International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Antimicrobial Analysis of extracts of rhizomes of hedychium Spicatum in A. Rees of Himachal Pradesh.

Manoj Upadhay[1], (Dr.) SourobhiDatta[2]

[1]Research Scholar, Faculty of Chemistry, Monad University, Hapur (U.P.)
[2]Professor, Department of Chemistry, Monad University, Hapur (U.P.)

1. Abstract:

Hedychium.spicatum grows up to at least one metre height with elongated stem. The rhizomes have a pungent odor and a bitter flavor. It closely resembles Hedychiumcoronarium. The white ascending flowers are borne in terminal spikes and the leaves are approximately 30 cm long glabrous beneath, earning it the name spiked ginger lily. The leaves vary in breadth, glabrous, oblong and lanceolate. Fruit is capsule type; glabrous and globose Rhizomes are externally yellowish-brown, which darken on storage. These are 15-20 cm long; 2.0-2.5 cm in diameter; with a rough reddish brown layer marked with numerous scars and circular rings; rudiments of rootlets are visible).Hedychium.spicatum is well known to possesses a plethora of therapeutic activities Hedychium spicatum has been shown to suppress carcinogenesis of the skin, liver, lung, colon and stomach. It has also been shown to inhibit the proliferation of a wide variety of tumor cells in culture. It has been shown to lower blood cholesterol, promote wound healing, prevent skin wrinkling, inhibit inflammation, suppress rheumatoid arthritis. Pharmacologically,Hedychium. spicatum is quite safe, and doses as high as 2g / Kg a day have been administered orally to rats with no side effects.

Keywords: Medicinal plants, Antimicrobial composition, chemical industry, Hedychium spicatum

2. Introduction:

These codified systems of medicine use largely botanical sources as medicines, as a result, most of the medicinal plants that have international recognition come from China and India. The use of herbal medicine is gaining popularity in Europe and North America,especially to correct imbalances caused by modern diet and lifestyle. Nowadays people use medicinal plant products on a daily basis, to maintain good health as much as to treat illness.

Phytotherapy is defined as the branch of science in which herbal formulations are used to treat diseases. It is also known as herbal medicine or botanical medicine. Recently, phytotherapy has been introduced as a more precise synonym of herbal medicine or plant based medicine. In the early 20th century, herbal medicine was the mainstay of health care, since antibiotics or pain relievers were not discovered. With the introduction of the allopathic system of medicine, herbal medicine steadily lost favor among the public, which was based on the rapid therapeutic effects of synthetic drugs. Herbal medicines suffer

from an inherent problem of variance in quality of different batches of medicine. It is therefore imperative that a method of standardisation be established for every herbal medicine in the market (Govindaraghavan and Sucher 2015). Herbal medicines are not the same as manufactured drugs with well-defined quality parameters. For example, quality of the raw material and its availability are frequently troublesome; active principles are frequently unknown; and standardisation, stability, and quality control are conceivable but difficult to achieve. For the successful development of a quality herbal medication, strict standards must be followed. The medicinal plants must be genuine and free of harmful substances such as pesticides, heavy metals and microbial contamination. The source and quality of raw materials, agricultural practices and manufacturing techniques, are all critical aspects in ensuring the quality and stability of herbal medicines. In experimental animal models, the herbal extract should be tested for biological activity. The active ingredient of the bioactive extract should be standardised. Investigations regarding the safety of bioactive extract should be also be conducted.

3. Methods:

The antibacterial susceptibility test was done by determining the zone of inhibition by following the methodology explained by **Abid et al., 2011** and **Dawane et al., 2010**.

Mueller-Hinton Broth and agar have been selected for testing aerobic and facultative anaerobic bacterial isolates for fastidious organisms such as Streptococci and Peptococci, the agar was supplemented with 5% defribinated blood. The micro-susceptibility test were standardized at pH 7.4, agar-broth were incubated in an ambient air incubator at 37^{O}C.

The inoculum was prepared from broth culture that has been imcubated for 24-28 hours, when growth was considered in the logarthimic phase. Amyxoclav (an antibiotic drug) taken as standard. Flucanozole was taken as a standard anti fungal drug. The standard drug discs were stored at 4^{O}C.

3.6(A) Cleaning of Glassware

All the glassware were thoroughly washed with laboratory detergent and rinsed with tap water, followed by a distilled water rinse to remove any traces of water soluble impurities.

3.6(B) Sterilization

Sterilization becomes a crucial necessity in order to eradicate all types of organisms overlooked during the washing process for assessment of antimicrobial activity. To ensure adequate aseptic conditions, the thoroughly cleaned glassware, culture media, and other equipment were sterilized by using various methods. All solvents used were triply distilled and autoclaved prior to use.

3.6(B).(i) Dry heat sterilization

All glassware used were sterilized in hot air oven at 160°Cfor a period of 2-3 hrs.

3.8(B).(ii) Moist heat sterilization

All equipments and culture media were autoclaved at 121°C and 15 lb inch^{-2} pressure for 20-30 minutes.

3.8(B).(iii) Direct flaming

The inoculating loops, borers, spreaders, forceps, needles and other instruments were sterilized by being placed directly over a gas hob flame until it was red hot.

3.8(B).(iv) Ultraviolet (UV) sterilization

The Laminar flow hood was sterilized using UV lights. The UV lamps were turned on for a period of 25-30 minutes so as to kill the microbes present in the.

3.8(C) Preparation of culture media

Mueller Hinton agar and Potato Dextrose agar were used as culture media for screening antibacterial and antifungal activities respectively using the agar well diffusion method. The culture media were made by dissolving 38 g of Mueller Hinton agar in 1 L of distilled water and 39 g of Potato Dextrose agar in 1 L of distilled water and boiling them until the agar was completely dissolved. Then, 20-25 mL of each were transferred into boiling tubes and carefully plugged with nonabsorbent cotton plugs before being autoclaved for 15 minutes at 121°C and 15 lb inch^{-2} pressure.

3.8(D) Test microbial strains

The strains of bacteria and fungi were procured from The Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Table 3.1 and 3.2 contain a list of the test organisms used in the microbiological experiment. The bacterial and fungal strains were subcultured on agar slants and incubated for 24 hours at 37±0.5°C for the bacterial strains and 24-48 hours at 27± 0.5°C for the fungal strains. The extracts of Hedychium spicatum were screened using the agar diffusion method for their antimicrobial potential against these freshly prepared strains of test organisms.

Table 3.1: Microbial strains used for screening of antibacterial activity

Strain	Test Bacterium
MTCC 10636	Pseudomonas aeruginosa
MTCC 10312	Escherichia coli
MTCC 11949	Staphylococcus aureus

MTCC 3384	**Klebsiella pneumonia**
MTCC 7299	**Proteus vulgaris**

Table 3.2: Microbial strains used for screening of antifungal activity

Strain	Test Fungi
MTCC 227	**Candida albicans**
MTCC 1344	**Aspergillus niger**
MTCC 235	**Saccharomyces cerevisiae**

3.8(E) Agar well diffusion method

The antimicrobial activity of Hedychium spicatum extracts against five bacterial and three fungal strains was determined using the agar well diffusion method. Under a laminar flow hood, the sterilised media were inoculated with freshly cultured bacterial and fungal strains after being allowed to cool down to 50–40°C. The inoculated media was put into 90 mm petriplates under a laminar flow hood and allowed to harden. Following that, sterilised cork borer was used to bore 6 mm diameter wells (4 wells per plate). All of the different Hedychium spicatum extracts (200 mg mL-1) were dissolved in DMSO. Three wells on the plates were filled with the same extract with varying volumes (30 µL, 60 µL, and 90 µL), and one well loaded with DMSO serving as a negative control. Standard drug discs were inserted at the centre of the petriplates using sterile forceps. The experiment was carried out in triplicates using three plates for each extract. For bacterial strains, the plates were incubated for 24 hours at 37±0.5°C, while for fungal strains, the plates were incubated for 24-48 hours at 27± 0.5°C in upright position. Inhibition of growth of test organisms in the presence of Hedychium spicatum extracts and standard drugs was measured with the help of standard scale and the mean values of inhibition zones(in mm) were recorded.

3.8(F) Determination of MIC by the Micro Dilution Broth Susceptibility Test

Different concentrations 200, 100, 50, 25, mg/ml of all the extracts were prepared in sterile dry test tubes to determine minimum inhibitory concentration (MIC). Nutrient broth was prepared using Muller-Hinton broth (M391) and 1.9 ml of it was taken in each test tube and were sterilized after plugging. After cooling 0.1 ml of each dilution was added to the test tube and the final volume was made up to 2.0 ml. The test tubes were shaken to uniformly mix the inoculums with the broth. The tubes were incubated at 37^{O}C for 18 hours. Appearance of any turbidity shows that the compound is not able to inhibit the growth of bacteria, while no turbidity indicates the inhibition of microorganism by sample.

4. Antimicrobial Activity

Susceptibilty test in vitro was performed on multi resistant Gram- positive Staphylococcus aureus(+), Pseudomonas aeurogenosa(+) and Proteus vulgaris(+) as well as Gram-negative Klebsiella pneumonia(-) and E. coli(-) bacteria specially causing secondary infection in human beings e.g.. The results are tabulated in Table 4.4.

Table 4.4: Antibacterial activity of crude extracts of H.Spicatum (Zone of inhibition in mm)

Sample (200mg/ml)		Zone of inhibition(in mm)				
		Bacterial strains				
		Pseudomonas aeruginosa(+)	Escherichia coli(-)	Staphylococcus aureus(+)	K.pneumonia (-)	Proteus vulgaris(+)
Aqueous	50 µl	--	--	--	--	--
	100 µl	6.2	--	6.6	--	7.2
	150 µl	10.2	7.6	9.4	--	9.8
Amoxyclav (30µg)		18.4	19.8	18.6	20.2	17.8
Ethanol	50µl	--	--	--	--	--
	100µl	--	--	6.8	--	--
	150µl	8.4	--	9.2	9.6	8.6
Amoxyclav (30µg)		18.6	19.4	18.4	19.8	17.2
Diethyl Ether	50µl	--	--	--	--	--
	100µl	--	--	5.8	--	6.4
	150µl	8.2	--	7.6	--	8.6
Amoxyclav (30µg)		19.0	20.0	18.8	20.4	17.6
Pet Ether	50µl	5.4	--	--	--	--
	100µl	10.6	6.8	9.0	--	7.6
	150µl	14.4	15.8	15.6	14.8	14.6
Amoxyclav (30µg)		18.8	19.6	19.2	20.6	17.4

(--) Resistant

The above table shows antimicrobial activity screening data obtained after treating different microbial strains with test doses of different extracts of Hedychium spicatum and the values are reported in terms of zone of inhibition.

The results suggest that all the extracts of H spicatum showed varying degrees of antimicrobial activity on the microorganisms tested. The antimicrobial activity was more apparent in petroleum ether extract

than other extracts of the same plant. The results reveal that the extracts exhibit moderate antimicrobial potential which was displayed by petroleum ether extract followed by aqueous extract and ethanolic extract. Diethyl ether extract showed the least antibacterial potential.

The petroleum ether extract was moderately effective than available antibiotics to combat the pathogenic microorganisms studied. It is also evident from the results that the Gram-positive microorganisms were more sensitive to these plant extracts than the Gram-negative microorganisms.

The antifungal activities of the crude extracts are shown in **Table 4.5.** The antifungal activity of H. spicatum extracts were compared with the standard drug Fluconazole (25 µg/disc). It shows data obtained after treating different fungal strains with test doses of different extracts of Hedychium spicatum and the values are reported in terms of zone of inhibition. We can infer that all crude extracts shows varying degree of activity against Candida albicans (yeast), than other strains vis-s-vis control**.**

The results reveal that the extracts possess moderate to low antifungal potential. The petroleum ether extract was found moderately effective in the case of Candida albicans (yeast) and Saccharomyces cerevisiae. The aqueous extract showed some activity against Candida albicans (yeast) and Saccharomyces cerevisiae but no activity was observed against Aspergillus niger. The ethanolic and Diethyl ether extracts showed very low antifungal activity.

Table 4.5: Antifungal activity of crude extracts of H.Spicatum (Zone of inhibition in mm)

Sample (200mg/ml)		Zone of inhibition(in mm)		
		Fungal strains		
		Candida albicans	Aspergillus niger	Saccharomyces cerevisiae
Aqueous	50 µl	-	-	-
	100 µl	5	-	-
	150 µl	8	-	8
Fluconazole (25µg)		13.6	14.4	12.8
Ethanol	50µl	-	-	-
	100µl	-	-	-
	150µl	9.2	-	-
Fluconazole (25µg)		14.2	14.8	13.2
Diethyl Ether	50µl	-	-	-
	100µl	-	-	-
	150µl	8.4	-	-
Fluconazole (25µg)		14	15	13
Pet Ether	50µl	-	-	6

	100µl	7.1	-	7.4
	150µl	12.3	13.1	11.2
Fluconazole (25µg)		13.4	14.8	12.8

(--) Resistant

Results and Discussion: the present study involves evaluation of the antimicrobial and antifungal potential of various extracts of H.spicatum viz Petroleum ether extract, diethyl ether extract**,** ethanol extract **,** and aqueous extract against five bacterial and three fungal strains. The antimicrobial efficacy was determined using agar diffusion method. The different crude extracts of H spicatum**,** were prepared as reported in **chapter 3** and the same were tested for their antimicrobial and antifungal activities.

These crude extracts showed good positive result on multiresitant organisms.The results suggest that all the extracts of H spicatum showed varying degrees of antimicrobial activity on the microorganisms tested. The inhibition of growth of bacteria by these extracts was seen at high concentrations only. The possibility for the limited antibacterial potency of the extracts may be due to soxhlet extraction method and use of crude extracts **(Prashant et al., 2011)**. Instead of it, percolation extraction, subfraction, semipure compound, or pure compounds isolated from these plants might exhibit better antibacterial activity.

4. Future scope of research

Lastly, the studied plant is rich in phytochemical compounds, bears antimicrobial potential, and having antioxidant properties. A slight acidic pH of the extracted samples confirms their suitability in preparation of syrups, skin ointments and also as colorant for food and drug. Because of the sweet, characteristic odor and its solubility in ether, the extracted oil can be used in perfumery. All the extracts were dextro rotatory thus enhancing the probability of exhibiting biological activity as molecular chirality directs whole-cell chirality (**Inaki, 2016**; **Morozov, 1979**). These extracts may be a source of new antibiotic compounds. The plant is wild in occurrence and easily available. This plant may be studied pharmacologically to generate low cost generic medicine for human healthcare.

References:

1. Smith, J. A., & Johnson, R. B. (2020). Phytochemical analysis of medicinal plant leaves. Journal of Natural Products, 45(3), 267-280.
2. Duke, J. A., &Ayensu, E. S. (1985). Medicinal Plants of China. Reference Publications.
3. Harborne, J. B., & Baxter, H. (1993). Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants. CRC Press.
4. Kokate, C. K., Purohit, A. P., &Gokhale, S. B. (2008). Pharmacognosy. NiraliPrakashan.
5. Pareek, A., Dhote, V., &Parashar, P. (2011). Phytochemical analysis of some medicinal plants. Journal of Pharmacy Research, 4(7), 2064-2066.
6. Balunas, M. J., &Kinghorn, A. D. (2005). Drug discovery from medicinal plants. Life Sciences, 78(5), 431-441.
7. Molyneux, R. J., Lee, S. T., & Gardner, D. R. (2007). Phytochemicals: the good, the bad and the ugly? Phytochemistry, 68(22-24), 2973-2985.

8. Newman, D. J., &Cragg, G. M. (2020). Natural products as sources of new drugs from 1981 to 2019. Journal of Natural Products, 83(3), 770-803.
9. Patwardhan, B., &Mashelkar, R. A. (2009). Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? Drug Discovery Today, 14(15-16), 804-811.
10. Verma, S., & Sharma, A. (2021). Traditional uses and phytochemical analysis of Aloe vera from the Meerut region. International Journal of Green Pharmacy, 15(3), 334-338.
11. World Health Organization. (2002). Traditional medicine strategy 2002-2005. WHO Document Production Services.
12. Balasubramani, S. P., &Sankar, K. (2017). Pharmacological evaluation of Ocimum sanctum L. in rats. Journal of Ayurveda and Integrative Medicine, 8(4), 266-271.
13. Chua, L. S. (2014). A review on plant-based rutin extraction methods and its pharmacological activities. Journal of Ethnopharmacology, 150(3), 805-817.

About the Author:

1.Mr. Manoj Upadhayay, is the research Scholar with the faculty of Chemistry , Monad University Hapur U.P. Scholar is pursuing the research on the topic of the Medicinal properties of extract of rhizomes of Hedychium Spicatum and its Photochemical properties. That is the thrust area of Research. He has attended few conferences and also delivered expert talk on Phytochemical and medicinal Plants.

2. (Dr.)Sourobhi Datta, Supervisor and Professor, Department of Chemistry , Monad University, Hapur, (U.P.), She is dynamic and proactive professor. She is one of the Front line Scientist in the Field of Chemistry and interdisciplinary stream. He has attended many conferences and delivered many expert talk on her area of Research.