ANTI-FUNGAL ACTIVITY OF CALLUS BODY OF VERBESINA ENCELIOIDES LEAVES

Chhaya Nenarsia, Career Point University Kota Rajasthan

Monika Karnawat*, Career Point University Kota Rajasthan

ABSTRACT

Verbesina encelioides belongs to the Asteraceae family and also known as golden crown beard and wild sunflower. In present study callus was raised from the leaf explant of *V. encelioides*. Callus was obtained on MS medium. The callus was used for antifungal activity identification. In the *in-vitro* study of the anti-fungal activity *Candida albicans* gave the most effective response out of all the studied fungi, with best activity being displayed in methanol extract followed by hexane and ethyl acetate. With the best activity in ethyl acetate and hexane, and the least activity in methanol extract, *Penicillium chrysogenum* was the least responsive fungal species. In comparison to ethyl acetate and hexane extracts, *Aspergillus niger* displayed the greatest activity in methanol extract. In the presence of an ethyl acetate extract, *Trichoderma reesei* was effectively active, however, its activity decreased when exposed to methanol and hexane.

Keywords: *Verbesina encelioides,* Asteraceae, Callus culture, antifungal activity,

INTRODUCTION

Plants have always been important components of both conventional and modern medicine, for their utility in health and wellness. Around 80% of the world's population relies on plant-derived ingredients (**Winter *et al*., 2012; Yuan *et al*., 2016**).

Nature has provided a plethora of remedies to cure all of humanity's ailments. Herbal medicines have a history as old as human civilization. Today, there is a wealth of information available about the therapeutic properties of various plants. Plants continue to be a major source of drugs in both modern and traditional medical systems **(Rupasinghe *et al,* 2003; Savithramma *et al,* 2011)**. India and China, two of the biggest nations in Asia, have the widest selections of officially recognized and well-known medicinal plants (**Raven *et al*., 1998**).

Callus culture is crucial in the use of biotechnology to harness plant products for human benefit. The process entails the development of plant tissues into an undifferentiated mass that can later be modified to produce beneficial products **(Eibl *et al.,* 2018)**.

The antimicrobial properties of phytochemicals have been stimulated by the high demand on the pharmaceutical and food industries to develop new food preservatives to avoid synthetic preservatives and majorly to novel therapies for the treatment of various microbial infections to combat microbial resistance against conventional antibiotics (**Alamgir *et al.,* 2017; O'Connor *et al*., 2015; Katz *et al*., 2016**).

The Asteraceae family is one of the largest flowering plant families, with over 23,600 species and approximately 1620 genera **(Funk *et al.,* 2009)**. *Verbesina encelioides,* also known as wild sunflower or golden crown beard, is an exotic invasive weed

that is thought to have originated in the United States and Mexico. Golden crown beard is an erect, annual, wild herb with a wide range of tolerance to climatic conditions and a competitive growth habit **(Singh *et al.*, 2017)**.

MATERIAL AND METHOD

Plant Materials

The experimental plant material of *Verbesina encelioides* leaf was collected from Campus of University of Rajasthan, Jaipur and voucher specimen was deposited to the herbarium of Department of Botany, University of Rajasthan, Jaipur for authentication (RUBL no. 211480). MS medium was used for all tissue culture studies **(Murashige and Skoog, 1962).**

Antimicrobial Activities from callus in plant extracts- The antimicrobial activity of *Verbesina encelioides* leaves and callus extracts was studied with methanol, ethyl acetate and hexane extracts. Four fungal strains were selected for the antimicrobial activity.

Preparation of Extracts - The leaves and callus of *Verbesina encelioides* have been washed thoroughly with tap water and are subjected to air-drying in the shade at room temperature (32-37˚C) for about 2-3 weeks. The dried samples were ground into powder form by using a homogenizer. About 50gm of sample (50gm/250ml) were extracted in a Soxhlet extractor for 8 to 10 hours, sequentially with methanol, ethyl acetate and hexane. The extracts obtained were then concentrated and finally dried to a constant weight. Dried extracts were kept at 20˚C until further tests were carried out.

Microorganisms Used- Clinical laboratory fungal isolates viz. *Candida albicans, Aspergillus niger, Trichoderma reesei* and *Penicillium chrysogenum* were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, India. .

Antifungal Assay

The antifungal activity of the experimental plant was investigated by the agar well diffusion method (**Bonjar et al., 2005**). The fungal strains were sub-cultured onto Sabouraud's Dextrose Agar, (SDA, Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile phosphate buffer saline (PBS) and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 6 mm in diameter were punctured in the culture media using a sterile glass tube. 20, 40, 60 and 80 µl of extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). Ketoconazole (40 µl) was used as antifungal positive control. The All experiments were made in triplicate and means were calculated.

Result and observation

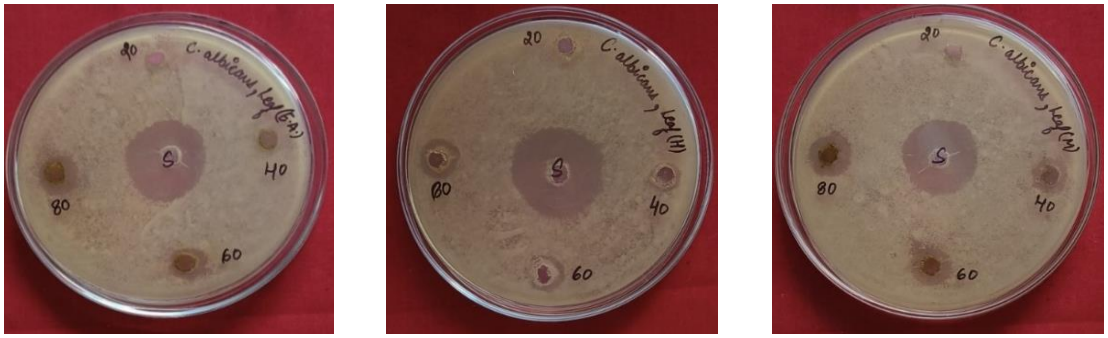
a) Anti-fungal activity of leaf extract

In the result of *in-vitro* study of anti-fungal activity of *Verbesina encelioides* leaf extracts with the standard Ketoconazole being 25 µL, *Candida albicans* gave the most efficient response out of all the studied fungi with best activity been displayed in methanol extract (IZ= 15mm) followed by hexane and ethyl acetate with IZ 13mm. *Penicillium chrysogenum* was the least responsive fungi with best activity in ethyl acetate and hexane (13mm and 12mm, respectively) and least in methanol extract (9mm). *Aspergillus niger* gave the best activity in methanol extract (12mm) followed by ethyl acetate (11mm) whereas no activity was visible in hexane extract. *Trichoderma reesei* was efficiently active under methanol extract (11mm) followed by ethyl acetate and hexane, both having an IZ of 10mm (table 1).

Table 1. Antifungal activity of *Verbesina encelioides* leaves extract against pathogenic fungi

Fungus	Extract	Inhibition zone (mm)				
		Standard	20 µL	40 µL	60 µL	80 µL
Candida albicans	Ethyl acetate	25	-	-	12	13
	Methanol	25	-	12	13	15

	Hexane	25	-	8	10	13
Penicillium chrysogenum	Ethyl acetate	25	-	-	10	13
	Methanol	25	-	-	-	9
	Hexane	25	-	-	9	12
Aspergillus niger	Ethyl acetate	25	-	-	10	11
	Methanol	25	-	7	10	12
	Hexane	25	-	-	-	-
Trichoderma reesei	Ethyl acetate	25	7	8	9	10
	Methanol	25	-	7	9	11
	Hexane	25	-	-	8	10



(a) (b) (c)

Figure 1. Antifungal activity of *Verbesina encelioides* leaves against, *Candida albicans*
(a) Ethyl acetate extract (b) Hexane extract (c) Methane extract

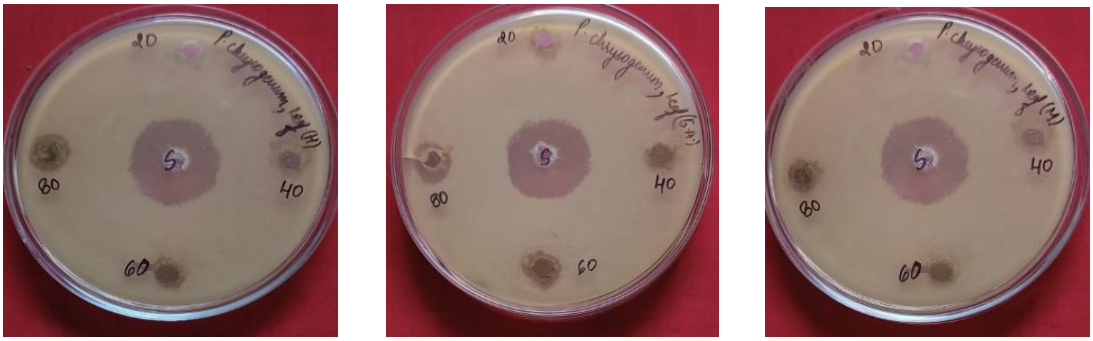


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Figure 2. Antifungal activity of *Verbesina encelioides* leaves against, *Penicillium chrysogenum*
(a) Ethyl acetate extract (b) Hexane extract (c) Methane extract

(a) (b) (c)

Figure 3. Antifungal activity of *Verbesina encelioides* leaves against, *Aspergillus niger*
(a) Ethyl acetate extract (b) Hexane extract (c) Methane extract

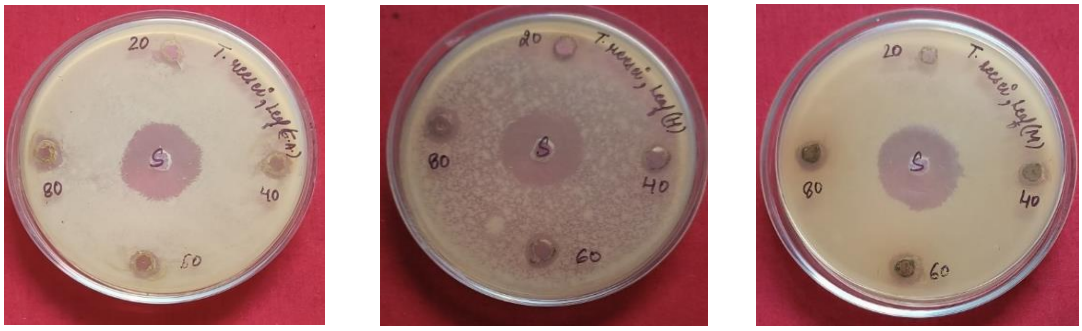

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Figure 4. Antifungal activity of *Verbesina encelioides* leaves against *Trichoderma reesei* (a) Ethyl acetate extract (b) Hexane extract (c) Methane extract

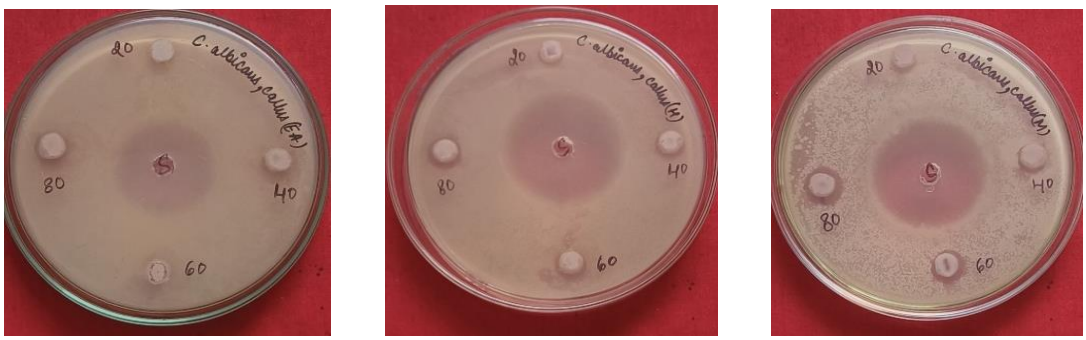
b) Anti-fungal activity of callus extract

In the result of *in-vitro* study of anti-fungal activity of *Verbesina encelioides* callus with the standard Ketoconazole being 25 µL, *Penicillium chrysogenum* gave the most efficient response out of all the studied fungi with best activity been displayed in methanol extract (IZ= 14mm) followed by hexane at 12 mm and ethyl acetate at 8mm. *Candida albicans* was best responsive with methanol extract (13mm) followed by hexane and ethyl acetate at 11mm. *Aspergillus niger* and *Trichoderma reesei* gave an inhibition zone of 12 and 13 mm with methanolic extract whereas in ethyl acetate extract it was 9 and 10mm and in hexane it was 10 and 7mm, respectively (Table 2).

Table 2. Antifungal activity of callus extract of *Verbesina encelioides* against pathogenic fungi

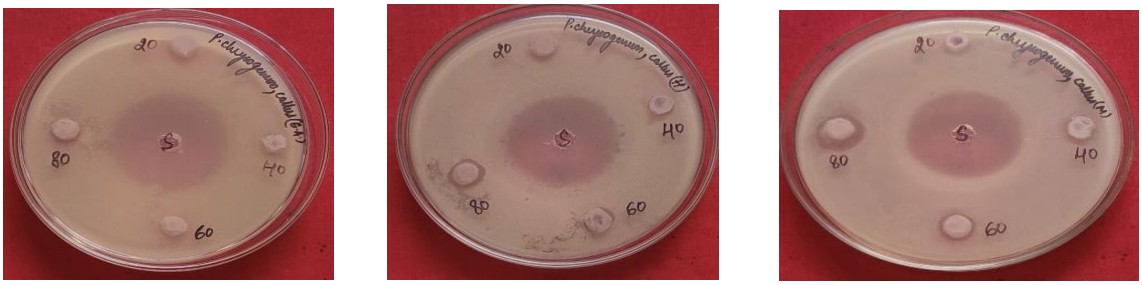
Fungus	Extract	Inhibition zone (mm)				
		Standard	20 µL	40 µL	60 µL	80 µL
Candida albicans	Ethyl acetate	25	-	-	7	11
	Methanol	25	-	9	12	13
	Hexane	25	7	9	10	11
Penicillium chrysogenum	Ethyl acetate	25	-	-	-	8
	Methanol	25	7	10	11	14
	Hexane	25	7	9	10	12
	Ethyl acetate	25	-	7	8	9

Aspergillus niger	Methanol	25	-	7	9	12
	Hexane	25	-	8	9	10
Trichoderma reesei	Ethyl acetate	25	-	8	9	10
	Methanol	25	-	-	10	13
	Hexane	25	-	-	-	7



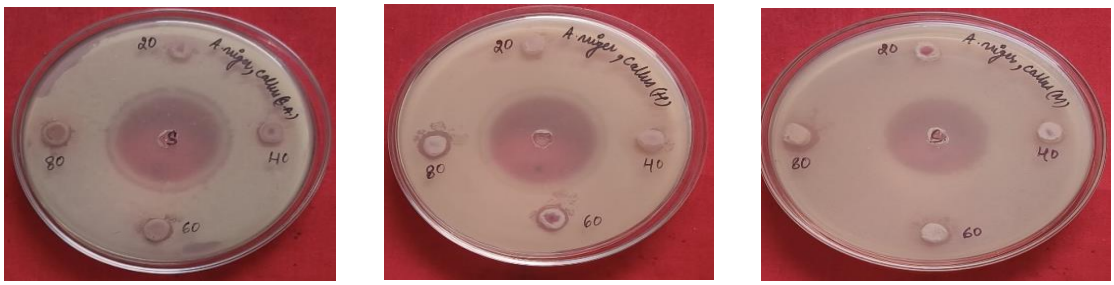
(a) (b) (c)

Figure 5. Antifungal activity of *Verbesina encelioides* callus against *Candida albicans* (a) Ethyl acetate extract (b) Hexane extract (c) Methane extract



(a) (b) (c)

Figure 6. Antifungal activity of *Verbesina encelioides* callus against *Penicillium chrysogenum* (a) Ethyl acetate extract (b) Hexane extract (c) Methane extract



(a) (b) (c)

Figure 7. Antifungal activity of *Verbesina encelioides* callus against *Aspergillus niger* (a) Ethyl acetate extract (b) Hexane extract (c) Methane extract

(a) (b) (c)

Figure 8. Antifungal activity of *Verbesina encelioides* callus against *Trichoderma reesei*

(a) Ethyl acetate extract (b) Hexane extract (c) Methane extract

Conclusion

In the *in-vitro* study of the anti-fungal activity of *Verbesina encelioides* leaf extracts with the standard ketoconazole being 25µL, *Candida albicans* gave the most effective response out of all the studied fungi, with best activity being displayed in methanol extract (IZ = 15mm), followed by hexane and ethyl acetate. With the best activity in ethyl acetate and hexane (IZ of 13 mm and 12 mm, respectively), and the least activity in methanol extract, *Penicillium chrysogenum* was the least responsive fungal species. In comparison to ethyl acetate and hexane extracts, *Aspergillus niger* displayed the greatest activity in methanol extract (12mm). In the presence of an ethyl acetate extract, *Trichoderma reesei* was effectively active, however, its activity decreased when exposed to methanol and hexane.

In callus *Penicillium chrysogenum* gave the most efficient response out of all the studied fungi with best activity been displayed in methanol extract (IZ = 14mm) followed by hexane at 12 mm and ethyl acetate at 8mm. *Candida albicans* was best responsive with methanol extract (IZ = 13mm) followed by hexane and ethyl acetate at 11mm. *Aspergillus niger* and *Trichoderma reesei* gave an inhibition zone of 12 and 13 mm with methanolic extract, respectively whereas in ethyl acetate extract it was 9 and 10mm respectively and in hexane it was 10 and 7mm, respectively.

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