PHYTOCHEMICAL EVALUATION AND ANTIFUNGAL POTENTIAL OF *TARAXACUM OFFICINALE* (WIGG.) & *RUMEX OBTUSIFOLIUS* (L.)

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Abstract

Taraxacum officinale (Wigg.) and *Rumex obtusifolius* (L.) were examined for their antifungal activity against *Penicillium glabrum* (Wehmer) and *Alternaria gaisen* (Nango). These phytopathogenic fungi cause pre and post-harvest diseases in large number of economically important plants. Methanolic leaf extracts of the two plants were used for in *vitro* screening against *P. glabrum* and *A. gaisen.* Leaf extract of *T. officinale* effectively inhibited the dry biomass of *A. gaisen* and *P. glabrum* upto 37 – 42 %. *R. obtusifolius* also caused upto 27 % reduction in dry biomass of both the test fungi. The leaf extract of *T. officinale* was separated amongst different organic solvents *viz., n*-hexane, chloroform, ethyl acetate and *n*-butanol. Four separated fractions and chemical fungicide (Mancozeb) were further investigated against *P. glabrum* and *A. gaisen* via Minimum inhibitory concentration (MIC) assay. Diverse concentrations of fungicide and separated fractions ranging from 0.01 g – 0.0000195 g mL^{-1} were applied in MIC bioassay. The data was noted after 24, 48 and 72 hrs. Fungicide and ethyl acetate fraction against *A. gaisen* and for *P. glabrum* fungicide and *n*-butanol fraction were found to be extremely inhibitory with MIC of 0.000019 g mL^{-1}. Phytochemical analysis of methanolic leaf extract of *T. officinale* showed the presence of glycosides, saponins, terpenoids and flavonoids.

Keywords: *Alternaria gaisen*, Antifungal, Bioasssay, MIC, *Penicillium glabrum*

Introduction:

Alternaria gaisen Nagano. a soil borne fungal pathogen, belongs to the class Ascomycota and is a causal agent of leaf blotch, fruit spot and ring spot plant disease (Harteveld *et al.*, 2013). *A. gaisen* survives in adverse conditions as resting bodies (microsclerotia) or resting spores (chlamydospores) in the soil. A great number of species of the genus *Alternaria* were recorded causing severe damage to crops and world-wide economic loss (Mamgain *et al.*, 2013). The fungus produces mycotoxin and attacks the young shoots, causing sunken elongated streaks which bring about the death of the shoot (give reference).

Penicillium glabrum (Wehmer) is a destructive soil borne fungal pathogen that belongs to the class Ascomycota and order Eurotiales. Numerous air borne asexual conidia are produced by this genus which are mostly ubiquitous in nature (Frisvad and Samson, 2004). *P. glabrum* is considered as an essential agent that causes post-harvest losses in different fruits and vegetables (Valiuskaite *et al*., 2006). Approximately 20-25 % of the harvested fruits are deteriorated by this fungal pathogen during post-harvest handling (Zhu, 2006).

These pathogens cause different kinds of diseases in plants so, different management techniques for diseases incited by *A. gaisen* and *P. glabrum* need combination of strategies including cultural practices and chemical practices. Use of synthetic fungicides, alteration of crop, host resistance varieties are major strategies for controlling fungal infections. To protect the crop from loss, increased life span, storage and quality of crop is maintained by using fungicide sprays by the farmers (Abawi and Widmer, 2000).

Plants have been known for their antimicrobial agents and are used medicinally as potential drugs in many countries (Mahesh and Satish, 2008). Plants excrete wide varieties of nontoxic materials or chemicals that may kill the pathogens or retard their growth. *In vitro* antimicrobial properties have been found in plants due to the wide spread occurrence of secondary metabolites *i.e.*, glycosides, terpenoids, tannins, flavonoids and alkaloids etc. in these plants (Dahanukar *et al.*, 2000). These metabolites act in defending plants against pathogens.

Taraxacum officinale (Wigg.) is a flowering herbaceous perennial weed that belongs to the family Astreaceae. It is commonly known as Dandelion. Dandelion contains minerals like potassium, iron and zinc and is rich in vitamins A, B, C and D. *T. officinale* has been used widely to treat various diseases (Rasool and Sharma, 2014).

Rumex obtusifolius L. is a perennial herbaceous flowering weed that belongs to the family Polygenaceae. *Rumex* genus possesses antioxidant, anti-cancer, antiviral and antifungal properties (Abad *et al.*, 1999; Guarrera, 2003). So, this study is intended to check the antifungal potential of *T. officinale* and *R. obtusifolius* against *A. gaisen* and *P. glabrum.*

Materials and Methods:

Collection of plant material: *T. officinale* and *R. obtusifolius* leaves were obtained from Fatehpur District Layyah. After extensive cleaning with a piece of cloth, the mud or litter was removed from the plants collected and allowed to be dried in open air. The dried plants without the presence of any moisture were grinded to form powder.

Collection of test fungus species: Pure culture of *A. gaisen* and *P. glabrum* test fungal species were procured from Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Malt extract agar (MEA) medium was used for sub culturing and storage was done at 4°C in a refrigerator.

Antifungal bioassay Antifungal bioassay was done by following the protocol of Waheed *et al.* (2016). 100 mL of methanol was used to dip the plant materials (twenty grams each) at ±25 °C. Plant materials were strained through muslin cloth (autoclaved). Filtered plant extracts were dried in an electric oven at 35°C to reduce the volume up to 20 g. For making 20 % stock solution in 20 g methanolic extract of both test plants 80 mL of distilled water was added and stored at 4 °C.

Malt extract (2 %) was prepared by adding 2 g of malt extract in 100 mL of distilled water in 250 mL flask and was autoclaved at 121°C for half an hour. In 8% v/v concentration of each methanolic extract was prepared by pouring 20 mL of stock solution in 80 mL of the malt extract medium. Further concentrations of 7 %, 6 %, 5 %, 4 %, 3 %, 2 % and 1 % were prepared by adding 17.5, 15, 12.5, 10, 7.5, 5 and 2.5 mL of the stock solutions in 83.5, 85, 87.5, 90, 93.5, 95 and 97.5 mL of malt extract medium. No plant extract was added in control treatments. Chloromycetin capsule (50 mg 100 mL^{-1}) was added in each concentration to get rid of bacterial contamination. All the concentrations were replicated three times. Mycelial discs (5 mm in diameter) using sterilized cork borer were prepared from seven days old culture of *A. gaisen* and *P. glabrum* and were poured in each flask. Fungal growth was checked by filtering the solution of each concentration through pre-weighed Whatmann no. 1 filter paper after seven days by using the formula:

$$\text{Growth inhibition (\% age)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Phytochemical tests of *T. officinale*: Phytochemical test of methanolic extract of *T. officianale* was performed by using the methods and standard techniques given by Parekh and Chanda (2007).

Partitioning of plant material: 20 g leaves of *T. officinale* were soaked in 100 mL methanol for 7 days. After 7 days the extract was filtered and partitioned with organic solvents including *n*-hexane, chloroform, ethyl acetate and *n*-butanol by means of separating funnel (Sadiqa *et al.,* 2015). Final gummy mass after partitioning with organic solvents were obtained as *n*-hexane (0.4 g), chloroform (0.1 g), ethyl acetate (0.1 g) and *n*-butanol (0.1 g) fractions respectively.

Minimum inhibitory concentration (MIC) assay: The MIC activity of four isolated fractions and a commercial synthetic fungicide (Mancozeb) was performed using serial dilution method in test tubes (Karim *et al.,* 2015). For this purpose maximum 0.01 g of every separated fraction was dissolved in 1 mL of distilled water and dimethyl sulphoxide (DMSO). After adding, this was further serially diluted upto the minimum tested concentration *i.e.,* 0.000019 g mL^{-1}. 2 % ME medium was freshly prepared and to it *A. gaisen* and *P. glabrum* seven days' old cultures were added to make a final volume of 0.001 mL. All the experiment was conducted in 1.6 cm diameter and 15 cm length test tubes. 100 µL of fungal biomass was added in each test tube while test tubes comprising DMSO and distil. Water was used as control. After 24, 48 and 72 hours of incubation period MIC value of each fraction were calculated to check fungal mycelia growth suing inverted microscope.

Statistical analysis: Data were analyzed statistically by using ANOVA followed by DMRT (Duncan's multiple range tests) (Steel & Torrie, 1980).

Results and Discussion:

In the present study methanolic leaf extracts of *T. officinale* and *R. obtusifolius* were investigated against target fungi *A. gaisen* and *P. glabrum.* Different concentrations of leaf extracts (1-8 %) significantly reduced fungal growth. Leaves of *T. officinale* showed maximum antifungal activity (27 %) against *A. gaisen* at 8 % (Figure 1) and *P. glabrum* (42 %) at 8 % (Figure 2). Other applied concentrations of *T. officinale* leaves *viz*. 1 %, 2 %, 3 %, 5 %, 6 %, 7 % and 8 % also effectively retarded the biomass of *A. gaisen* upto 9 %, 24 %, 20 %, 17 %, 24 %, 17 % and 17 % respectively as compared to control treatment. In case of *P. glabrum* the applied concentrations (1-7 %) of *T. officinale* leaves caused 21 % – 33 % reduction. Literature also supported these findings as Odintsova *et al*. (2010) reported that *T. officinale* showed inhibitory activity against *Phytophtora infestans.* Valenzuela *et al*. (2018) reviewed that *T. officianle* contains various phytochemicals that might responsible for the antifungal activity against large number of economically important fungi.

Leaf extract of *R. obtusifolius* at 8 % concentration effectively inhibited the growth of *A. gaisen* and *P. glabrum* (27 %) (Figure 3 & 4). Other applied concentration also effectively inhibited the growth of both the test fungi. Earlier Wegiera *et al.* (2011) also documented that *R. obtusifolius* has strong antimicrobial properties against various tested bacteria and fungi.

Leaf methanolic extract of *T. officinale* was also phytochemically analyzed. Results confirmed the presence of flavonoids, glycosides, terpenoids and saponins whereas tannins, phlobatanins and alkaloids were absent (Table. 1). Results of Hu and Kitts (2005) also revealed that *T. officinale* contains terpenoids and flavonoids. Many studies from previous literature has suggested that flavonoids, phenolic compounds, terpenes and glycosides are responsible for the inhibitory/antifungal activity of *T. officinale* (Jassim *et al.*, 2012; Chadwick et al., 2013; Mir *et al*., 2013).

MIC (0.01 mg – 0.0000195 g mL^{-1}) of various isolated portions of methanolic leaf extract of *T. officinale* and commercial fungicide (Mancozeb) were tested against *A. gaisen* and *P. glabrum.*

Fungicide and ethyl acetate fraction were found to be highly effective against *A. gaisen* as compared to other fractions (Table 2) while fungicide and *n*-butanol fraction were found to be the most effective against *P. glabrum* in comparison to other fractions (Table 2). Khan *et al*. (2011) reported that ethyl acetate fraction of *T. officinale* potentially retarded the growth of *Fusarium oxysporum* and *Rhizoctonia solani*. Dandelion also caused significant inhibition in mycelial growth of *A. alternata*, *P. expansum* and *M. piriformis* (Parveen *et al.*, 2013). Iqbal *et al*. (2014) also studied the MIC of *T. officinale* against different bacterial strains in the range of 0.30 mg mL^{-1} and found promising results. On the basis of these findings present study concluded that *T. officinale* has strong antifungal potential against *A. gaisen* and *P. glabrum*.

Table 1: Phytochemical constituents of *Taraxacum officinale.*

Sr. no.	Phytochemical constituent	Observations
1.	Alkaloids	_
2.	Phlobatannins	_
3.	Glycosides	+
4.	Tannins	_
5.	Saponins	+
6.	erpenoids	+
7.	Flavonoids	+

+ referred to presence
_ referred to absence

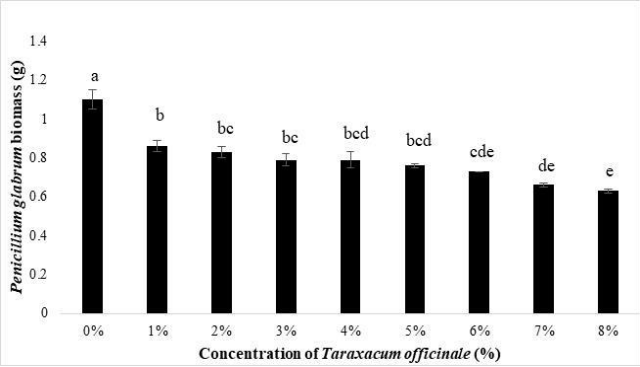

Fig. 1

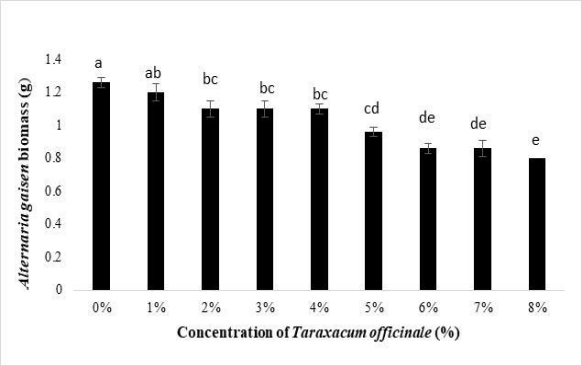

Fig. 2

Figure 1: *In vitro* effects of methanolic extract of *T. officinale* on the growth of *A. gaisen.* Standard errors of means of three replicates are shown by vertical bars. Significant differences are shown by different letters as calculated by Duncan's multiple range tests.

Figure 2: Effects of methanolic extract of *T. officinale* on *in vitro* growth of *P. glabrum.* Standard errors of means of three replicates are shown by vertical bars. Significant differences are shown by different letters as determined by DMR Test.

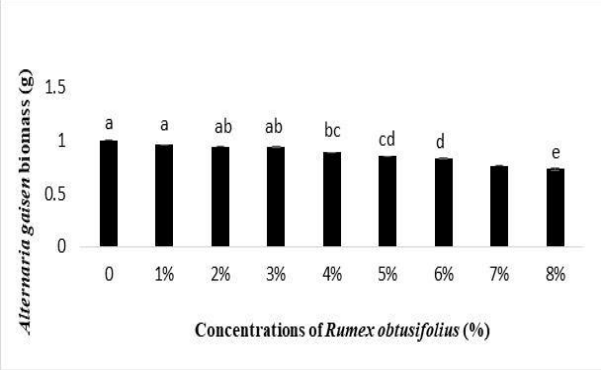

Fig. 3

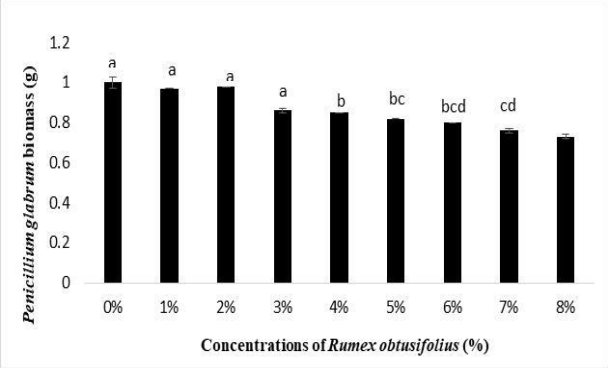

Fig. 4

Figure 3: *In vitro* effects of methanolic extract of *R. obtusifolius* on the growth of *A. gaisen.* Standard errors of means of three replicates are shown by vertical bars. Significant differences are represented with different letters as determined by DMR Test.

Figure 4: *In vitro* effects of methanolic extract of *R. obtusifolius* on the growth of *P. glabrum.* Standard errors of means of three replicates are shown by vertical bars. Significant differences are represented as values with different letters as determined by DMR Test.

Table 2: MIC values of different organic fractions isolated from *T. officinale* and synthetic fungicide (Mancozeb) against *A. gaisen* after 24, 48 and 72 hours incubation periods.

Con. mg mL^{-1}	Fractions																			
	24 hours after incubation							48 hours after incubation							72 hours after incubation					
	H_2O	DMSO	*n*-Hexane	Chloroform	Ethyl acetate	*n*-Butanol	Mancozeb	H_2O	DMSO	*n*-Hexane	Chloroform	Ethyl acetate	*n*-Butanol	Mancozeb	H_2O	DMSO	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Mancozeb
10	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-
5	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-
2.5	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	+	-
1.25	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	+	-
0.625	+	-	-	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+	-	+	-
0.3125	+	-	-	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+	-	+	-
0.156	+	-	-	-	-	-	-	+	+	+	-	-	+	-	+	+	+	+	-	+	-
0.078	+	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-
0.039	+	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-
0.019	+	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-

Mycelium present: +
Mycelium absent: -

Table 3: MIC values of different organic fractions isolated from *T. officinale* and synthetic fungicide (Mancozeb) against *P. glabrum* after 24, 48 and 72 hrs incubation periods.

Con. mg mL^{-1}	Fractions																			
	24 hours after incubation							48 hours after incubation							72 hours after incubation					
	H_2O	DMSO	*n*-Hexane	Chloroform	Ethyl acetate	*n*-Butanol	Mancozeb	H_2O	DMSO	*n*-Hexane	Chloroform	Ethyl acetate	*n*-Butanol	Mancozeb	H_2O	DMSO	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Mancozeb
0.01	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-
0.05	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-
0.0025	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-
0.00125	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-
0.000625	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+	+	-	-	-
0.000312	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+	+	+	-	-
0.000156	+	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	+	+	+	-	-
0.000078	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-
0.000039	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-
0.000019	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-

Mycelium present: +, Mycelium absent: -

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