Pharmacogn J. 2017; 9(6)Suppl: s64-s68
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Phytochemical Screening and Antimicrobial Activity of Rhizomes of *Hedychium spicatum*

Ritu Arora*, Avijit Mazumder

ABSTRACT

Background: The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. **Objective**: In this study the rhizomes of *Hedychium spicatum* (Zingiberaceae) were evaluated for phytochemical parameters & antimicrobial activity by determining its MIC (by checker board method) and zone of inhibition (by cup plate method). Phytochemical parameters were studied with the aim of drawing the pharmacopoeial standards for this species. This study was also designed to evaluate the antimicrobial efficacy of the methanolic extract of the rhizomes of *H. spicatum* against various diarrhea and dysentery causing drug resistant microorganisms isolated from patients admitted in hospitals. **Methods and Material:** The raw materials of *H. spicatum* were procured from the local supplier. Various Microbial strains included various drug resistant hospital isolates collected and characterized in the Dept. of Pharmaceutical Technology, Jadavpur University, India. **Results**: It was evident from the results that the extract was highly active against *Shigella boydii*, *Shigella. soneii*, *Shigella flexneri*, *B. cereus, Vibrio cholera, E. coli, S. aureus, Ps. aeruginosa and K. pneumoniae*. The result of determination of zone of inhibition was compared with that of standard drug (Ciprofloxacin). This study has pointed to the potential application of *H. spicatum* as a bactericide and fungicide. **Conclusions:** The findings of this study further reinforces the importance of *H. spicatum* rhizomes in traditional healthcare practice and its use in culinary. Further investigation is however needed to isolate and purify the bioactive antimicrobial principles for potential development into generic antimicrobials.

Key words: Bactericide, MIC, Fungicide, Diarrhea. *H. spicatum*.

Ritu Arora*, Avijit Mazumder

Pharmacy Institute Noida Institute of Engineering and Technology, 19, Knowledge Park-II, Greater Noida, Uttar Pradesh 201 306.INDIA.

Correspondence

Ritu Arora

Pharmacy Institute, Noida Institute of Engineering and Technology, 19, Knowledge Park-II, Greater Noida, Uttar Pradesh 201 306, INDIA.

Phone no: 9910291959

E-mail: ritu.wadhwa84@gmail.com

History

- Submission Date: 17-08-2017;
- Review completed: 11-09-2017;
- Accepted Date: 02-11-2017

DOI : **10.5530/pj.2017.6s.159**

Article Available online
http://www.phcogj.com/v9/i6s

INTRODUCTION

H. spicatum (Ham-ex-Smith) is a perennial rhizomatous herb belonging to the family Zingiberaceae. It grows throughout the subtropical Himalaya in the Indian state of Assam, Arunachal Pradesh and Uttarakhand within an altitudinal range of 1000–3000 m.[1,2] *H. spicatum* rhizome is mentioned as Shati in Ayurvedic classics and has been used in various dosage forms to treat cough, wound ulcer, fever, respiratory problems and hiccough. The rhizomes have a strong aromatic odor and bitter taste. In local language, the rhizomes are commonly known as kapur kachari or ban Haldi.[3] Rhizomes are also used as perfume in tobacco and insect repellent. The rhizome extract has been reported to contain essential oil, starch, resins, organic acids, glycosides, albumen and saccharides, which has been advocated for blood purification and treatments of bronchitis, indigestion, eye disease and inflammations.[4,5] Rhizome is reported to contain sitosterol and its glucosides, furanoid diterpene-hedychenone and 7-hydroxyhedychenone and essential oil contains cineole, terpinene, limonene, phellandrene, p-cymene, linalool and terpeneol. The plant rhizomes possess hypoglycemic, vasodialator, spasmolytic, hypotensive, antioxidant properties.[6] Powdered rhizome of *H. spicatum* has been used clinically for the treatment of asthma[7] and tropical pulmonary eosinophilia [8] and as anti-inflammatory and analgesic.[9]

An extensive search of the literature reveals no reports on the antimicrobial activity of the plant. Thus, present investigation was planned to find out the therapeutic level of methanolic extract of H. spicatum plant in antimicrobial activity.

MATERIALS AND METHODS

Plant Material

The raw materials of H. spicatum were procured from the supplier (S.S. Herbal, 485/2, Katra Ishwar Bhavan, Khari Baoli, Delhi) and the sample was identified and authenticated. 100 gm of air dried powdered plant material was extracted with methanol in soxlet apparatus for 96 hrs. After that the extract was filtered and again suspended in the above mixture for 24 hrs. Finally extracts was filtered and concentrated over water bath at a temperature of 40°C. The extract was cooled and kept in desiccator overnight. The extracts was weighed and used for antibacterial and antifungal potentiality.

Cite this article: Arora R, Mazumder A. Phytochemical Screening and antimicrobial activity of rhizomes of *Hedychium spicatum*. Pharmacog J. 2017;9(6)Suppl:s64-s8.

Test microorganisms

The test bacteria used were *Shigella flexneri* type 36 NK 381, *Sh. flexneri* type 6B 999, *Sh. flexneri* type BCH 995, *Sh. boydii* 22461, *Sh. boydii* 16552, *Sh. boydii* 8, *Sh. soneii* BCH 397, *Sh. soneii* E08869, *Sh. soneii* NK 840, *Sh. soneii* BCH 937, *Sh. soneii* I, *Sh. soneii* DN3, *Sh. soneii* F11001, *Sh. soneii* NK 29, *Sh. dysenteriae* 1, *Sh. dysenteriae* 9, *Vibrio cholerae* 1023, *V. cholerae* BD 1/81, *V. cholerae* 1341, *V. cholerae* 452, *V. cholerae* 1033, *V. cholerae* 575, *V. cholerae* 765, *V. cholerae* 1311, *V. cholerae* 756, *V. cholerae* DN6, *V. cholerae* A 26, *Escherichia. coli* AP600, *E. coli* 383, *E. coli* RH 07/12, *E. coli* 18/9, *E. coli* 597, *E. coli* 798, *E. coli* 35B, *E. coli* 306, *E. coli* K88, *E. coli* 872, *Enterobacter* spp. AP596 , *S. typhii* Type 2, *S. aureus* ML 267, *Staphylococcus. aureus* ATCC 6538, *S. aureus* MTCC 96, *S. aureus* 381, *Bacillus. subtilis* MTCC 441, *B. cereus* MTCC 1305, *B. pumilus* 8241, *Pseudomonas putida* MTCC 2252, *P. aeuriginosa* AP585 NLF, *Klebsiella pneumoniae* and *Proteus vulgaris* AP679 NLF. These microbial strains included various drug resistant hospital isolates collected and characterized in the Department of Pharmaceutical Technology, Jadavpur University, India. All strains were maintained on Nutrient Agar (NA) for bacteria and Sabourauds's Dextrose Agar (SDA) slants for fungi at 4°C prior to use for antibacterial and antifungal tests respectively.

Physicochemical Analysis

All parameters were applied on rhizome physicochemical analysis i.e., percentage of ash values and extractive values, were performed according to the official methods prescribed in Indian Pharmacopoeia, 1996 and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines).[10]

Preliminary Phytochemical Screening

Chemical tests were performed in the preliminary phytochemical screening to identify various secondary metabolites such as tannins and phenols, carbohydrates, glycosides, saponins, alkaloids, flavonoids and sterols using standard methods.[11,12]

Determination of MIC by Serial Dilution technique

The rhizome extract (stock solution) was reconstituted with a minimum amount of dimethyl sulfoxide (DMSO). This solvent did not possess any antimicrobial activity of its own. Calculated volumes of this stock solution were dispensed in a series of McCartney bottles previously containing calculated volume of sterile cooled molten nutrient agar media (40-45°C) to prepare final volume of 30 ml each with dilutions of 5, 10, 25, 50, 100, 200, 400, 800 and1000 µg/ml. The stock solution were dispensed into molten SDA to prepare varying dilutions of 100, 200, 400, 800, 1500 and 2000 µg/ml while determining the MIC against the fungi. Then these molten media containing varying concentration of extract were poured aseptically in pre-sterilized Petri dishes (70 mm) to give sterile nutrient agar plates with varying dilution of extract. These plates were then kept in refrigerator at 4°C for 24hrs to ensure uniform diffusion of the extract. Then these plates were dried at 37°C for bacteria and 25°C for fungi for 2 hours before spot inoculations. One loopful (loop diameter: 3mm) of an overnight grown bacterial strain suspension (105 CFU/ml) was added in each quadrant as marked by checker board technique. The spotted plates were incubated at 37°C and 25°C for 24 hours for bacteria and fungi respectively, in an incubator and MIC values were obtained.[13,14,15]

Determination of Mode of Action of the Extract

To determine whether the extract was bacteriostatic or fungistatic and bactericidal or fungicidal in nature, plugs from the zone of inhibition were taken out and reincubated into fresh media which were then examined for their growth after 96 hours incubation at 37°C and 25°C in an incubator, respectively.[15,16]

RESULTS AND DISCUSSION

Physicochemical Studies

Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The alcohol soluble extractive was high in rhizomes of *H. spicatum*. The results of physicochemical constants of the drug powder are presented in (Table 1).

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extract revealed the presence of tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar in it (Table 2).

Table 1: Physicochemical Studies of *H. spicatum*

Parameter	Result
Ash value	6.9%
Acid insoluble value	1.03% w/v
Water soluble Ash	2.61% w/v
Water insoluble Ash	2.10 w/v
Moisture content	5.25%w/w
Extractive value(water soluble)	13.9 w/v
Extractive Value (alcohol soluble)	5.24 w/v
pH of 1% suspension	5.41 w/v

Table 2: Physicochemical Constants

Phytoconstituents	Petroleum Ether	Chlorofom	Acetic acid	Acetone	Methanol	Aqueous	Benzene
Alkaloids	_	_	+	_	+	+	-
Carbohydrate	_	_	+	+	+	+	+
Flavonoids	+	–	-	+	+	+	-
Gums& Mucilage	–	–	-	+	–	+	+
Glycosides	+	_	-	–	+	+	-
Phenolic compounds	–	+	-	+	_	_	-
Protein	+	+	-	+	+	+	+
Amino acid	_	_	-	+	_	_	+
Saponins	+	–	+	+	+	+	-
Steroids	+	+	-	–	+	-	-
Triterpenes	–	–	-	–	–	–	-
Tannins	–	+	-	–	+	+	+

Table 3: Determination of MIC of the rhizomes extracts of *H. spicatum* against different bacterial strains:

Name of M/O	0	5	10	25	50	100	200	400	600	800	1000
Shigella flexneri type 36 NK 381	+	+	+	+	+	+	-	-	-	-	-
Sh. flexneri type BCH 995	+	+	+	±	-	-	-	-	-	-	-
Sh. flexneri type 6BCH 999	+	+	±	±	-	-	-	-	-	-	-
Sh. boydii 22461	+	+	+	+	+	+	+	+	-	-	-
Sh. boydii 16552	+	+	+	+	+	+	±	±	±	-	-
Sh. boydii 8	+	+	+	±	±	±	±	±	-	-	-
Sh. sonnei BCH 397	+	+	+	+	+	+	+	-	-	-	-
Sh. sonnei E08869	+	+	+	+	+	+	-	-	-	-	-
Sh. sonnei NK 840	+	+	+	±	±	±	±	-	-	-	-
Sh. sonnei BCH 937	+	+	+	+	+	+	±	±	±	±	-
Sh. sonnei 1	+	+	+	±	±	±	-	-	-	-	-
Sh. sonnei DN3	+	+	+	+	+	+	+			-	+
Sh. sonnei F11001	+	+	+	+	+	-	-	-	-	-	-
Sh. sonnei NK 29	+	+	+	+	+	+	+	-	-	-	-
Sh. dysenteriae 1	+	+	+	+	+	+	+	±	-	-	-
Sh. dysenteriae 9	+	+	+	+	+	+	+	+	+	±	±
Vibrio cholerae 1023	+	+	+	+	+	+	-	-	-	-	-
V. cholerae BD 1/81	+	+	+	+	±	±	±	-	-	-	-
V. cholerae 1341	+	±	±	-	-	-	-	-	-	-	-
V. cholerae 452	+	+	+	+	+	+	+	+	+	+	+
V. cholerae 1033	+	+	+	+	+	+	+	+	-	-	-
V. cholerae 575	+	+	+	+	+	+	-	-	-	-	-
V. cholerae 765	+	+	+	+	+	+	+	±	-	-	-
V. cholerae 1311	+	+	+	+	+	+	-	-	-	-	-
V. cholerae 756	+	+	+	±	±	-	-	-	-	-	-
V. cholerae DN6	+	+	+	+	+	+	+	+	+	+	+
V. cholerae A 26	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli AP600	+	+	+	+	+	+	+	+	+	+	+
E. coli 383	+	+	+	+	+	+	+	+	+	+	+
E. coli RH 07/12	+	+	+	+	+	+	+	±	-	-	-
E. coli 18/9	+	+	+	-	-	-	-	-	-	-	-
E. coli 597	+	+	+	+	+	±	-	-		-	-
E. coli 798	+	+	+	+	+	±	-	-	-	-	-
E. coli 35B	+	+	+	+	±	-	-	-	-	-	-
E. coli 306	+	+	+	+	+	±	-	-	-	-	-
E. coli K88	+	+	+	+	+	+	±	±	±	-	-
E. coli 872	+	+	+	+	+	±	-	-	-	-	-
Proteus vulgaris AP679 NLF	+	+	+	+	±	±	-	-	-	-	-
Pseudomonas putida MTCC 2252	+	+	+	+	+	±	+	-	-	-	-
Ps. aeruginosa AP585 NLF	+	+	+	±	±	±	-	-	-	-	-
Enterobacter spp. AP596	+	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae	+	+	+	+	±	±	±	±	±	-	-
Salmonella typhii Type 2	+	+	±	-	-	-	-	-	-	-	-
Staphylococcus aureus ML 267	+	+	+	+	+	+	+	±	-	-	-
S. aureus ATCC 6538	+	+	+	±	±	±	±	±	-	-	-
S. aureus MTCC 96	+	+	+	+	±	±	-	-	-	-	-
S. aureus 381	+	+	±	±	-	-	-	-	-	-	-
Bacillus subtilis MTCC 441	+	+	+	+	+	+	±	-	-	-	-
B. cereus MTCC 1305	+	+	+	+	+	+	-	-	-	-	-
B. pumilus 8241	+	+	+	+	+	±	±	±	±	±	-

Table 4: Determination of diameter of zone of inhibition (in mm) produced by the methanolic extract of the rhizomes of *H. spicatum* and its comparison with that of Ciprofloxacin against selected sensitive bacterial strains*

NAME OF BACTERIA	ZONE OF INHIBITION PRODUCED BY EXTRACT OF H. spicatum					ZONE OF INHIBITION PRODUCED BY Ciprofloxacin				
	200 µg/ml	400 µg/ml	800 µg/ml	1000 µg/ml	1200 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	1000 µg/ml	1200 µg/ml
Pseudomonas putida MTCC 2252	6.5	9.0	11.5	15.0	17.5	10	12	14	18	20
Ps. aeruginosa AP585 NLF	6.5	7.0	7.5	8.0	8.5	11.5	12.5	14	16	18
V. cholera 1023	5.5	6.5	8.5	9	15	12	21	24	28	30
V. cholera 1341	4.5	6.5	7	11	18	11	19	21	27	29
V. cholerae 1311	6.0	7.7	8	10.5	16	12	18	22	26	28
V. cholerae 575	4	5	6	9	15	10	15	21	30	32
V. cholerae 756	9.0	11.5	13.5	14.5	16	9	9.5	11	13	16
E. coli 18/9	8.4	9.0	9.5	10.0	10.5	11.5	12.0	12.5	13.0	13.5
E. coli 798	8.5	9.0	10.0	11.5	12.5	11.5	12.5	13.0	13.5	14.5
E. coli 872	6.5	6.5	8.0	10.0	13.5	11	17	21	28	29
E. coli 358	6.5	8.0	9.5	13.0	18.5	11	20	24	26	28
Enterobacter spp. AP596	7.0	7.5	9.5	10.5	11.0	9.0	9.5	11.5	12.0	13.5
Proteus vulgaris AP679 NLF	7.5	8.5	10.0	11.5	12.0	10	13	15	16.5	18.0
Salmonella typhii Type 2	8.0	8.5	10.0	10.5	11.0	11.5	12.0	13.5	14.5	15
Bacillus subtilis MTCC 441	6.0	7.5	8.0	9.0	10	11	12.5	13.5	14.5	16
B. subtilis MTCC441	8.0	8.5	11.5	14.5	18.5	10	18	20	27	32
Staphylococcus aureus 381	7.5	9.0	11.0	12.5	13.0	9.5	11.0	12.5	14.0	14.5
Shigella flexneri type 36 NK 381	6.5	7.0	7.5	8.5	9.0	10.0	10.5	11.0	11.5	12.5
Sh.soneii F11001	9.5	10.2	11.8	13.5.	15.0	17	25	29	34	31
Sh. flexneri type BCH 995	9.5	10.5	12.0	13.0	13.8	12.0	12.5	13.4	14.0	14.7

(* Average of two plates)

Antibacterial Activity

The result in Table 3 depicted the MIC values of the methanolic extract of the rhizomes of *H. spicatum* against various tested bacterial pathogens.

It was evident from the results shown in the Table 3 that the extract was highly active against *Shigella boydii*, *Sh. soneii*, *Shigella flexneri, B. cereus, Vibrio cholera*, *E. coli*, *S. aureus*, *Ps. aeruginosa* and *K. pneumoniae*. The result of determination of zone of inhibition of the crude extract of the rhizomes of the plant and their comparison with those of standard antibacterial agent Ciprofloxacin against the tested bacterial strains is recorded in Table 4.

The sensitivity pattern of the bacterial organisms to the extract was found to decrease in the following order: *E. coli* 358, *B. subtilis MTCC441, V. cholera 1341, Pseudomonas putida* MTCC 2252, *V. cholerae 1311, V. cholerae 756, V. cholerae 575, V. cholera 1023, E. coli 872, E. coli* 798, *E. coli* 18/9, *Ps. aeruginosa AP585 NLF*, as evident from table 5.

CONCLUSION

The pharmacognostical and phytochemical evaluation of *H. spicatum* (Zingiberaceae)) rhizome provided useful information for identification and authentication of the plant. The antibacterial study of the methanolic extract of the rhizomes of *H. spicatum* showed the maximum activity against *Shigella boydii*, *Sh. soneii*, *Shigella flexneri, B. cereus, Vibrio cholera*, E. *coli, S. aureus, Ps. aeruginosa and K. pneumoniae*. The results of phytochemical analysis and antimicrobial activity studies of the plants extracts confirmed its therapeutic usage, as depicted in the literature. The active plant extract may be further subjected to biological and pharmacological investigations for isolation of antibacterial and therapeutic compounds.

ACKNOWLEDGEMENT

The authors are grateful to extend special thanks to Dr. G. S. Chakraborthy, Pharmacy Institute Noida Institute of Engineering and Technology, Greater Noida, for his constant encouragement & support throughout the work.

CONFLICTING INTEREST

The authors are declared no conflict of interest.

ABBREVIATION USED

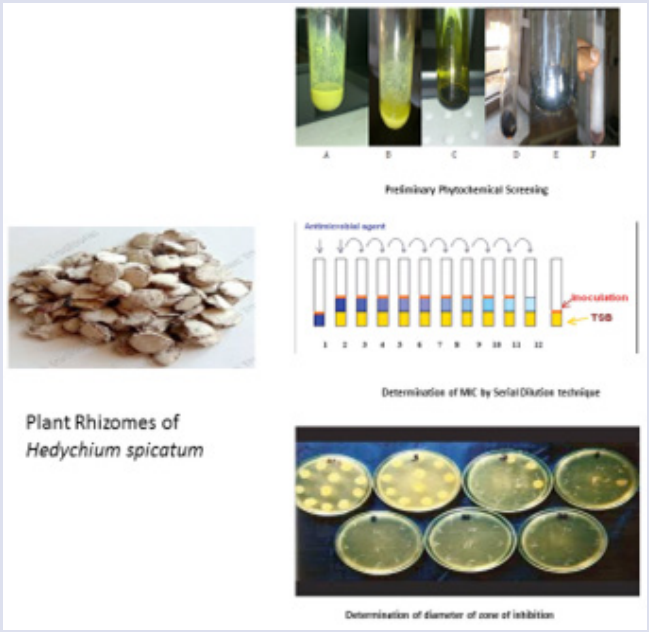
H. spicatum: *Hedychium spicatum;* ***(B.):*** *Bacillus;* ***(S.):*** *Staphylococcus;* ***(P.):*** *Pseudomonas;* ***(E.):*** *Escherichia;* ***(V.);*** *Vibrio.*

REFERENCES

1. Samant SS, Dhar U. Diversity, endemism and economic potential of wild edible plants of Indian Himalaya. Int J Sust Dev World 1997;4(1):79-91.
2. Thakur RS, Puri HS, Hussain A. Major medicinal plants of India. CIMAP Publication, Lucknow; 1989. p. 50-2.
3. Chopra RN, Nayar SL, Chopra LC. Glossary of Indian medicinal plants, New Delhi: CSIR Publication; 1956. p.13-131.
4. Srimal RC, Sharma SC, Tandon JS. Anti-inflammatory and other pharmacological effects of *Hedychium spicatum* (Buch-Hem). Indian J Pharmacol 1984;16(3):143–7.

5. Sravani T, Paarakh MP. *Hedychium spicatum* Buch. Ham.-An overview. Pharmacologyonline 2011;2:633–42.
6. Giri D, Tamta S, Pandey A. A review account on medicinal value of *Hedychium spicatum* Buch-Ham ex Sm. Vulnerable medicinal plant. J. Med. Plants Res 2010; 4: 2773–7.
7. Chaturvedi GN, Sharma BD. Clinical studies on *Hedychium spicatum* (Shati): An antiasthmatic drug. J res indian med. 1975;10(2):941.
8. Sahu RB. Clinical trial of *Hedychium spicatum* in tropical pulmonary eosinophilia. Journal of Nepal Pharmaceutical Association, 1979;7:65–72.
9. Tandon SK, Chandra S, Gupta S, J. Lal: Analgesic and anti-inflammatory effects of *Hedychium spicatum*. Indian J. Pharm. Sci 1997;59(3):148–50.
10. WHO/QCMMPM. Quality Control Methods for Medicinal Plant Material, Organisation Mondiale De La Sante, Geneva; 1992. p.22-34.
11. Khandelwal KR. Practical Pharmacognosy. 7th Ed. CBS publishers and distributors, 2004;149-56.
12. Kokate CK. Practical Pharmacognosy, 4th Ed. Vol. I, Vallabh Prakashan; 2005.p. 16.
13. Mazumder R, Mendiratta T, Mandal SC, Mazumder A. Antimicrobial potency of the leaf stalk extract of *Curcuma longa* Roxb. Anc Sci Life 2000;(1-2): 92-6.
14. Mazumder A, Singh SK, Mazumder R, Basu SP, Saha BP. Antimicrobial action of the leaf extract of *Lagerstroemia parviflora* Roxb. Anc Sci Life 2001; XXI (3):198-201.
15. Karuba RM, Siboe GM and Dossaji SF. Antifungal activity of *Schizozygia coffaeoids* Bail. (Apocynaceae) extract. J Ethnopharmacol 2001;74(1):41-4.

GRAPHICAL ABSTRACT



SUMMARY

- *Hedychium spicatum* is a good source for flavonoid compounds.
- Methanolic extract had shown strong positivity in preliminary phytochemical screening.
- Antimicrobial activity performed by determining its MIC (by checker board method) and zone of inhibition (by cup plate method)
- Antibacterial study of the methanolic extract of the rhizomes of *H. spicatum* showed the maximum activity against *Shigella boydii, Sh. soneii, Shigella flexneri, B. cereus, Vibrio cholera, E. coli, S. aureus, Ps. aeruginosa* and *K. pneumoniae.*

ABOUT AUTHORS

Ritu Arora: Is a Research Scholar at Dr. Abdul Kalam University, Lucknow. Her research is focused on Phytochemical analysis of plants and herbal products, pharmacological screening of plant and plant products.

Dr Avijit Mazumder: Is working as Professor and Director in the NIET Pharmacy Institute in Noida Institute of Engineering and Technology since October 2009. He had a total of more than one hundred eighty national and international publications to his credit. He had contributed eight book chapters and is the author of three books of repute.

Cite this article: Arora R, Mazumder A. Phytochemical Screening and antimicrobial activity of rhizomes of *Hedychium spicatum*. Pharmacog J. 2017;9(6)Suppl:s64-s8.