinalplantsof theisland SoqotraJ. Ethnopharmacol.96:177-181.
[15]. DhanabalSP,MohanMarugaRajaMK,RamanathanM,SureshB,(2007).Hypoglycemic activityof Nymphaea stellate leaves ethanolicextractinalloxan induceddiabeticrats.Fitoterapia.78: 288–291.
[16]. .Savithramma N, Linga Rao M and Bhumi G (2011). Phytochemical screening ofThespesia populnea (L.) Soland and Tridax procumbens. L J Chem Pharm Res. 3(5):28-34.
[17]. Hari Babu R and Savithramma N (2014). Screening of Secondary Metabolites ofUnderutilizedSpeciesofCyperaceae.IntJ PharmSciRev Res.24(2):182-187.