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PHARMACOGNOSTIC STANDARDIZATION AND PRELIMINARY PHYTOCHEMICAL STUDIES OF RHIZOME OF *DORONICUM HOOKERI*

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Abstract

The tendency of any microorganism that can interfere with Immune System which can lead to any negative or deleterious effect to the body organs is known as Infection. Diseases caused by infections are known as Contagious Diseases. Infections can be both acute and chronic. This research is based upon the Pharmacognostic and Pharmacological (Anti-microbial) activity of the plant *Doronicum hookeri*. This plant shows various activities like- Cardio tonic activity, Nerve tonic activity, Liver tonic activity, Antioxidant, and antifungal and antibacterial activity. *Doronicum hookeri* was subjected to various Pharmacognostic and Pharmacological evaluations. The Pharmacognostic evaluations investigated were Macroscopic and Microscopic evaluations and Physico-chemical evaluations like Loss of Drying, Ash values, Extractive values and pH determination. The phytochemical screening tests were done to investigate the presence of various secondary metabolites like tests for flavonoids, saponins, tannins, fixed oils and proteins. The Isolation and characterisation that are Thin Layer Chromatography, UV-Spectroscopy.

Keywords: Ethno-Medicinal Herbs, Pharmacognostic Studies, Phytochemical Studies.

1. INTRODUCTION

For a long time, Ethno-medicinal herbs have been known for numerous bioactivities such as antiviral, antibacterial & antifungal, painkiller & anti-inflammatory, anticancer, fever-reducing, and hypotensive activity. They are an excellent contributor to several bioactive components. (1)

A considerable portion of the world's population has used herbal remedies for patient care and treatment of illness over the last two millennia. This evidence revealed a solid and positive linkage between ethnomedicinal plant application and clinical trials. (2)

The Plant kingdom is crucial for the survival of humans and other living organisms in the Universe. Over half of the population in several emerging nations like Ibero-America and the eastern countries uses and acknowledges herbal medicine as one essential medical treatment.

Medicinal plants also increased in developed countries to treat various disorders. On the other hand, classical drugs, also known as conventional drugs, are crucially involved in the healthcare system of the majority of rural and tribal communities. In most circumstances, folk medication is the most economical and convenient approach for illness treatment. (3,4)

Although the availability of Modern medications, ancient and traditional drugs are becoming more popular in wealthy nations. As a result, it is critical to conserve herb's particular characteristics and provide a more excellent scientific knowledge of how certain plants may heal specific conditions. (5)

AYUSH is becoming more popular all over the globe. Nature has provided a wealth of medicinal plants. As a result, the worldwide trend of allopathic medicines has shifted to herbal medications. We may refer to this as a back to the environment for preventing illness and pain. Plant-based past knowledge has emerged as a valuable resource in the hunt for novel pharmacological and nutraceutical sources. (6,7)

Doronicum hookeri is one of the essential but unknown drugs of USM, which belongs to the family Asteraceae. This plant shows various activities like- Cardio tonic activity, Nerve tonic activity, Liver tonic activity, Antioxidant, and antifungal and antibacterial activity. The term Doronicum took from the Arabic word "Darawnay." In English, *Doronicum hookeri* called as Leopard's Bane, Hindi as Toos, Tarang, and Urdu as Darunaj Aqrabi. There are few references to the plant *Doronicum hookeri* in the literature. However, extensive and systematic evidence is unavailable. Some experimental data shows that this plant's parts have antioxidant, antifungal, antibacterial properties, and cardio protective and hepatoprotective properties. Different plant parts examine for various phytoconstituents and therapeutical properties. Still, very little information publishes on the standardization and pharmacological investigation of the dried rhizome of *Doronicum hookeri*. (8–11)

As a result, an initiative took to conduct a pharmacognostic and phytoconstituents analytical study of the dried rhizome of *Doronicum hookeri*. The resulting evidence or information uses as a reference for future research studies on

adulteration, pharmacognostic parameters, and phytoconstituents examinations of this plant.

2. MATERIALS AND METHODS

2.1 Collection, and Authentication of plant part: Collection of the plant's part, i.e., the rhizome, was collected from the garden of H.N.B.U.M.E. University, Uttarakhand (India) and authenticated by H.N.B.U.M.E. University, Uttarakhand (India). After authentication, the collection process of plant parts starts-first, collected rhizomes shade-dried. Then, the rhizomes were powdered, shifted through a 60# sieve, and kept inside airtight jars for further studies.

2.2 Chemical: All the chemicals used in research work are of laboratory grade. Glycerin, Safranin solution, Ethanol, Gallic acid, Methanol, HCl, Quercetin, Mayer's Reagent, Dragendroff's reagent, Tannic acid, Ferric chloride, Gelatin, Vanillin HCl, Acetic anhydride, Sodium hydroxide, Lead acetate solution, Sulphuric acid, Chloroform, Ninhydrin solution, Fehling solution A& B, Distilled water, Silica gel G, Toluene, Ethyl acetate, Glacial acetic acid, Folin- ciocalteu reagent, Aluminium chloride, Potassium acetate solution, BHT, DPPH solution, Trolox, Beef extract, Yeast extract, Peptone, Sodium chloride (NaCl), Sodium carbonate,& Cefotaxime.

2.3 Pharmacognostical Studies

2.3.1 The Macroscopic study of the dried rhizome of *Doronicum hookeri*:

The dried rhizome was examined macroscopically for verification and identification, and the specifications correlated to the literature. (12)

2.3.2 The Microscopic study of the powder of dried rhizome of *Doronicum hookeri*:

For TS of powder of the rhizome, dried rhizomes were powdered using a mortar pestle and strained through a sieve screen of mesh size 60#;

1) Pour a few drops of glycerine on a slide containing powdered drugs and then cover it using a glass cover. Now slide was mounted over a compound microscope for examination.
2) A stained slide provides better microscopic results than a regular slide. So for a stained slide, the powdered drug was stained with safranin solution (1% solution in 50% ethanol solution) for 2 min and then covered it using a glass cover. Now slide was mounted over a photo display microscope to recognize lignified cells.

This microscopic study detected various known and unknown cells like vascular bundles, xylem & phloem tissue components, trichrome, parenchymatous cells, Etc. and their pictures collect for the reference purpose. (12)

2.4 Extraction of the dried rhizome of *Doronicum hookeri*

Continuous Hot Percolation

This process is also known as Soxhlet extraction or soxhelation process, in which continuous extraction of solids with a high-temperature solvent (mainly an organic solvent, e.g., methanol). The extractor is a unique glass reflux device comprising a condenser, round bottom flask, and extraction chamber.

Placed 14g of powdered drug in a filter paper thimble and place it in a Soxhlet extractor. The condenser mounts on an extractor chamber, and a round bottom flask (RBF) containing the solvent is present below the chamber. The solvent in the RBF boils, and the vapour rises and reaches the condenser via the side tubes,

the condenser cause condensation of vapours. Then the condensed liquid falls into the thimble containing the drug and fills the extraction chamber. When condensed vapours fill the extractor tube with the solvent attached, the solvent passes into the flask (RBF) through a siphon tube, and the extract collects in the flask. At least 25 cycles shall be done to get a proper extract. (13)

2.5 Physicochemical Evaluation

2.5.1 Description

Various characteristic features like color, odor, taste, size, and shape of the plant part and its powder examine by naked eyes and noted. (12)

2.5.2 Loss on drying (LOD)

Properly weigh 1g powder of dried rhizome (without initial desiccating) and put it in a tarred Petri plate. Then the drug was dried at 105℃ for almost 2 hours and weighed. We continued drying, cooling and weighing at the time interval of 10 minutes until we reached a constant weight. After drying for 10 minutes in the hot air oven and then cooling for 10 minutes in a desiccator, and it achieves a stable weight when two successive weights displayed a difference of NMT 0.01g. Now, calculate the % LOD by using the formula mentioned below: (12,14)

$$\text{(LOD \%)} = \frac{\text{(Weight of sample)}_{\text{before dry}} - \text{(Weight of sample)}_{\text{after dry}}}{\text{(Weight of sample)}_{\text{before dry}}} \times 100$$

production of high-quality natural remedies. These natural products are safe and effective for the application.

a) Macroscopic study of the dried rhizome of *Doronicum hookeri*

It is also known as the organoleptic study, which is the first stage of plant identification. Naked eyes and microscope visually studied the dried rhizome and its powder for their colour, shape, taste, odour, and size.

Figure 1: Dried rhizome of Doronicum hookeri

Table 1: Organoleptic characters of rhizome of Doronicum hookeri

Colour	Brownish
Shape	Irregular
Taste	Starchy and Astringent
Odour	Non-specific
Size	Length- 2.5 to 5 cm, Width- 0.1 to 0.7 cm

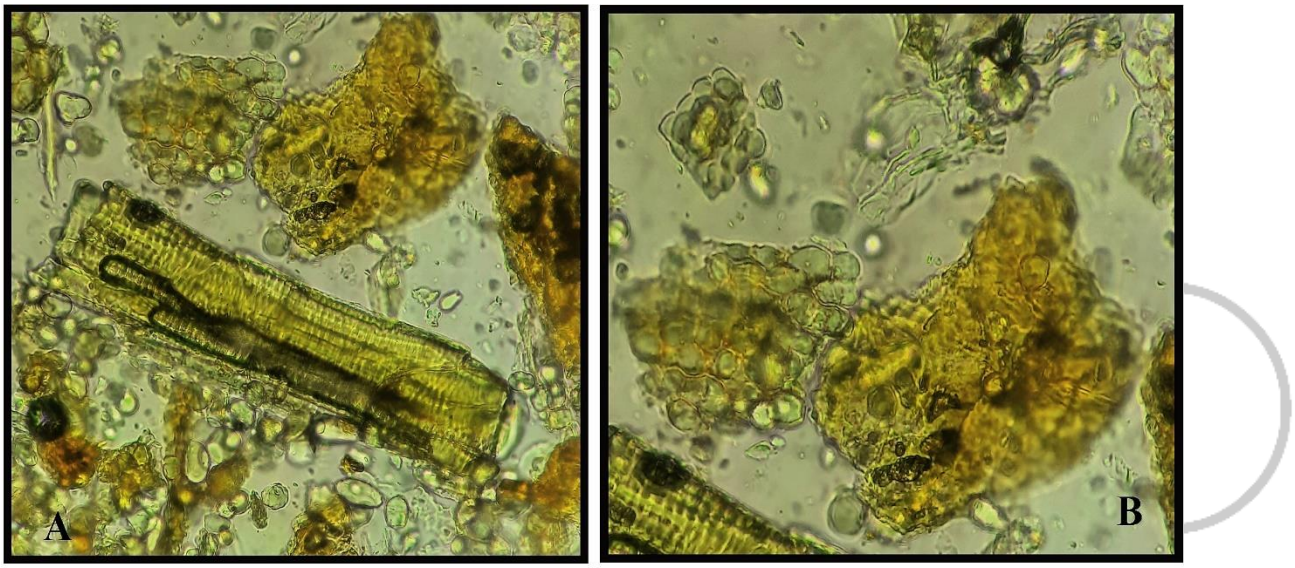
Figure 2: Powder of dried rhizome of Doronicum hookeri

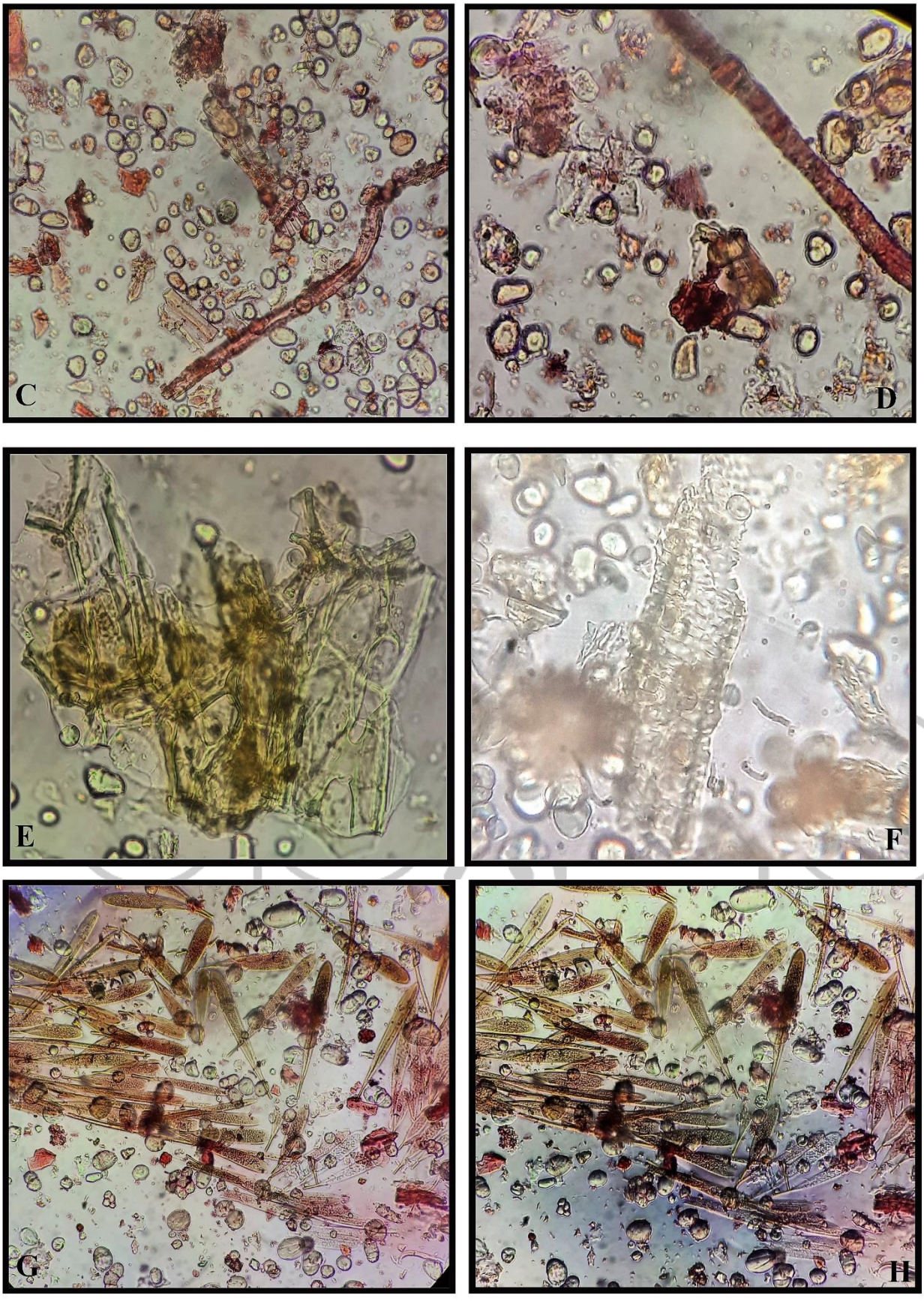
Table 2: Powder characteristics of dried rhizome of Doronicum hookeri

Colour	Dull brownish
Taste	Astringent
Odour	Present & Non-specific

b) Microscopic study of the powder of the dried rhizome of *Doronicum hookeri*

The analysis has been performed to know the plant's anatomy and get knowledge of various cells in the plant's rhizome.

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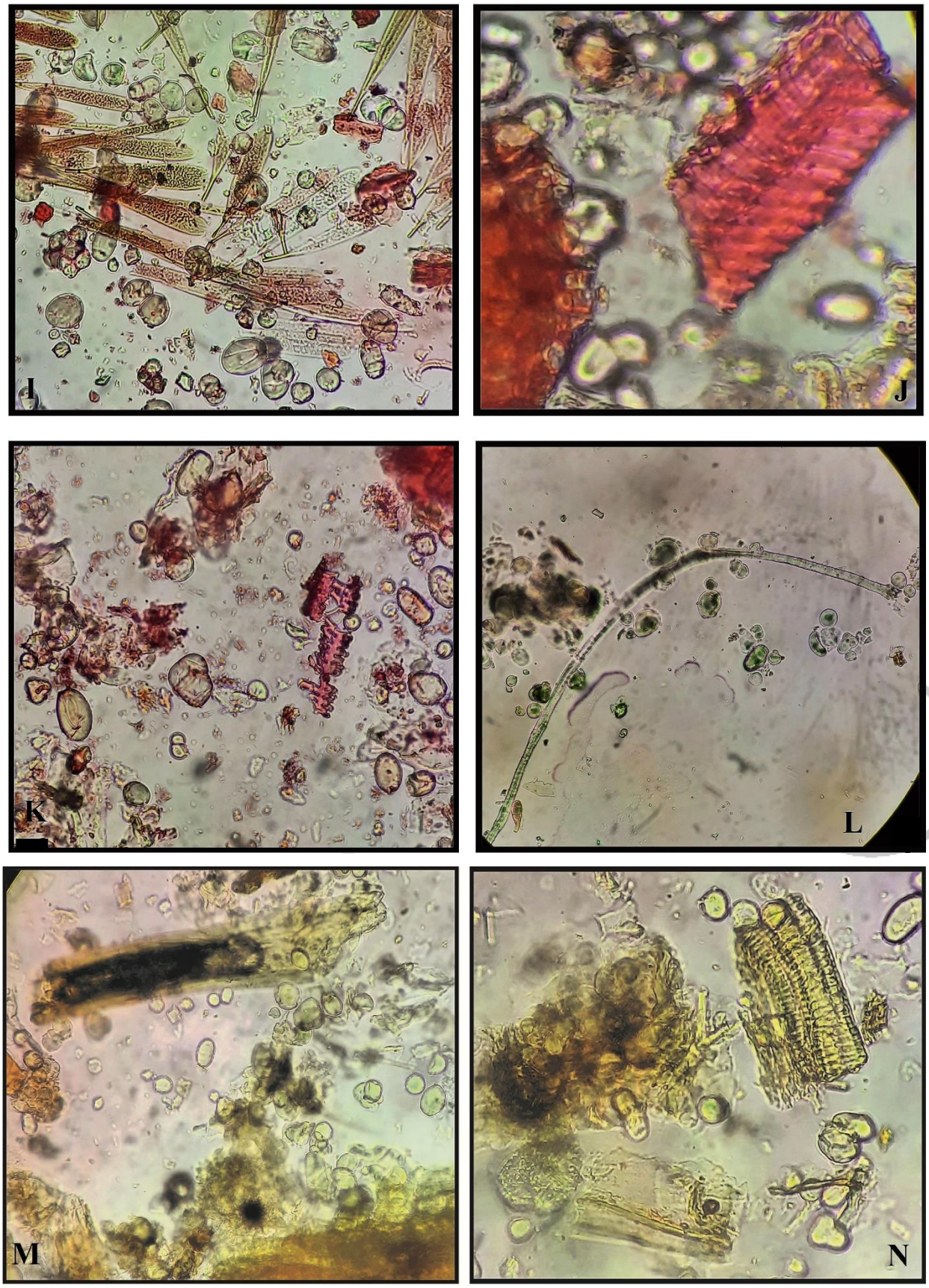
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Figure 3: A- Xylem vessel with reticulate thickening; **B-** Parenchyma cells with oil cell; **C& D-** Lignified xylem fibers; **E-** Wavy parenchyma cells with starch; **F-** Xylem vessel with spiral thickening; **G, H& I-** Starch grain; **J-** Cork cell; **K-** Isolated trichome; **L-** Unicellular trichome with glandular trichome; **M-** Monosclereids; **N-** Spiral xylem vessel; **O-** Bundle sheath with xylem element.

3.2 Extraction of dried Rhizome of *Doronicum Hookeri*

Continuous Hot Percolation

The Soxhlet apparatus uses to perform the extraction process, and the solvent used is 200ml methanol and powdered drug (14g) placed inside a thimble prepared from filter paper. The process started at 12:50 PM. After 25 extraction cycles process ended at 03:10 PM. The extract collected is Brownish in appearance. The volume collected at the end of the process is 70ml.

Figure 4: Soxhlet apparatus

Figure 5: Methanolic extract of Darunaj

Table 3: Information of the extract obtained

Colour of methanolic extract collected	Brownish
Amount of methanolic extract collected	70 ml

3.3 Physicochemical Evaluation

Description

The dried rhizome was brownish and had an irregular shape. It has an odour present but undefined. The rhizome's length varies from 2 to 5.5 cm, and its width varies from 0.1 to 0.7 cm. The powder of the dried rhizome is dull brownish in appearance. Its taste is starchy and astringent.

Table 4: Physicochemical test results

S. no.	Physicochemical Test	Result
	LOD	9%
	Alcohol soluble extractive content	40%
	Total Ash content	8%
	Ash content (Water soluble)	3.5%
	Ash content (Acid insoluble)	1.5%
	The pH of the extract	5

3.4 Phytochemical Screening

The methanolic extract of the dried rhizome of the plant has undergone various chemical tests to get information regarding the chemical constituents present in the plant extract.

Table 5: Results of phytochemical analysis

S. No.	Test	Result
	Test for Alkaloids	
	Mayer's Test	+ve
	Dragendroff's Test	+ve
	Tannic acid Test	+ve
	Test for Tannins	
	$FeCl_3$ Test	-ve
	Gelatin Test	-ve
	Vanillin Test	-ve
	Test for Steroids	
	Liebermann Buchard Test	-ve
	Test for Flavonoids	
	Alkaline Reagent Test	+ve
	Lead Acetate Test	+ve
	Test for Terpenoids	
	Salkowski Test	+ve
	Test for Proteins	
	Ninhydrin Test	-ve
	Test for Reducing sugar	
	Fehling Test	+ve
	Test for Saponins	
	Frothy Test	-ve

****NOTE: (+ve) =Positive and (–ve) = Negative**

The phytochemical analysis of the methanolic extract of the plant provided information for the presence of Alkaloids, Flavonoids, Terpenoids and reducing sugar, whereas tannins, protein and saponins were absent.

3.5 Isolation & characterization of extract of the dried rhizome of *Doronicum hookeri*

a) Thin-layer chromatography (TLC)

After the whole process the TLC examined in the UV chamber in which number of spots and R_f value find out. The

R_f value is mentioned in the table below and figure-------- shows the TLC.

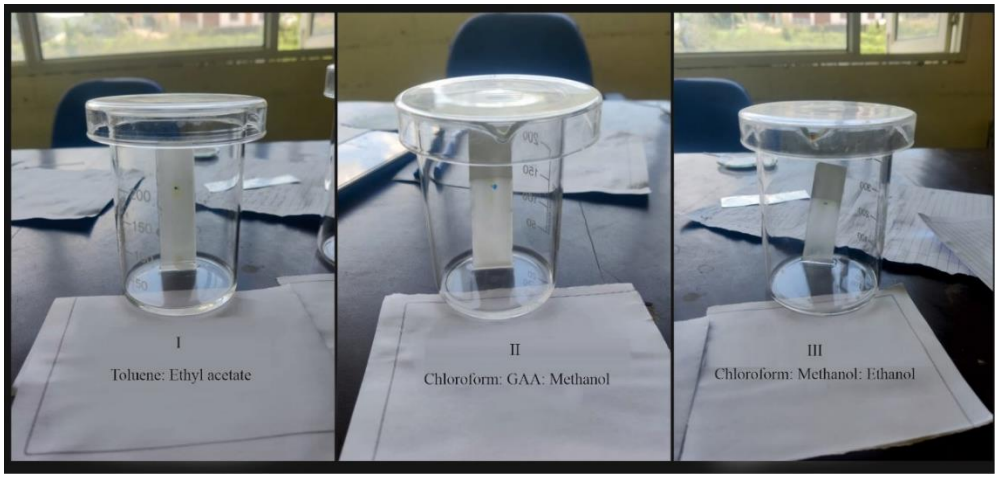

Figure 6: TLC plate in different moblie phase

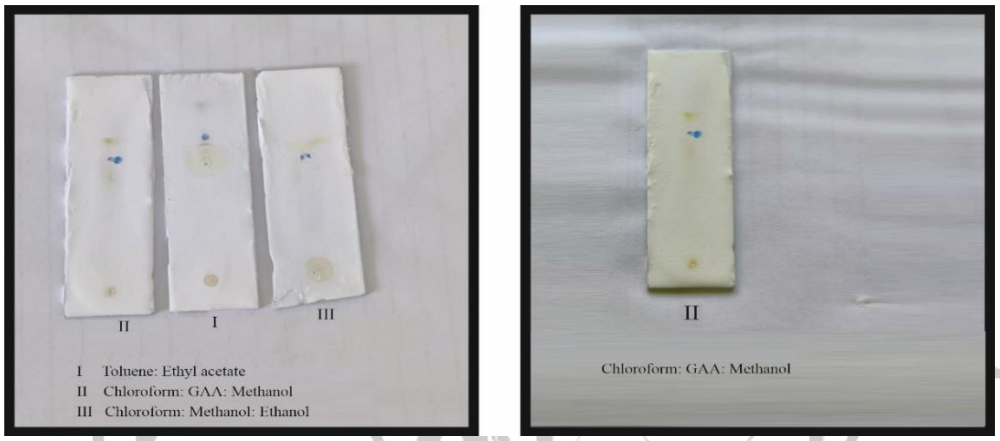

Figure 7: TLC plate observation of the spots

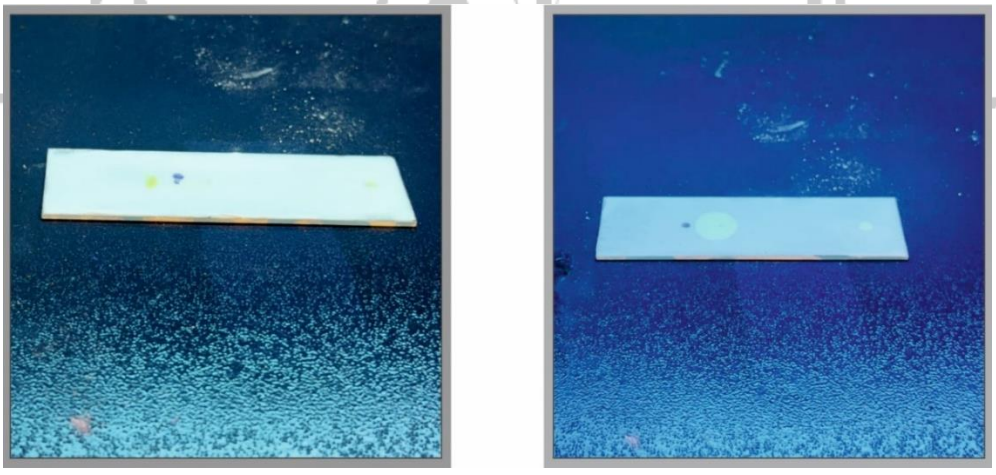

Figure 8: Observation of the TLC plate in the UV chamber

Table 6: R_f value and no. of spot observed in a TLC

S.no.	Extract Used	Solvent or mobile phase used	No. of spots observed	R_f value
	Methanolic extract of the dried rhizome of Doronicum hooker	Chloroform: Glacial acetic acid: Methanol	01	0.77

		(4:5:1)		
	Methanolic extract of the dried rhizome of Doronicum hooker	Toluene : Ethyl alcohol **(5:4)**	01	0.73
	Methanolic extract of the dried rhizome of Doronicum hooker	Chloroform: Methanol: Ethyl alcohol **(2:2:0.5)**	01	0.70

The extract shows 1-1 spot in all the solvents used in UV chamber. The R_f value 0.77, 0.73& 0.70 is due to therapeutic active constituents.

b) UV Spectroscopy

The extract dilutes with distilled water, and a UV spectrophotometer examined this diluted extract in a wavelength range of 200-800nm. Now, observe the maximum peak value and found to be λ_{max} 281.00 nm.

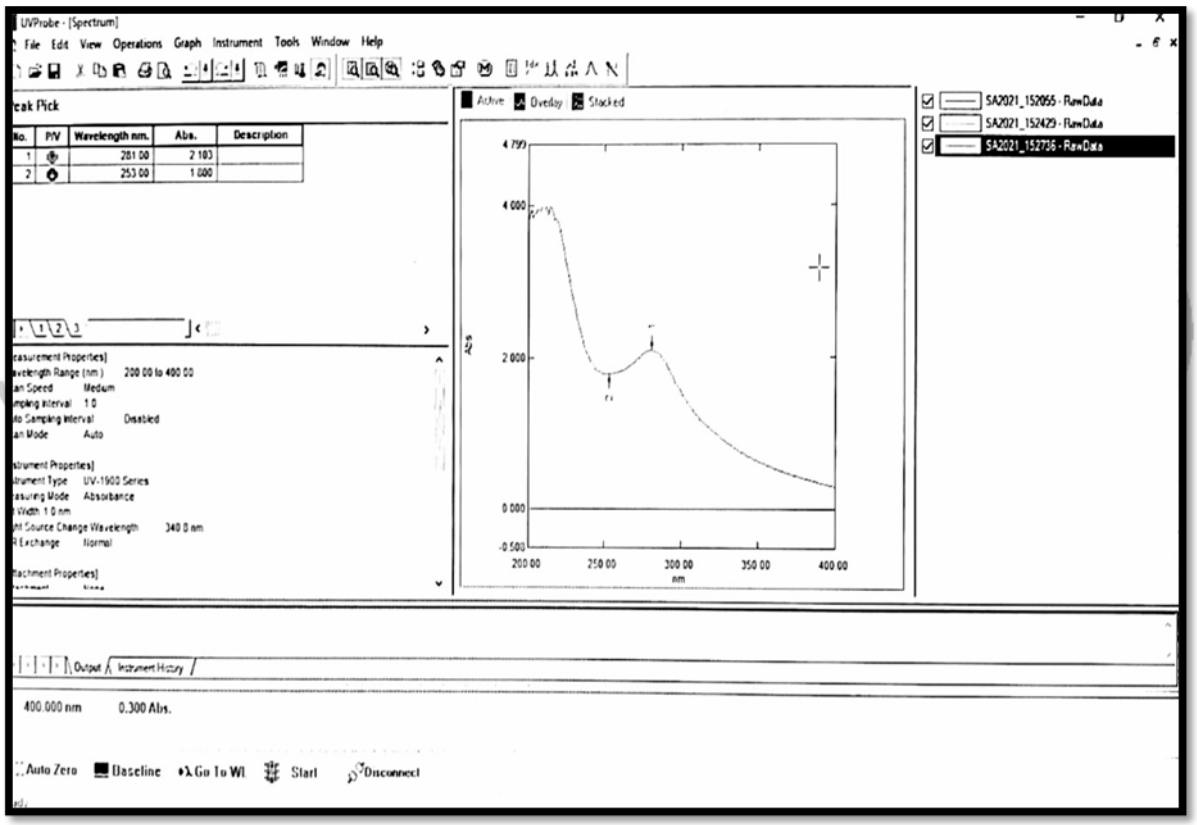

Figure 9: λ_{max} Observed in the UV- spectrophotometer

c) FTIR Spectroscopy

The extract examines with the help of an Everest ATR spectrometer. Now, observe the characteristic peaks in a wavenumber ranging from 400-4000 cm^{-1}, and possible functional groups and types of bonds are given in the table below.

Table 7: Possible function group present in Extract of Doronicum hookeri

S. no.	Wave number	Frequency Range	Group	Functional Group

1.	3270.746	3550-3200	O-H Stretching	Alcohol
2.	2921.920	3000-2800	N-H Stretching	Amine Salt
3.	1626.690	1662-1626 1650-1600 1650-1580 1650-1566	C=C Stretching C=C Stretching N-H Bending C=C Stretching	Alkene Conjugated alkene Amine Cyclic alkene
4.	1332.730	1420-1330	O-H Bending	Alcohol
5.	1147.449	1390-1310 1150-1085 1250-1020	O-H Bending C-O Stretching C-N Stretching	Phenol Aliphatic ether Amine
6.	1074.929	1085-1050	C-O Stretching	Primary Alcohol
7.	761.415	750±20	C-H Bending	Mono-substituted
8.	573.421	600-500 690-515 850-550	C-I Stretching C-Br Stretching C-Cl Stretching	Halo compound
9.	526.249	600-500 690-515	C-I Stretching C-Br Stretching	Halo compound
10.	511.001	600-500	C-I Stretching	Halo compound

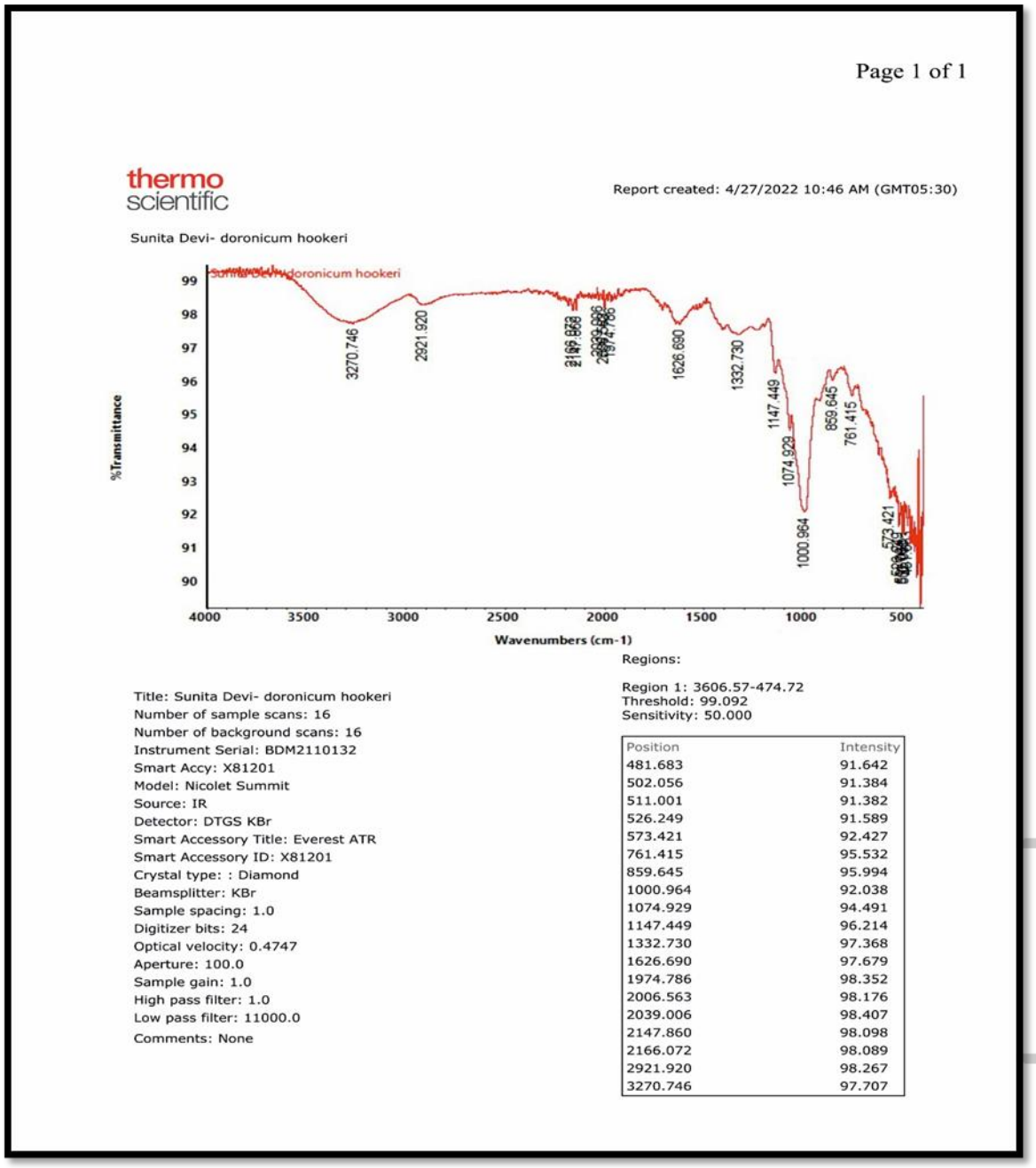

Figure 10: FT-IR of the extract of Doronicum hookeri

SUMMARY AND CONCLUSION

In conclusion, the results of the present studies indicated that the *Doronicum hookeri* is an excellent source of bioactive compounds. In the present study, the herbal drug *Doronicum hookeri* is selected and authenticated by morphological and organoleptic properties. Detailed Microscopical characters and detailed powdered Microscopical characters of *Doronicum hookeri* rhizome and photographs of their unique characters were taken.

Several physicochemical parameters were established for the selected drugs. This drug was extracted with methanol solvent using the soxhlet apparatus. The alcohol-soluble extractive values are indicating the presence of more polar constituents such as flavonoids, alkaloids, saponins, sterols, etc. The ash values were performed to know the presence of any earthy particles or inorganic matter and

are committed to obtaining total ash, insoluble acid ash, and water-soluble ash. The drug shows that ash values are within limits during ash value content. The pH value hints at the presence of a type of constituents as it describes the fundamental nature, i.e., acidity or basicity of the components. The *Doronicum hookeri* contains components belonging to a somewhat acidic range. The TLC with mobile phase Chloroform: GAA: Methanol shows the Rf value of 0.77. The UV-spectrophotometer examined the dilute extract of Doronicum hookeri, and λ max was observed at 281.00 nm. Several functional groups have reported the dilute extract of Doronicum hookeri examined by the IR-spectrophotometer like alcohol, amine salt, Phenols, etc.

Phytochemical screening revealed that *Doronicum hookeri* was found positive for alkaloids, flavonoids, terpenoids, and reducing sugar and negative for tannins, Steroids, proteins, and saponins.

References:

1. Kang CG, Hah DS, Kim CH, Kim YH, Kim E, Kim JS. Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. Toxicol Res. 2011;27(1):31–6.
2. Attimarad M, Mueen Ahmed KK, Aldhubaib BE, Harsha S, Cuvelier ME, Berset C, et al. a Contribution To the Medicinal Plants of Sahastradhara, District Dehradun, Uttarakhand (With Ethenobotanical Notes). Molecules. 2016;26(3):216–9.
3. Nanoparticles P, Improved FOR, Of B. Int J Pharm Pharm Sci , Vol 9 , Issue 4 , 129-139 Original Article. 2017;7(12):233–7.
4. Prakash A, Janmeda P, Pathak P, Bhatt S, Sharma V. Development and standardization of quality control parameters of different parts of Trianthema portulacastrum L. SN Appl Sci. 2019;1(9):1–14.
5. Afaka S, State E. Ethnomedicinal uses of Polyherbal mixtures in the treatment of skin infections : A Review. 2021;8(6):9–21.
6. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and future perspective. J HerbMed Pharmacol. 2018;7(1):1–7.
7. Holley J, Cherla K. The Medicinal Plants Sector in India : A Review. 1998;1–98.
8. Hooker JD. The flora of British India /By J. D. Hooker assisted by various botanists. Published under the authority of the secretary of state for India in council. Vol. Part 3, LONDON: L. REEVE & CO., 5 HENEIETTA STREET, COVENT GARDEN. 1882. London : L. Reeve,; 1875. 1–724 p.
9. Swamy MK, Akhtar MS. Natural bio-active compounds: Chemistry, pharmacology and health care practices. Vol. 2, Natural Bio-active Compounds: Chemistry, Pharmacology and Health Care Practices. 2019. 1–492 p.
10. Aleem M, Imran Khan M, Islam Usmani Q, Ahmad A. Review on Darunaj-aqrabi (Doronicum hookeri C.B. Clarke): an Unexplored Medicinal Plant of Unani System of Medicine. J AYUSH Ayurveda, Yoga,Unani ,Siddha Homeopath. 2020;(October).
11. Khare CP. Indian Medicinal Plants. Springer. Springer; 2007. 1–836 p.
12. Anonymus. THE AYURVEDIC PHARMACOPOEIA OF INDIA THE AYURVEDIC PHARMACOPOEIA OF INDIA Part -I Volume- IV THE AYURVEDIC PHARMACOPOEIA OF INDIA PART-I VOLUME-VI First Edition GOVERNMENT OF

INDIA MINISTRY OF HEALTH & FAMILY WELFARE DEPARTMENT OF AYUSH NEW DELHI 2008. 1st ed. Vol. VI. Central Council for Res, New Delhi; 2008. 01–492 p.
13. Becker FG, Cleary M, Team RM, Holtermann H, The D, Agenda N, et al. No 主観的健康感を中心とした在宅高齢者における

健康関連指標に関する共分散構造分析Title. Syria Stud. 2015;7(1):37–72.
14. Grover N. Physiochemical Evaluation, Phytochemical Screening and Chromato-Graphic Fingerprint Profile of Woodfordia Fruticosa (L.) Kurz Extracts. Pap Knowl Towar a Media Hist Doc. 2014;5(2):40–51.
15. Evans WC. Trease and Evans' Pharmacognosy: Sixteenth Edition. Trease Evans' Pharmacogn Sixt Ed. 2009;1–603.
16. Nurnasrina PAP. Scanned by CamScanner ىرازمك. A Psicanal dos contos fadas Tradução Arlene Caetano. 2013;466.
17. Alebiosu CO, Yusuf AJ. Phytochemical Screening, Thin-layer Chromatographic Studies and UV Analysis of Extracts of Citrullus lanatus. J Pharm Chem Biol Sci. 2015;3(2):214–20.
18. Ameerah Shaeroun AR, Hamed Alqamoudy ABA, Mohamed K., Nouri Kushlaf NA, Akram Almabrouk misbah AME, Zuhur rajab Almes STO. Thin Layer Chromatography (TLC) and Phytochemical Analysis of Moringa Oleifera Methanol, Ethanol, Water and Ethyl Acetate Extracts. Saudi J Med Pharm Sci. 2019;05(10):817–20.
19. Modern GTO, Analysis OFP, Edition T. Techniques of Plant Analysis Third Edition.
20. Kalaichelvi K, Dhivya SM. Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of Micrococca mercurialis (L.) Benth. Int J Herb Med. 2017;5(6):40–4.
21. Rajeshkumar R, Jeyaprakash K. Screening of UV-VIS, TLC and FTIR spectroscopic studies on selected red seaweed (Acanthophora specifera) collected from Gulf of Mannar. World J Pharm Sci. 2016;4(10):28–33.
22. Rani N, Sharma S, Sharma M. Phytochemical analysis of Meizotropis pellita by FTIR and UV- VIS spectrophotometer. Indian J Sci Technol. 2016;9(31):1–4.
23. Gupta D, Bleakley B, Gupta RK. Phytochemical analysis and antioxidant activity of herbal plant Doronicum hookeri hook F. (Asteraceae). J Med Plants Res. 2011;5(13):2736–42.
24. Syed S. Study to Evaluate the Antioxidant and Hepatoprotective Activities of Roots Extracts of Doronicum hookeri in CCl4 Treated Rats. European J Med Plants. 2014;4(6):675–85.
25. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
26. Ankalge R, Jagdale M, Ghaytidak S, Patil K, Manani L, Desai S. Pharmacognostic evaluation of leaves of Dysoxylum binectariferum. Phytomedicine Plus. 2021;1(4):100149.