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ANTIFUNGAL ACTIVITIES OF LEAF EXTRACTS OF *SPHAGENTICOLA CALENDULACEA (L.)* PRUSKI OF ASTERACEAE FAMILY

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ABSTRACT:

Antifungal activities of leaf extracts of *Sphagneticola calendulacea* (L.) Pruski of Asteraceae family were investigated by using agar well diffusion method. Fungi viz. *Aspergillus Niger, Aspergillus flavus*, *Fusarium oxysporum* and *Rhizophus stolanifer* were cultured on PDA agar medium separately in petri plates of 11cm diameter. Wells of 10mm diameter were prepared in the agar medium with cork-borer Fresh leaf extracts of 10, 20, 30 and 40% were prepared, sterilized and used. Sterile distilled water was added in the central well and treated as control. Plates were incubated art 37^{0}C. Inhibition zones were measured after 5 days. Well agar diffusion method: Leaf extract of 10%, 20%, 30% &40% concentration added in separate well. Central well was added with water was treated as a control. Extract inhibited the fungus growth around the wells. The inhibited zones around the wells were measured in millimetre. Readings were recorded in tabular form. Photographs were taken by using digital camera.

Keywords: *Leaf extracts, aqueous, methanol Sphagneticola calendulacea (L.) Pruski, Aspergillus*.

INTRODUCTION

Study Area: Ahmednagar is one of the largest districts of the Maharashtra states of India. It occupies an area of 17.035 sq.km. It is located between 180 2'and 190 9'North latitude and 700 9'and 750 5'East longitude. *Sphagneticola calendulacea* (L.) Pruski of Asteraceae family were collected from college campus of Ahmednagar city for the present work. *Sphagneticola calendulacea* (L.) Pruski (= Wedelia chinensis (Osbeck0 Merr.): Hook. f. Fl. Brit. India 3-306, 1881; Cooke, Fl. Pres. Bombay 2:98, 1958 (Repr); Almeida, Flora of Maharashtra Vol. 3(A), 146, 2001. Singh, Fl of Maharashtra state, Vol. 2, 250, 2001.Procumbent perennial herb; rooting at nodes; leaves simple, opposite, trinerved, subpetiolate, hispid; heads solitary, involucral bracts longer than disc florets, ligulate ray florets, yellow; cultivated in gardens.

Many workers have done work on antifungal properties of plant species of Asteraceae family.Some of theseare: Adjibode *et al* (2015) worked on *Synedrella nodiflora* (L.) Gaertn. ;Jalander and Gachande (2012) worked on effect of Pigeon pea [*Cajanus cajan* (L.) Millsp.] on the growth of *Fusarium oxysporum* sp udam; Jiang *et al* (2016) Identified and worked on allelochemicals in *Chrysanthemum indicum* L. and their fungicidal potential against *Sclerotium rolfsii*

Sacc. And *Atractylodes macrocephala* Koidz.; Kamble and Moon (2015) studied antifungal activity of crude extracts of *Tridax procumbens* L. against potentially pathogenic fungal species; Karunambigal and Gayathri Devi (2014) studied antibacterial activity of leaves and roots of *Eclipta alba;* Krishnaswamy and Christina (2015) worked on antibacterial activity of different parts of *Tridax procumbens* L. against human pathogens.; Malarkodi and Manoharan (2013) Antifungal activity of *Parthenium hysterophorus* L; Mares *et al* (2004) worked on antifungal activity of *Tagetes patula* extracts on some phytopathogenic fungi like *Pythium ultimum;* Shankar and Thomas (2014) studied antibacterial activity of flower heads of *Wedelia trilobata* (L.) ; Toppo *et al* (2013) Antimicrobial activity of *Sphagneticola trilobata* (L)Pruski, against some human pathogenic bacteria and fungi .

MATERIAL AND METHODS

Rhizopus stolanifer Vuillemin: Saprophytic as well as parasite, causes rot of fruits; aseptate mycelium; sporangiophores grouped; cosmopolitan. It is included in Mucoraceae family of order Mucorales, class Zygomycetes of Division Zygomycotina.

Aspergillus Niger Van Tieghem : Cosmopolitan; Saprophytic as well as pathogenic; known from fields before and after postharvest, stored grains, fruits; cause human diseases like aspergillosis; septate branched hyphae,; produce condiophores and conidia. It is included in Trichomaceae family of order Eurotiales, class Eurotiomycetes of Division Ascomicotina. *Aspergillus flavus* Link : Cosmopolitan; green, yellowish, reddish in color; Saprophytic as well as pathogenic; known from fields before and after post harvest, well developed on cereals, pulses, legumes, nuts, stored grains, fruits; septate branched hyphae,; produce condiophores and conidia. It produces aflatoxins which is toxic to mammals. It is included in Trichomaceae family of order Eurotiales, class Eurotiomycetes of Division Ascomicotina. *Fusarium oxysporium* Schlecht, Synder & Hanson.: Soil borne saprophytic as well as pathogenic, it causes wilt disease of red gram; septate mycelium; form two types of conidia viz. microconidia and macroconidia. It is included in Nectiriaceae family of order Hypocreales, class Sordariomycetes of Division Ascomicotina.

Bioassay experiments were performed in the research laboratory of New Arts, Commerce & Science College; Botany Department; district Ahmednagar (Maharashtra State) at room temperature 250 c to28 0 c. Plant materials were collected from college campus. Stock solutions of 10%, 20%, 30% and 40% concentration of leaf samples of selected plant were prepared by using solvents like water and methanol. Extract solutions were obtained by washing and crushing leaves in mortar and pestle (Narval and Turo, 1994).

Preparation of PDA media: Potato dextrose Agar Potato -200 gm Dextrose -20 gm Agar -20 gm Water -1 liter . Peeled potatoes were cut into small pieces. 200 gm of potato pieces were weighed, washed quickly in running water and put in one litre of water and boiled for nearly one hour till a mass is formed. The mass was then squeezed through double layer of muslin cloth to obtained as much of the pulp as possible. Agar was dissolved separately in small volume of water 250 ml. Potato mass, Agar solution and Dextrose were mixed and final volume was made up to 1 litre in suitable glass container (conical flask) and sterilized in an autoclave at 120 c and 15 pounds pressure for

15 minutes. This PDA medium is poured in sterilized Petri plates under aseptic conditions of laminar air flow for culturing fungi. Cultures of *Aspergillus Niger, Aspergillus flavus* and *Fusarium oxysporium* and *Rhizhopus stolanifer*

Well agar diffusion method: Petri dishes of 11 cm diameter containing freshly prepared PDA medium were used for fungus culture. Many Petri plates with fully grown fungi were prepared. Then with the help of cork borer five wells per Petri plates were prepared. Leaf extract of 10%, 20%, 30% &40% concentration added in separate well. Central well was added with water was treated as a control. Extract inhibited the fungus growth around the wells. The inhibited zones around the wells were measured in millimetre. Readings were recorded in tabular form. Photographs were taken by using digital camera.

RESULTS AND DISCUSSION:

Effect of aqueous leaf extracts of *Sphagneticola calendulacea* (L.) Pruski of family Asteraceae family (Refer table 1 and graphs 1 to 5): Fresh leaf extracts viz 10% to 40% inhibited growth of *Aspergillus Niger* Van Tiegheum, *Aspergillus flavus* Link, *Fusarium oxysporum* Shlecht, Synder and Hanson and *Rhizhopus stolanifer* Vuillemin in a concentration correlated manner.

Inhibition was in an order of: *Fusarium oxysporum* > *Aspergillus flavus* > *Rhizhopus stolanifer* >*Aspergillus Niger*. *Aspergillus Niger* was least inhibited as compared to other three fungi while *Fusarium oxysporum* was maximally inhibited.

Effect of methanol leaf extracts of *Sphagneticola calendulacea* (L.) Pruski of Asteraceae family (Refer table 1 and graphs 1 to 5): All Methanol leaf extracts inhibited growth of *Aspergillus Niger* Van Tiegheum, *Aspergillus flavus* Link, *Fusarium oxysporum* Shlecht, Synder and Hanson and *Rhizhopus stolanifer* Vuillemin . Inhibition was concentration correlated.

Inhibition was in an order of: *Aspergillus flavus* > *Fusarium oxysporum* > *Rhizhopus stolanifer* >*Aspergillus Niger*. *Aspergillus Niger* least inhibited as compared to other three fungi while Aspergillus flavus was maximally inhibited.

SUMMARY AND CONCLUSION:

Aqueous leaf extracts of *Sphagneticola calendulacea* (L.) Pruski of family Asteraceae inhibited growth of *Aspergillus Niger* Van Tiegheum, *Aspergillus flavus* Link, *Fusarium oxysporum* Shlecht, Synder and Hanson and *Rhizhopus stolanifer* Vuillemin in a concentration correlated manner. *Aspergillus Niger* was least inhibited as compared to other three fungi while *Fusarium oxysporum* was maximally inhibited.

Methanol leaf extracts of *Sphagneticola calendulacea* (L.) Pruski of Asteraceae family inhibited growth of *Aspergillus Niger* Van Tiegheum, *Aspergillus flavus* Link, *Fusarium oxysporum* Shlecht, Synder and Hanson and *Rhizhopus stolanifer* Vuillemin . Inhibition was concentration correlated. *Aspergillus Niger* least inhibited as compared to other three fungi while Aspergillus flavus was maximally inhibited. Shankar and Thomas (2014) demonstrated similar antibacterial results of flower heads of *Wedelia trilobata* (L.)

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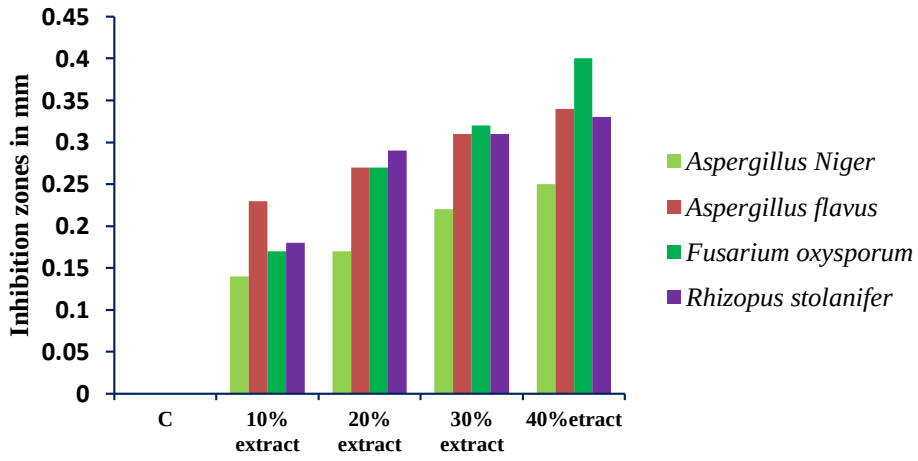
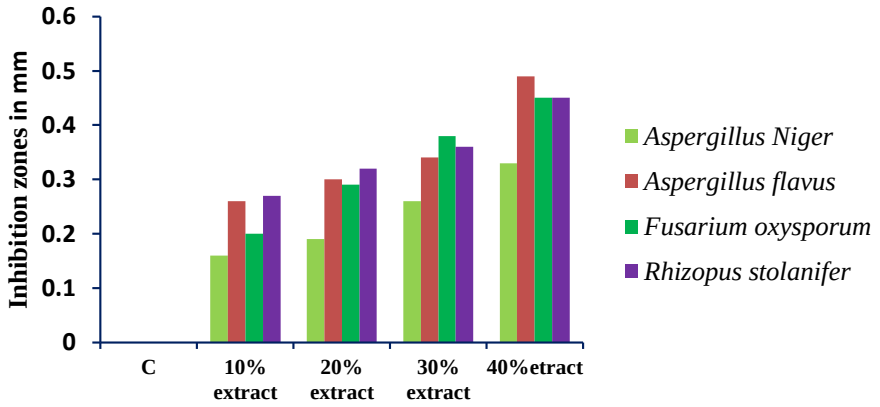
Table No1: Effect of leaf extracts of *Sphagneticola calendulacea* (L.) Pruski (= *Wedelia chinensis* (Osbeck) Merr):

Plant	Leaf Extract	Name of the fungus	Inhibition zone in mm					CD at 0.05%	P value at 0.05%
			control	10%	20%	30%	40%		
Sphagneticola calendulacea (L.) Pruski	Aqueous	*Aspergillus niger*	0.00a±0.00	0.14b ± 0.018	0.17b ±0.024	0.22c ±0.020	0.25d ±0.03	0.038	7.55E-10
	Aqueous	*Aspergillus flavus*	0.00a±0.00	0.23b ±0.026	0.27bc ±0.021	0.31bd ±0.023	0.34e ±0.048	0.05	6.54E-12
	Aqueous	*Fusarium oxysporum*	0.00a±0.00	0.17b ±0.026	0.27c ±0.021	0.32 ±0.025	0.40c ±0.37	0.044	5.53E-12
	Aqueous	*Rhizhopus stolanifer*	0.00a±0.00	0.18b ±0.25	0.29c ±0.23	0.31d ±0.23	0.33d ±0.037	0.046	7.37E-12
Sphagneticola calendulacea (L.) Pruski	Methanol	*Aspergillus Niger*	0.00a±0.00	0.16b ±0.014	0.19c ±0.024	0.26d ±0.03	0.33e ±0.023	0.026	4.29E-11
	Methanol	*Aspergillus flavus*	0.00a±0.00	0.26b ±0.03	0.30 ±0.024	0.34 ±0.022	0.49 ±0.031	0.031	2.99E-16
	Methanol	*Fusarium oxysporum*	0.00a±0.00	0.020b ±0.025	0.29c ±0.024	0.38d ±0.033	0.45e ±0.037	0.035	1.13E-14
	Methanol	*Rhizhopus stolanifer*	0.00a±0.00	0.27b ±0.031	0.32c ±0.026	0.36 ±0.026	0.45e ±0.034	0.034	2.30E-14

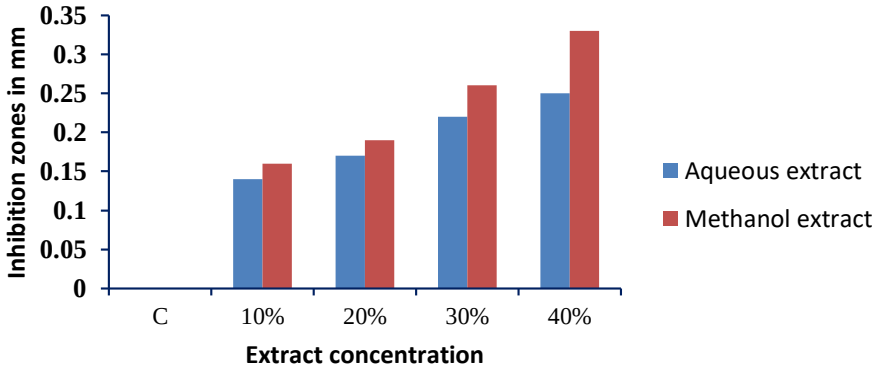
Graph 1: Effect of aqueous leaf extracts of *Sphagneticola calanendulaca* (L.)Pruski of fungal growth.



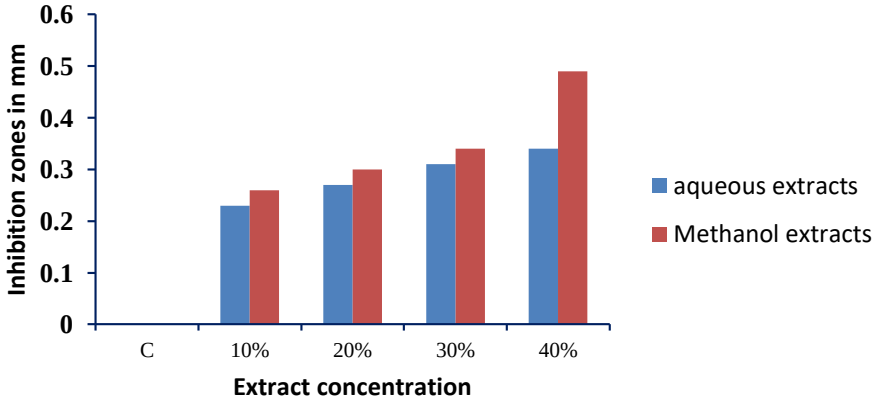
Graph 2: Effect of methanol leaf extracts of *Sphagneticola calendulacea* (L.)Pruski on fungal growth



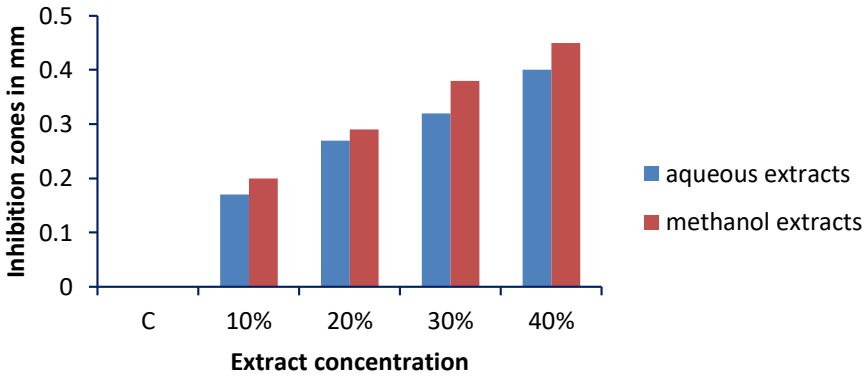
Graph 3:Effect of leaf extracts of *Sphagneticola calendulacea* on *Aspergillus Niger*



Graph 4:Effect of leaf extractsof *Spagneticola calendulacea* on growth of *Aspergillus flavus*



Graph 5:Effect of leaf extracts *of Spgagneticola calendulacea* on growth of *Fusarium oxysporum*



Graph 6: Effect of leaf extracts of *Sphagneticola calendulacea* on growth of *Rhizopus stolanfer*