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Effect of Antifungal activities of some Angiospermic sources against *Cladosporium herbarum* isolated From *Oxalis corniculata* L.

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Abstract

Oxalis corniculata L. is a medicinally important plant. Its medicinal usage is reported in different traditional systems of medicine such as Ayurveda , Unani & Sidha. It possesses important activities like antioxidant, anticancer, antimicrobial, antifungal, antihelmic, anti inflammatory, Kumar, *et . al.*[14]. In Chhattisgarh, *O. corniculata* L. popularly known as Tinpania bhaaji is being consumed as a leafy vegetable crop by the local masses. A periodic survey was made to collect the infected leaves of *O. corniculata L.* for pathological investigations. *Cladosporium herbarum* was isolated as a fungal pathogen from the leaves with large dark brown necrotic spots.. The pure culture was maintained on PDA slants for further studies. The presence of antifungal compounds, in higher plants, has long been recognized as an important factor in disease resistance. Four Angiospermic plant extracts viz: *Cassia fistula* (*Cf*) , *Annona squamosa* (*As), Bauhinia variegata* (*Bv*), *and Aegle marmelos* (*Am*), were tested *in vitro* against *Cladosporium herbarum* on the solid and broth media both. *A. marmelos* extract was found to be most effective in inhibiting the mycelial growth of *C. herbarum* whereas *C. fistula* extract exhibited minimum inhibitory effect.

Oxalis corniculata L. is an important plant of family Oxalidaceae and identified by its narrow, yellow green stem and cluster of rounded leaves which produces bright yellow flowers. *O. corniculata* is a natural occurring weed that have been used in traditional medicine for the cure of dysentery and diarrhoea in India[11]. Paste of crushed leaves prevent dysentery disorders. Bioactivity profiling of extracts from *O. corniculata* identified several compounds that showed antiamoebic activity against *Entamoeba histolica* common causal organism of dysentery.[1]. Traditional healers of Chhattisgarh applied the aqueous paste of herb externally on affected part to remove the wounds and therefore popularly known as dadmari.

Herbs and herbal extracts have been used as medicine since the beginning of human civilization. People have great faith on them for their effectiveness and their inherent medicinal properties.

Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases. There are many methods which are presently being used to control various plant pathogens. Traditional medicines and ethnobotanical information play vital role in scientific research, especially when the literature and field work has been properly evaluated[27]. Several workers have correlated the trace element contents of the herbal drugs with therapeutic action and described "inorganic switches. Bangniwar *et. al*.[3] demonstrated the effect of leaf aqueous extracts of various plants. Present day research necessitate to explore the use of natural products for crop protection,[24]. Bioassay can play an important role in the standardization in herbal drugs[5].

O. corniculata is a wonderful plant and this paper indicates the demand of scientific research to enhance the condition of its cultivation, awareness towards the medicinal properties and appropriate usage of crop, preparation of byproduct and commercialization the crop in the world of medicine , for which the disease free crops are needed. The present investigation deals with the isolation of follicolous necrotic fungi, which severely damages the crop. *Cladosporium herbarum* was isolated as a dominant fungal pathogen from its leaves (1a). Due to increased awareness about the risks involved in use of pesticides, much attention is being focused on the alternative methods of pathogen control. Therefore, today scientists look for methods which are safe and specific for pathogen. Keeping in view present study was under taken to evaluate angiospermic plant extracts for the ecofriendly management of plant diseases.

Infected leaves of *Oxalis corniculata* L. (Fig. 1a) were collected from different crop field locations of Raipur city viz: Kushalpur, Mahamaya Mandir, Mahadevghat and Changorabhata barriers of approximately one acre size of barries, during September to February periodically in 2012 & 2013. The plants were collected, photographed, identified and specimens were prepared for herbarium. The pathogenicity was proved by leaf detached method to ensure Koch's postulate technique and the pathogen was identified as. *Cladosporium herbarum*. The degree of infection ranged from moderate to severe. Spore size was measured micrometrically and its average size was 4.5µm. in length and 45µm. in width (Fig 1c).

One gm of required plant leaves were thoroughly washed with sterile water and grind separately with the help of mortal & pestle using 100 ml of sterilized distilled water the mixture was squeezed with double layered sterilized cheese cloth and finally filtered through filter paper. (0.01, 0.02, and 0.03) percent concentration were prepared from the stock solution, 2 ml. of each treatment was poured in semi solid and broth media both . The effect of plant extracts against mycelial growth of *C. herbarum* was tested by poisoned food technique in semi solid and broth media both[12,19]. The plant extract was mixed thoroughly in melted PDA, Just before pouring in sterilized petriplates and were allowed to solidify and

inoculated by 7 days old culture of *C. herbarum*, in triplicates. The inoculated petri plates were incubated at room temperature 28 +2ºC for 7 days. A control was maintained where medium was not supplemented with any extract. Colony diameter was measured after 7 days of incubation and percent growth inhibition was calculated.

For broth studies Richard's broth medium was selected with pH - 4.5. 25ml of medium was taken in 100 ml. conical flask. From the stock solution of each treatment 2 ml was added to 25 ml. sterilized Richard's broth, then one loopful of *C. herbarum* was transferred to the flask. After inoculation by *C. herbarum* the properly sealed flask were inoculated for a 7 days and then were filtered over a filter paper, dried and biomass was measured. The broth without the treatment solution. Served as control. Percentage growth inhibition was calculated by the formula.

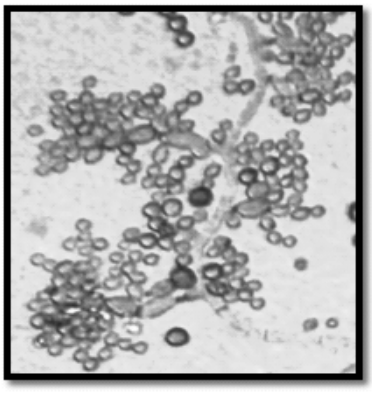
$$\% \text{ of growth inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

C. herbarum, was isolated as a dominant fungal pathogen from the leaves of *Oxalis corniculata* L. showing dark brown necrotic spots. Degree of infection ranged from moderate to severe. During survey of selected crop fields, it was found that leaves collected from Kushalpur barri were more infected than the leaves collected from the other areas. Pathogenicity test was done to ensure Koch' postulates. The spore size was measured micrometriically and its average size was found to be 4.5 µm x 45µm, (Figure-1c),. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory conditions,[6,18]. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases,[26]. Bhardwaj[4] investigated the potential of twenty plant extracts against plant fungal pathogen. After these facts were known, the present work was done to investigate the antifungal activity of the leaves of these plants against *C. herbarum*. Four angiospermic plants viz: *C. fistula, A. squamosa, B. variegata, A. marmelos* were tested in three different concentrations (0.01,0.02, 0.03 percentage) on semisolid & broth media both by poisoned food technique against the mycelial growth of *C. herbarum*. The data presented in (Table-1 and 2), revealed that all four extracts were effective in inhibition. *A. marmelos* extract was found to be most effective where as *Cassia fistula* extract showed the least inhibition. In all plant extracts 0.03% concentration was found to be most effective concentration to check the mycelial growth of *C. herbarum*. The effectiveness of angiospermic sources against the radial size and biomass growth of *A. alternata*., can be equated as *Am > As > Bv > Cf.*

The present work was done to investigate the antifungal activity of the leaves of selected plants against *C. herbarum*. *A. marmelos*, a tree species belonging to the family Rutaceae, whose leaves, stem, bark and fruits have long been used in traditional medicine for its medicinal value . They contain terpenoids which act as an antifungal agent, Poonkothaj[2,21]. *A. marmelos*, contain active principles that can cause inhibition of fungal growth & used as potential source of natural pesticide[9]. The results obtained from *in vitro* studies of antifungal and antioxidant activities clearly suggest that the methanol, chloroform,

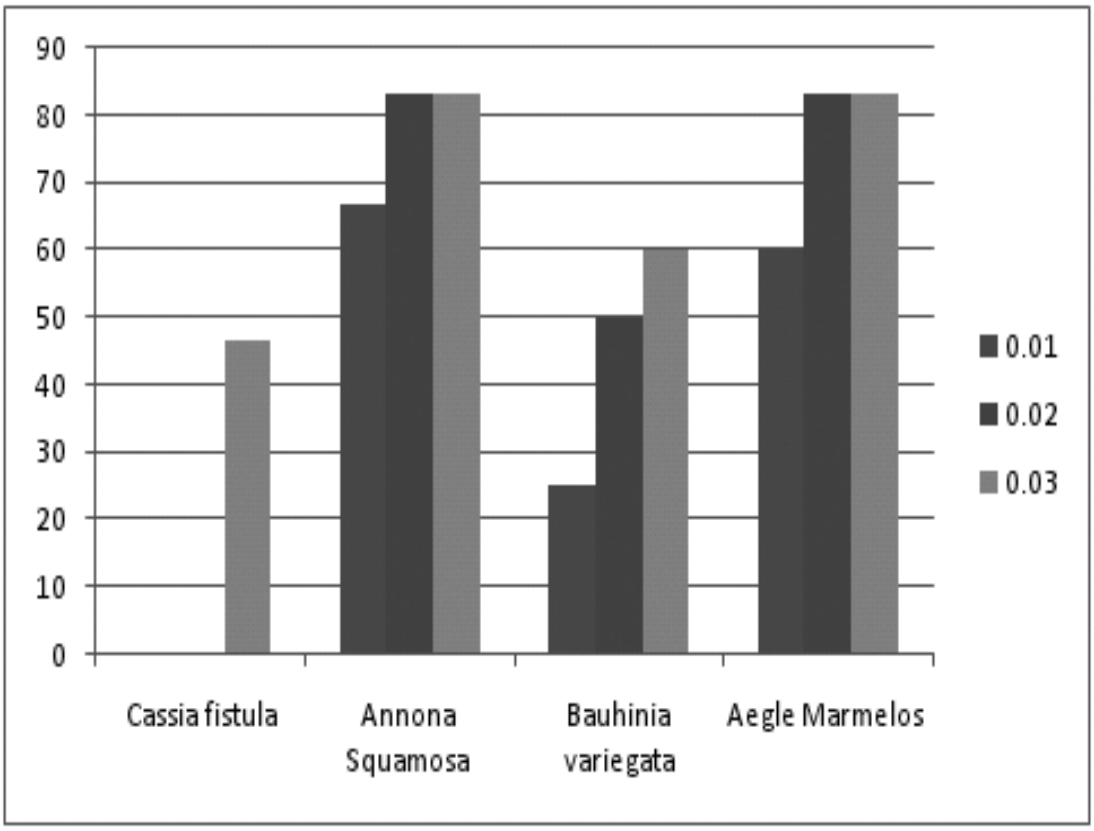
1(a) Symptoms on leaves

1(b) Culture plate



1(c)Spores

Figure of *Cladosporium herbarum*



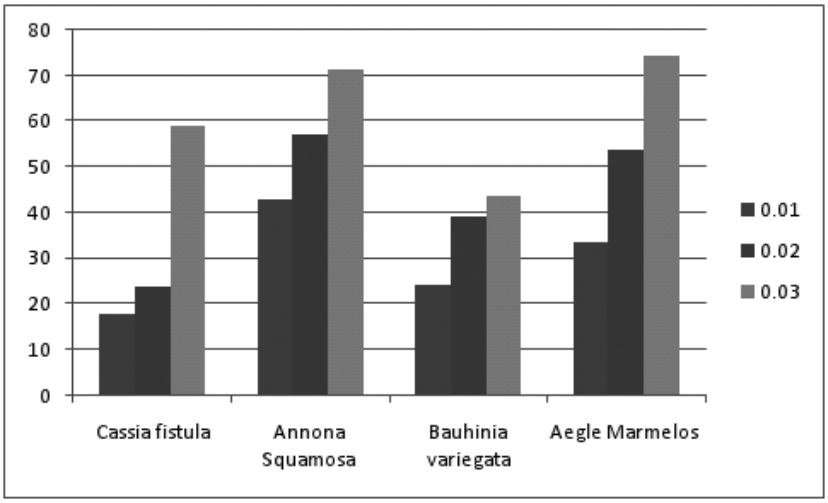
% Inhibition of mycelia growth on Solid Medium (PDA)

Table – 1. *In Vitro* evaluation of plant extract against the mycelial growth of *C. herbarum*.

Angiospermic sources & code	Plant extracts concentration percentage						
	0.01		0.02		0.03		Control
	Radial size	Inhibition %	Radial size	Inhibition %	Radial size	Inhibition %	
Cassia fistula (Cf)	15mm	0%	15mm	0%	8mm	46.6%	15 mm
Annona squamosa (As)	10mm	66.6%	5mm	83.3%	5mm	83.3%	30 mm
Bauhinia variegata (Bv)	15mm	60%	10mm	50%	8mm	60%	20mm
Aegle marmelos (Am)	10mm	66.6%	5mm	83.3%	5mm	83.3%	30mm

Table – 2. *In Vitro* evaluation of plant extract against the biomass of *C. herbarum*

Angiospermic sources & code	Plant extracts concentration percentage						
	0.01		0.02		0.03		Control
	Biomass	Inhibition %	Biomass	Inhibition %	Biomass	Inhibition %	
Cassia fistula (Cf)	0.28mg	17.6%	0.26mg	23.5%	0.14mg	58.8%	0.34mg
Annona squamosa (As)	0.20mg	42.8%	0.15mg	57.1%	0.10mg	71.4%	0.35mg
Bauhinia variegata (Bv)	0.31mg	23.9%	0.28mg	39.1%	0.26mg	43.4%	0.46mg
Aegle marmelos (Am)	0.26mg	33.3%	0.16mg	53.8%	0.10mg	74.3%	0.39mg



% Inhibition of mycelial growth in Broth medium

and aqueous extracts of *A. squamosa* leaves possess antifungal and antioxidant activity[10]. Similarl effect results from phytochemical analysis of *B. variegata* and *C. fistula*[34]. Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides. Synthetic pesticides may be dangerous for consumers[20,29,30]. The result of experiments also indicate that increasing percentage of the treatments enhances the percentage inhibition of. *C. herbarum*. Similar results have been reported by Jalander *et. al.*,[9], Koushik[13] in aqueous extracts of four *Datura* sp., were found to be effective in reducing the growth of two plant pathogenic fungi *F. oxysporum* f. sp. *udum* and *A. solani*, supported by Rajesh[22], Shinde and Dhale[25], Antifungal properties of extracts of *Ocimum tenuiflorum* and *Datura stramonium* against some vegetable pathogenic fungi. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides[28]. Some of the volatile oils, which often contain the principal aromatic and flavouring components of herbs and spices, have been recommended as plant based antimicrobials to retard microbial contamination and reduction in spoilage of food commodities[8].

The foliicolous fungi causes huge losses every year in different parts of world. The destruction caused by these enemies of leaves is a serious problem before us. The leaf borne fungi adversely affect the production and quality of vegetables.[17]. The focus of this research is identification and overcome from the severity of these enemies of nature as well as in the protection of floral diversity from the infection of these pathogens and also in the conservation of valuable flora of the area[16]. Based on the findings of the present study, there are great potentials in the control of fungal diseases using naturally occurring substances. The toxic effect of synthetic chemicals can be overcomed, only by persistent search for new and safer measures like angiospermic sources, which are eco-friendly and effective.

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