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In vitro Antimicrobial Activity of Leaf and Rhizome Extracts of *Hedychium flavescens*

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ABSTRACT: *Hedychium flavescens* or yellow ginger has innumerable medicinal uses in folk medicine mostly for the treatment of respiratory, skin, digestive, bone and joint diseases. We are reporting for the first time the antibacterial and antifungal activities of chloroform, acetone, methanol and aqueous extracts from the leaves and rhizomes of *H. flavescens*. Antimicrobial drugs are associated with side effects like hives, gastrointestinal effects, fatigue, secondary infections and may also lead to antibiotic resistance. Some plants which are rich in a wide variety of secondary metabolites have traditionally been used as antimicrobial agents and they also show minimal to no side effects. In this study it was seen that the leaf extracts showed better antimicrobial activity than the rhizome extracts. The leaf extracts showed strong activity against *Escherichia coli, Propionibacterium acnes, Streptococcus mutans, Streptococcus pyogenes, Aspergillus flavus, Aspergillus niger, Aspergillus tubigenesis* and *Sporothrix schenckii*. The rhizome extract showed significant activity against *Bacillus subtilis, Propionibacterium acnes, Pseudomonas aeruginosa, Aspergillus ustus, Cryptococcus neoformans* and *Sporothrix schenckii.* The extracts showed significant inhibition against *E. coli* as well as *P. aeruginosa,* thus indicating their broad spectrum of activity. Acetone, chloroform and methanol extracts of both leaf and rhizome showed better results compared to the aqueous extract. This study proves that the leaf and rhizome extracts of *H. flavescens* have significant antimicrobial activity and can thus be used as an antimicrobial agent.

Keywords: *Hedychium flavescens*, Antimicrobial , Antibacterial, Antifungal, Zingiberaceae, Medicinal plant.

INTRODUCTION

There has been renewed interest in screening high plants for novel biologically active compounds, particularly those that effectively intervene the human ailments (Malaviya and Mishra 2011). Zingiberaceae, commonly known as ginger family, comprises of a number of plants that show significant antimicrobial activities. Documentation of 23 ethnomedicinal plants belonging to Zingiberaceae family was done by Dalisay *et al.* (2018) which showed that 16 species were traditionally used by local folks and herbal healers of the Antique province to alleviate and cure ailments.

Hedychium is a genus of rhizomatous perennial flowering plants in the family Zingiberaceae, commonly growing 120–180 cm (47–71 in) tall and native to lightly wooded habitats in India, Southeast Asia and Madagascar. There are approximately 70-80 known species of *Hedychium. Hedychium flavescens* N. Carey ex Roscoe, commonly known as cream garland-lilyor yellow ginger is a coarse perennial herb with thick fleshy rhizomes and erect, leafy pseudostems of 1-3 m in height (CABI, 2022). It is native to the eastern Himalayas, including Nepal and north-eastern India (CABI, 2022). In India it is found at altitudes of between 1200 metres and 2000 metres in the states of Assam, Meghalaya, Sikkim, Kerala and Tamil Nadu.

The yellow ginger's rhizome has numerous medicinal uses in traditional medicine for the treatment of tonsilitis (Staples and Herbst 2005), bronchitis, throat swellings, chest congestion, cough, asthma, abdominal swellings, colic, hemorrhoids, various skin infections (Singh and Sharma 2018), fractures, stomach ache,back pain (Nurainas *et al.*, 2021), gastritis (Kom *et al.*, 2018), infected nostrils and body ache. Tribal groups of Bijar in India used its rhizome as febrifuge, antirheumatic, tonic and stimulant (Raphael and Madhavan 2013). Its flower buds are edible and can be used like a vegetable or in tea to add the unique ginger fragrance.

Phytochemical screening of *Hedychium flavescens*

rhizome yielded carbohydrates, starch, sugar, protein, cardiac glycoside, phenols, saponins, alkaloids, tannin, phlobatannin, and terpenoids and of flowers yielded carbohydrates, ketose protein, phenols, saponins, and terpenoids (Raphael and Madhavan 2013). GC-MS study of theessential oil of its rhizome showed monoterpene ß-pinene (43.6%) as the most abundant constituent while the number of sesquiterpenes and their derivatives in its rhizome oils were only 0.6% (Sabulal *et al.*, 2007). In the same study *H. flavescens* rhizome oil showed the strongest activity against *Salmonella typhi* (inhibition zone 23 mm against 12 mm of control), *Escherichia coli* (18/8 mm) and *Proteus vulgaris* (15/9 mm). It also showed strong activity against the fungi *Candida albicans* (13/8 mm) and *C. glabrata* (14/8 mm) (Sabulal *et al*., 2007).

This article reports about the antibacterial and antifungal activities of chloroform, acetone, methanol and aqueous extracts from the leaves and rhizomes of *Hedychium flavescens*. This is the first time report on the antibacterial and antifungal activities of leaf and rhizome extracts from *H. flavescens.*

MATERIALS AND METHODS

A. Collection and Authentication

The plant material was collected from Moolayar stream, Palani hills, Dindigul district, Tamil Nadu and authenticated by Dr. S. John Britto S.J. at the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli. The voucher specimen (RHT 68885) was deposited for future references.

B. Extraction

The leaves and rhizomes were washed with water and wiped with paper towels and then shade dried. They were then powdered using mechanical grinder and stored in air tight containers. The leaf and rhizome samples were immersed in different solvents such as Chloroform (CH), Acetone (AC), Methanol (ME) and Aqueous (AQ) for extraction and kept in shaker for 72 hours at room temperature. The extracts were filtered using muslin cloth. The extracts were concentrated in rotary evaporated. Dark, gummy solid extracts obtained were dissolved in dimethyl sulfoxide (DMSO) to make a concentration of 50 µg/µl and used for antibacterial and antifungal analysis.

C. Anti-bacterial Activity

Test Micro-Organisms. 10 bacterial strains were used in the study namely *Bacillus subtilis* (MTCC 441), *Corynebacterium diphtheria* (MTCC 116), *Escherichia coli* (MTCC 443), *Propionibacterium acnes* (MTCC 1951), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 902), *Streptococcus faecalis* (MTCC 439), *Streptococcus mutans* (MTCC 21575) and *Streptococcus pyogenes* (MTCC 1928). These pathogenic micro-organisms were purchased from MTCC, Chandigarh, India. All the test bacterial strains were maintained on nutrient agar media at 4°C. Nutrient agar medium, nutrient broth and Gentamicin antibiotic solution was purchased from Himedia, India.

Preparation of Disc. 6 mm discs were prepared and sterilized in autoclave. 62 µl of the different extracts – Chloroform, Acetone, Methanol and Distilled water were added to the discs. Gentamicin antibiotic was used as a positive control (PC).

Determination of Antibacterial Activity. Antibacterial activities of *Hedychium flavescens* extracts were determined by disc diffusion method as described by Bauer *et al.* (1996). The plates were prepared by pouring 20 ml of molten media into sterile petriplates. The plates were allowed to solidify. Each plate of nutrient agar was swabbed with different bacterial strain using sterile cotton swab. The soaked dried discs were placed on the surface of each inoculated plate. The plates were allowed for diffusion for half an hour and then transferred to incubator at 37˚C for 24 hours. Standard disc of Gentamicin was also placed as positive control. For the negative control 62 µl of the DMSO was added to disc. The antibacterial activity of *Hedychium flavescens* leaf and rhizome extracts was determined by measuring the diameter of the zone of inhibition using a transparent ruler in millimeter and recorded as mean ± standard deviation. The experiments were conducted in triplicate. All statistical analyses were calculated using Graph Pad Prism 6.0 software (USA). One-way ANOVA and two-tailed unpaired t-test were utilized to determine the significant difference in antibacterial activity. P value < 0.05 was determined as significant.

D. Antifungal activity

Fungal strains. *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Aspergillus tubigenesis, Aspergillus ustus, Candida albicans, Cryptococcus neoformans, Phialophora verrucosa* and *Sporothrix schenckii* were the fungal strains used for the antifungal analysis.

Determination of antifungal activity. The agar well diffusion method as described by Perez *et al.* (1990) was modified to conduct the antifungal study. Petri plates containing 20ml potato dextrose agar medium were seeded with 72 hours culture of fungal strains. Wells were cut using a sterile Cork Borer and 100µl of the different extracts CH, AC, ME and AQ of leaf and rhizome samples were added into the well. Amphotericin B was used as a positive control. For the negative control 100µl of the DMSO was added into the wells. The plates were then incubated at 28°C for 72 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well and recorded as the mean ± standard deviation. The experiments were conducted in triplicate. All statistical analyses were calculated using Graph Pad Prism 6.0 software (USA). One-way ANOVA and two-tailed unpaired t-test were utilized to determine the significant difference in antifungal activity. P value < 0.05 was determined as significant.

RESULTS AND DISCUSSION

In this study, the antimicrobial activity of the chloroform, acetone, methanol and aqueous extracts of the leaves and rhizomes of *H. flavescens* was studied using bacteria *Bacillus subtilis, Corynebacterium*

diphtheria, Escherichia Coli, Propionibacterium acnes, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis, Streptococcus mutans and *Streptococcus pyogenes* and fungi *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Aspergillus tubigenesis, Aspergillus ustus, Candida albicans, Cryptococcus neoformans, Phialophora verrucosa* and *Sporothrix schenckii* (Table 1-4). A concentration of 50 µg/µl was used in both *in vitro* antibacterial and *in vitro* antifungal studies.

It was seen that the inhibition displayed by the leaf extracts of concentrations 50 µg/µl was higher than the inhibition showed by the positive control in some cases like against *Escherichia coli* (18.5±0.7 mm), *Propionibacterium acnes* (14.5±0.7 mm) and *Streptococcus mutans* (14.5±0.7 mm) (Fig. 1). Overall the leaf extracts showed better antimicrobial activity than the rhizome extracts (Fig. 1-4). The most susceptible organisms to the leaf extract were found to be *Escherichia Coli, Propionibacterium acnes, Staphylococcus aureus, Streptococcus faecalis, Streptococcus mutans, Streptococcus pyogenes, Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Aspergillus tubigenesis* and *Sporothrix schenckii* (Fig. 1, 3). The least susceptible organisms to leaf extracts were *Bacillus subtilis* and *Proteus vulgaris* (Fig. 1, 3).

The most susceptible organisms to the rhizome extract were found to be *Bacillus subtilis, Escherichia coli, Propionibacterium acnes, Pseudomonas aeruginosa, Streptococcus mutans, Aspergillus flavus, Aspergillus niger, Aspergillus ustus, Candida albicans, Cryptococcus neoformans* and *Sporothrix schenckii* (Fig. 2, 4). The least susceptible organisms to rhizome extracts were *Corynebacterium diphtheria, Proteus vulgaris, Staphylococcus aureus, Streptococcus faecalis, Aspergillus fumigatus* and *Phialophora verrucosa* (Fig. 2, 4).

Table 1: Antibacterial activity of leaf extracts of *Hedychium flavescens.*

Sr. No.	Name of the test organism	Zone of inhibition (mm)				
		CH	AC	ME	AQ	PC
1.	*Bacillus subtilis.*	5.5±0.7	4.25±0.35	4.25±0.35	4.5±0.7	13.25±0.35
2.	*Corynebacterium diphtheria*	5.5±0.7	6.25±0.35	9.25±0.35	7.5±0.7	12.5±0.7
3.	*Escherichia coli*	15.25±0.35	18.25±0.35	14.25±0.35	18.5±0.7	17.5±0.7
4.	*Propionibacterium acnes*	12.75±1.06	5.5±0.7	14.5±0.7	7.5±0.7	14.5±0.7
5.	*Proteus vulgaris*	5.25±0.35	3.25±0.35	4.5±0.7	4.5±0.7	12.5±0.7
6.	*Pseudomonas aeruginosa*	5.5±0.7	7.25±0.35	4.25±0.35	5.25±0.35	12.25±0.35
7.	*Staphylococcus aureus*	7.5±0.7	8.25±0.35	9.25±0.35	5.25±0.35	12.25±0.35
8.	*Streptococcus faecalis*	9.5±0.7	7.5±0.7	4.5±0.7	5.5±0.7	13.5±0.7
9.	*Streptococcus mutans*	5.5±0.7	5.25±0.35	14.5±0.7	7.5±0.7	13.5±0.7
10.	*Streptococcus pyogenes*	15.25±0.35	16.25±0.35	11.25±0.35	14.25±0.35	17.25±0.35

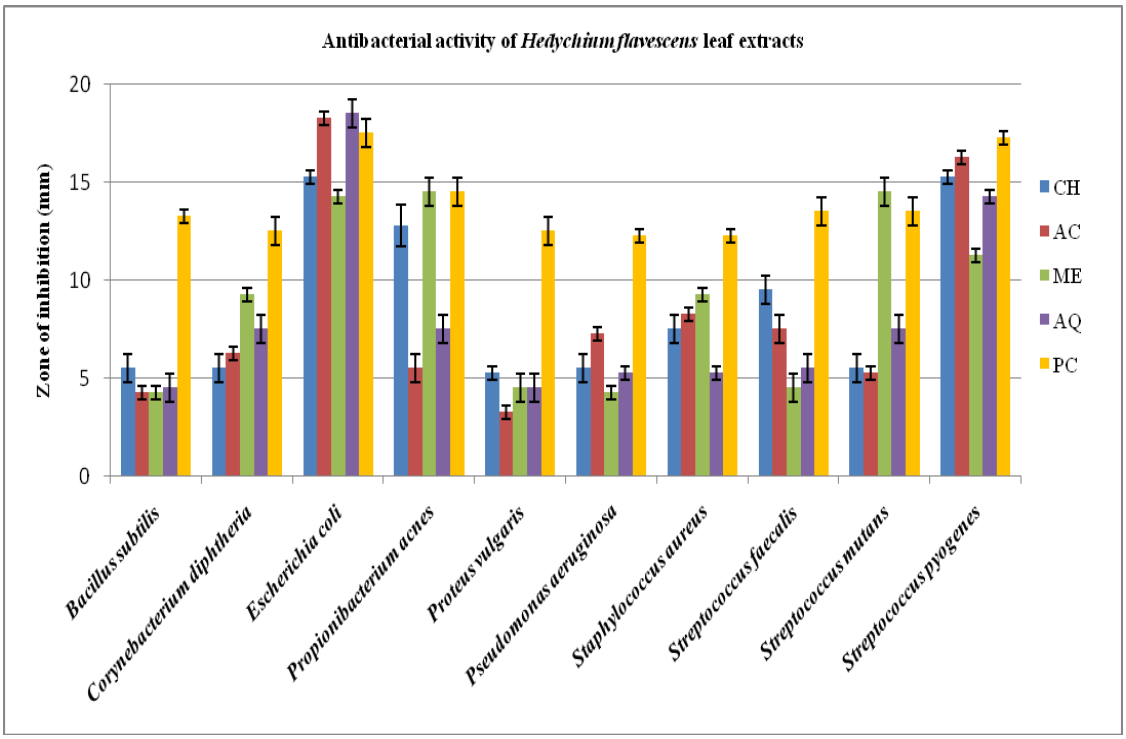

Fig. 1. Antibacterial activity of leaf extracts of *Hedychium flavescens.*

Table 2: Antibacterial activity of rhizome extracts of *Hedychium flavescens.*

Sr. No.	Name of the test organism	Zone of inhibition (mm)				
		CH	AC	ME	AQ	PC
1.	*Bacillus subtilis*	13.5±0.7	11.5±0.7	6.5±0.7	10.25±0.35	15.5±0.7
2.	*Corynebacterium diphtheria*	0	3.25±0.7	3.5±0.7	4.25±0.35	11.5±0.7
3.	*Escherichia coli*	7.5±0.7	8.25±0.35	9.25±0.35	0	15.25±0.35
4.	*Propionibacterium acnes*	4.5±0.7	4.5±0.7	4.25±0.35	0	6.5±0.7
5.	*Proteus vulgaris*	3.5±0.7	3.25±0.35	5.5±0.7	4.25±0.7	12.75±1.06
6.	*Pseudomonas aeruginosa*	5.5±0.7	5.25±0.35	3.25±0.35	3.5±0.7	6.5±0.7
7.	*Staphylococcus aureus*	0	3.25±0.35	3.25±0.35	0	15.5±0.7
8.	*Streptococcus faecalis*	6.5±0.7	3.5±0.7	5.25±0.35	4.25±0.35	14.5±0.7
9.	*Streptococcus mutans*	9.5±0.7	10.5±0.7	10.25±0.35	9.25±0.35	13.25±0.35
10.	*Streptococcus pyogenes*	10.5±0.7	11.25±0.35	9.5±0.7	4.25±0.35	30.5±0.7

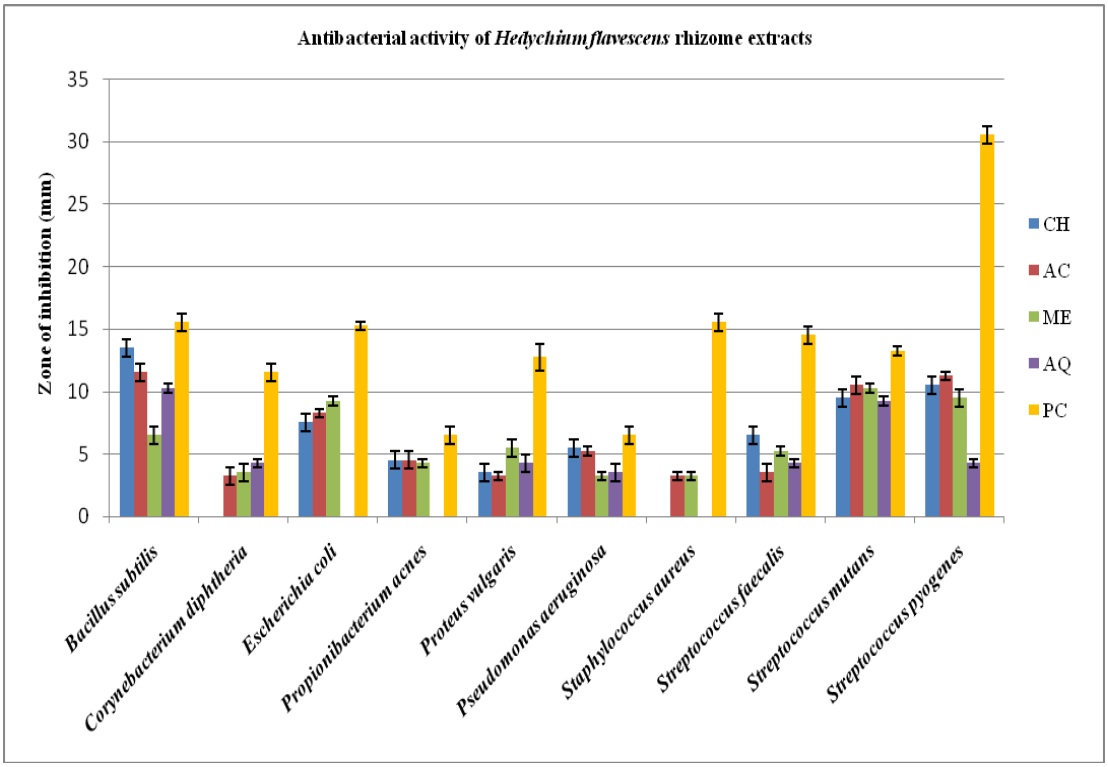

Fig. 2. Antibacterial activity of rhizome extracts of *Hedychium flavescens.*

Table 3: Antifungal activity of leaf extracts of *Hedychium flavescens.*

Sr. No.	Name of the test organism	Zone of inhibition (mm)				
		CH	AC	ME	AQ	PC
1.	*Aspergillus flavus*	9.5±0.35	15.5±0.7	13.25±0.35	12.25±0.35	16.75±1.06
2.	*Aspergillus fumigatus*	9.25±0.35	12.25±0.35	14.25±0.35	14.5±0.7	17.5±0.7
3.	*Aspergillus niger*	9.25±0.35	13.25±0.35	12.25±0.35	11.25±0.35	14.25±0.35
4.	*Aspergillus tubigenesis*	7.5±0.7	12.25±0.35	12.5±0.7	10.5±0.7	13.5±0.7
5.	*Aspergillus ustus*	5.5±0.7	12.25±0.35	9.5±0.7	6.5±0.7	16.75±1.06
6.	*Candida albicans*	7.5±0.7	10.25±0.35	10.5±0.7	5.5±0.7	15.25±0.35
7.	*Cryptococcus neoformans*	5.5±0.7	10.5±0.7	7.5±0.7	9.5±0.7	16.25±0.35
8.	*Phialophora verrucosa*	5.5±0.7	13.5±0.7	12.5±0.7	4.5±0.7	20.5±0.7
9.	*Sporothrix schenckii*	17.25±0.35	10.5±0.7	9.25±0.35	12.5±0.7	17.5±0.7

Acetone, chloroform and methanol extracts of both leaf and rhizome showed better activity compared to the aqueous extract (Table 1-4). Aqueous extract of rhizome showed no activity against *Escherichia coli, Propionibacterium acnes, Staphylococcus aureus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cryptococcus neoformans, Phialophora verrucosa* and *Sporothrix schenckii* (Table 2, 4). Similarly, chloroform extract of the rhizome did not produce significant zones of inhibition on *Corynebacterium diphtheria, Staphylococcus aureus* and *Phialophora verrucosa* (Table 2, 4). The methanol extract of the rhizome failed to inhibit growth of fungi *Aspergillus tubigenesis* and *Phialophora verrucosa* (Table 4).

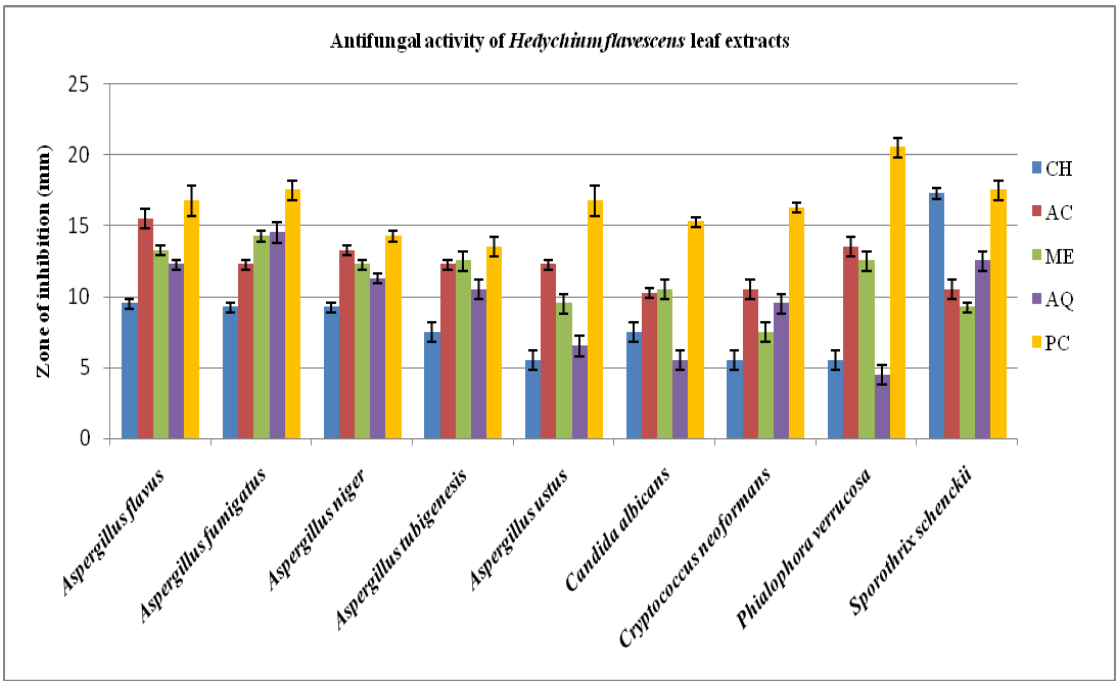

Fig. 3. Antifungal activity of leaf extracts of *Hedychium flavescens.*

Table 4: Antifungal activity of rhizome extracts of *Hedychium flavescens.*

Sr. No.	Name of the test organism	Zone of inhibition (mm)				
		CH	AC	ME	AQ	PC
1.	*Aspergillus flavus*	7.5±0.7	4.5±0.7	8.25±0.35	0	11.75±1.06
2.	*Aspergillus fumigatus*	5.5±0.7	5.25±0.35	5.25±0.35	0	16.5±0.7
3.	*Aspergillus niger*	5.5±0.7	4.5±0.7	6.5±0.7	0	9.5±0.7
4.	*Aspergillus tubigenesis*	7.5±0.7	6.5±0.7	0	4.5±0.7	13.5±0.7
5.	*Aspergillus ustus*	12.25±0.35	6.5±0.7	11.5±0.7	4.5±0.7	12.25±0.35
6.	*Candida albicans*	7.5±0.7	8.25±0.35	5.5±0.7	6.5±0.7	10.75±1.06
7.	*Cryptococcus neoformans*	11.25±0.35	12.25±0.35	14.25±0.35	0	13.25±0.35
8.	*Phialophora verrucosa*	0	5.5±0.7	0	0	14.5±0.7
9.	*Sporothrix schenckii*	10.25±0.35	11.25±0.35	9.5±0.7	0	12.25±0.35

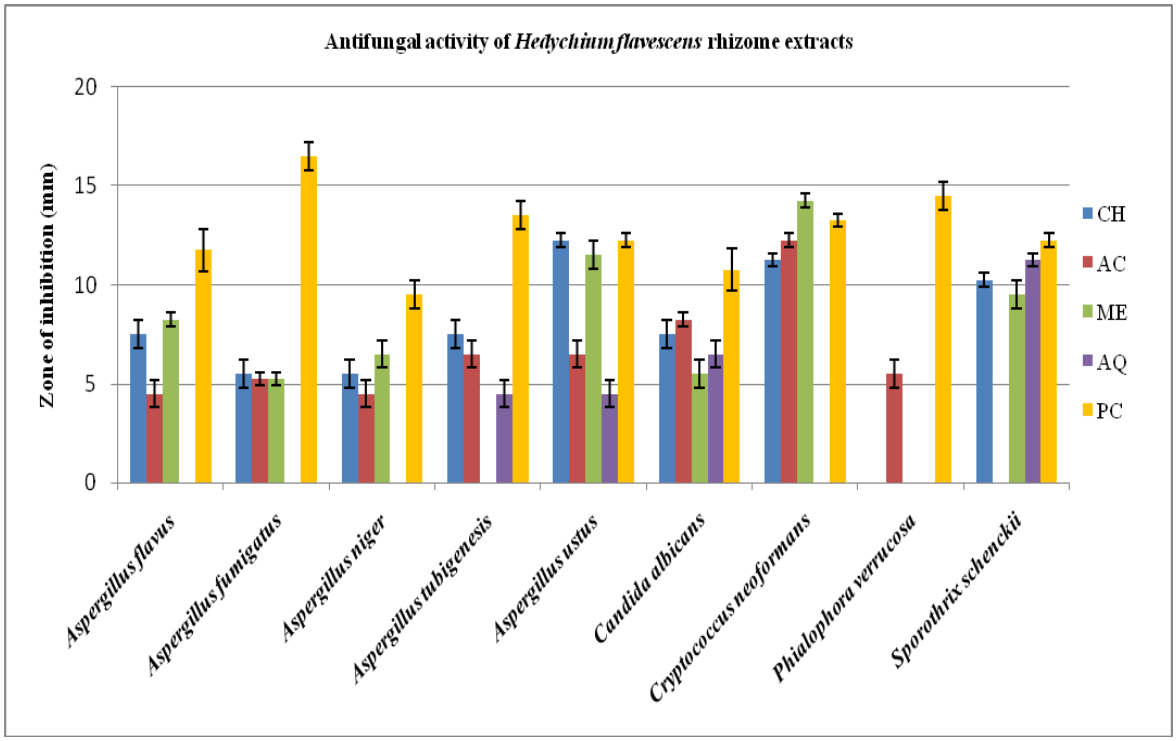

Fig. 4. Antifungal activity of rhizome extracts of *Hedychium flavescens.*

CONCLUSIONS

Antibiotics, the antidote to various bacterial infections, have greatly enhanced the quality of human life. But over the past few years, antibiotics have become less effective against certain diseases due to emergence of drug resistant bacteria. Besides some antibiotics can cause severe side effects. However, plant products have been used as powerful remedy against pathogenic microbes since ancient times. Therefore, antimicrobial screening of plant extracts and testing their antimicrobial capacity would lead to safer and effective antimicrobial drug discovery.

Earlier studies on *Hedychium flavescens* have reported that the essential oil of the rhizomes had high content of monoterpene β-pinene and the oil showed strong activity against bacteria *Salmonella typhi, Escherichia coli, Proteus vulgaris, Staphylococcus aureus* and *Bacillus subtilis* and *fungi Candida albicans* and *C. glabrata* (Sabulal *et al.*, 2007; Suksathan *et al.*, 2013).

In our investigation, for the first time we have documented the *in vitro* antimicrobial activity of the leaf and rhizome extracts of *Hedychium flavescens.* This study proves that the leaf and rhizome extracts of *H. flavescens* have significant antimicrobial activity especially the acetone, chloroform and methanol extracts. The extracts showed significant inhibition against *E. coli* as well as *P. aeruginosa* thus indicating their broad spectrum of activity. This study thus reveals that *H. flavescens* may serve as a natural alternative source of medicine for the treatment of microbial infections. The bacteria and fungi used in this study typically cause respiratory, skin, digestive, bone and joint infections. On this account, this report validates the traditional use of *Hedychium flavescens* for the treatment of tonsillitis (Staples and Herbst 2005), bronchitis, throat swellings, chest congestion, cough, asthma, abdominal swellings, various skin infections (Singh and Sharma 2018), stomach ache (Nurainas *et al.*, 2021), gastritis (Kom *et al.*, 2018), infected nostrils and arthritis (Raphael and Madhavan 2013).

FUTURE SCOPE

The antibacterial and antifungal activities of *Hedychium flavescens* reported in the present study may confirm its therapeutic use for its combating abilities against broad spectrum of microbes. Further study is required to evaluate the toxicity of these extracts. Additional research has to be conducted to identify the active compounds responsible for the microbial activity and the exact mode of action by which these extracts exert their antimicrobial effect.

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Conflict of Interest. None.

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