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Original Article

Evaluation of C*urcuma longa* and *Curcuma amada* against aflatoxin producing fungus A*spergillus flavus*

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ABSTRACT

Solvent extracts of rhizome of *Curcuma amada* and *Curcuma longa* were evaluated for antifungal potential against *Aspergillus flavus*. Amongst all the extracts, the hexane extracts of C. longa and chloroform extracts of C.amada showed promising results at higher dose of 0.8 mg/ml by Agar well diffusion technique. In Radical growth method, hexane extracts of C. longa showed 59.3 % inhibition, whereas C. amada chloroform extracts exhibited 66.7% reduction in growth at 1000microgram/ml. Significant activity of 76 percent inhibition of biomass was obtained in chloroform extract of *Curcuma amada.* Leaf extracts had mild activities against all the test models. Study has provided lead for the development of antifungal agent from *Curcuma amada* rhizome, which is mainly used for the preparation of salads and pickles.

Keywords: *Curcuma amada*, *Curcuma longa*, *Aspergillus flavus*, biomass, radical growth, antifungal

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INTRODUCTION

Aspergillus flavus is a pathogenic fungus which secretes most toxic aflatoxins hazardous to animals as well as human beings[1]. There are four major class of aflatoxins, aflatoxinsB1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), amongst these Aflatoxin B1 is the most common and the most widespread aflatoxin in the world and accounts for 75% of all aflatoxins contamination of food and feeds. Aflatoxin contaminated food and commodities pose serious hazards to the health of humans and animals. They are most commonly known for causing acute or chronic liver disease [2]. The ingestion of these contaminated materials leads to serious health problems, such as liver, kidney or nervous system damage, immunosuppression and carcinogenesis [3].

Aspergillus flavus is found globally as a saprophyte in soils and decaying vegetation which causes disease on many important agricultural crops. Common hosts of the pathogen are cereal grains, legumes and tree nuts. In order to prevent the menace of Fungus it is important to find a natural antifungal agent as use of synthetic compounds for control of these fungi has always raised concerns about the environmental impact and the potential health risks related to their use[4]. There are number of crops, rhizomes and tubers which successfully flourish in soil, it can be assumed that they have some kind of mechanism to save them from the attack of soil invading fungi. Keeping view of this fact two rhizomatous species, belonging to Zingiberaceae family, reported to have broad spectrum antimicrobial potential[5] were selected for antifungal activity against *Aspergillus flavus*, these were namely *Curcuma longa* and *Curcuma amada*.

MATERIALS AND METHODS

Collection and Processing of Plant Materials: Fresh rhizomes of *Curcuma amada* and *Curcuma longa* were collected from medicinal garden of Regional Plant Resource Center (RPRC), Bhubaneswar. Bulbs were washed with running tap water to remove dust and impurities, were sliced into thin chips for proper drying. After drying they were made into fine powder, which was used for preparation of solvent extracts as per the standard protocols[6].

Phytochemical Screening of the plant Extracts: Phytochemical analysis of rhizome of *Curcuma longa* and *Curcuma amada* were done by the standard phytochemical screening methods[7]. Samples were tested for presence of different phytochemicals like Alkaloids, Flavonoids, Anthraquinones, Saponins, Terpenoids, Cardiac glycosides and Tannins.

Antifungal activity of solvent extracts

A. By agar well diffusion method: Stock solutions of all the rhizome extracts were prepared in DMSO (20mg extract/1ml). Spore suspension (1×10^6/100 µl) was spread evenly over each PDA plates, Different doses (100µg, 200µg, 400µg and 800 µg) of extract was poured in the wells cut in plates. Control and experimental samples were kept for incubation at 32˚C for 72 hours. Zone of inhibition was measured for every sample. Each assay was performed in triplicates and experiment was repeated thrice for each solvent extract.

B. By radical growth method: The antifungal properties of the extracts were determined by using the modified radical growth method [8]. 50µl of each extracts (1000 µg/ml) were spread over solidified PDA plates. Centre of plates was inoculated with single spore. Plates were kept for incubation at 32˚C. Each assay was conducted in triplicate. Radical growth was measured on alternative days starting from day 2 to day 14 until the entire plate gets covered with fungal mat. Experiment was repeated thrice for every solvent.

Isolation and estimation of aflatoxin content and estimation of biomass of the control and experimental samples: Sabouroud liquid broth medium of control (without extract) and experimental samples (with solvent extracts) were inoculated with fungal spores ($1X10^6$). On day 15th of inoculation, samples were deactivated by autoclaving. Samples were filtered for removing the fungal mats, biomass was weighed for every extract and percentage inhibition was calculated. After removing the fungal mats from the broth by filtration, culture filtrate was transferred to a separatory funnel, extracted with equal volume of chloroform, shaken for about 30 minutes and allowed to stand for 30 minutes. The organic phase was separated and was left for drying at room temperature. The dried filtrates obtained, contains the crude aflatoxin content. Crude aflatoxin of control and experimental sample was estimated by taking absorbance at 400nm using microplate reader, Percentage inhibition was calculated comparing with the control samples. Fungal mats of all the samples were dried and weighed to estimate the biomass inhibition by the solvent extracts of C. amada and C longa.

RESULTS AND DISCUSSION

Phytochemical Screening: The phytochemical tests were conducted to depict the presence of secondary metabolites in the plant. As can be seen in Table 1, Although both the species belong to same genus Curcuma yet there was a difference in their pytochemical moieties. Flavonoids and sapponins were common to both, where as

Alkaloids and phlobotanin were present in *Curcuma longa*, the study is in comfirmation with earlier studies as a large number of alkaloids like curcuminoides have been isolated from the plant[9] Terpenoids, cardiac glycosides and tannin were found in *Curcuma amada* rhizome extracts. In earlier studies also terpenoids have been isolated from the oil extract for the species[10].

Table 1: Phytochemical analysis of solvent extracts of rhizomes of *Curcuma amada* and *Curcuma longa*

Phytochemical	Hexane		Chloroform		E.acetate		Methanol	
	C.amada	C.longa	C.amada	C.longa	C.amada	C.longa	C.amada	C.longa
Alkaloid	-	-	-	+	-	+	-	+
Flavonoid	+	+	+	+	+	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-
Saponin	+	-	+	-	+	+	+	+
Terpenoids	-	-	-	-	+	-	-	-
Cardiac glycosides	-	-	-	-	+	-	+	-
Tannin	-	-	-	-	-	-	+	-
Starch	-	-	-	-	-	-	-	-
Phlobatannin	-	-	-	+	-	-	-	+

Antifungal activity of solvent extracts:

Agar well diffusion method: Highest Zone of inhibition was observed in hexane extracts of *C. longa* at dose of 0.8 mg/ml followed by chloroform and ethyl acetate whereas, methanol extracts showed no zone of inhibition. *C. amada* chloroform and ethyl acetate extracts were active against A. flavus depicting homogenous readings on all the doses of chloroform whereas ethyl acetate showed dose dependent activity with better activity at higher doses. This indicated that activity reduced with increasing polarity. A large number of active principles have been isolated from *Curcuma longa*, which have broad spectrum of medicinal properties like anti inflammatory and anti oxidant activities[10].

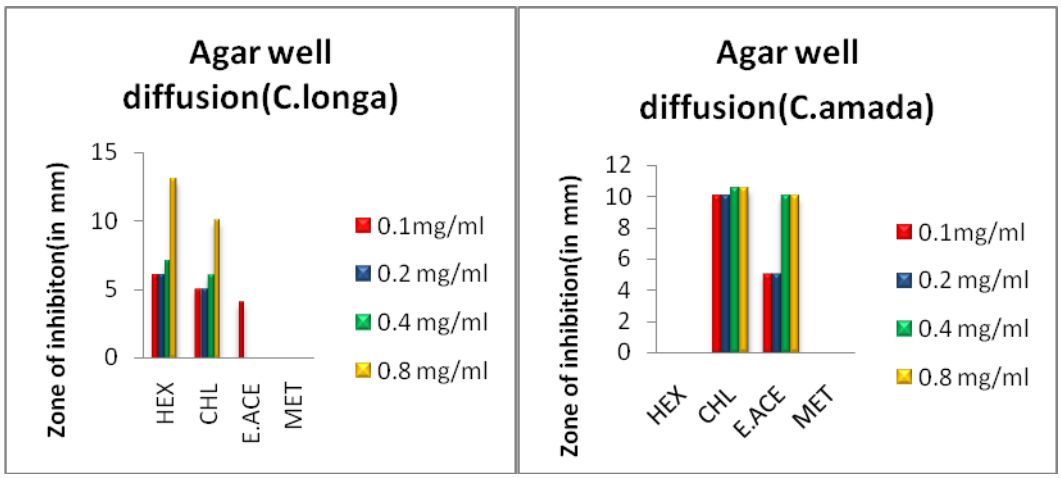

Fig 1: Effect of C.longa and C.amada on *A. flavus* using agar well diffusion method

Radical growth method: The radical growth is expressed in terms of percentage of growth inhibition. Growth inhibition was highest (59.25%) on day 6 in hexane extracts of C. longa, while other extracts showed mild growth inhibition as compared to the positive control(DMSO)(Fig 2).whereas in *C.amada* extracts growth inhibition was observed highest in chloroform extracts(66.66%) followed by ethyl acetate (53.33)% on 3rd day of observation(Fig 3). Remaining extracts showed insignificant activities. Thus results of both the methods confirmed the activity of non polar extracts.

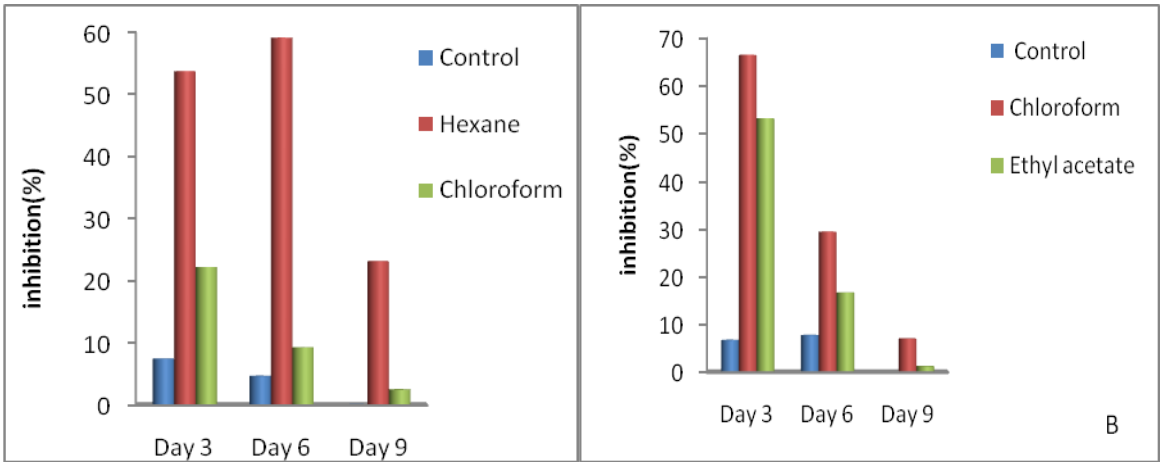

Fig 2: Effect of *C. longa*(A) and *C. amada*(B) active extracts on *Aspergillus flavus* radical growth.

Quantitative analysis of aflatoxin content: The dried filtrates obtained, contains the aflatoxin which were analyzed by taking absorbance at different dilutions i.e. 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml at 400 nm wavelength by micro plate reader along with control . Dilutions were made using water: methanol (1:1). Growth inhibition was calculated on the basis of absorbance observed at 400 nm wavelength. Highest inhibition was observed in hexane rhizome extracts of C. longa with 47.19%, followed by chloroform with 44.14% at 200 µg/ml doses(Fig 3), whereas in *C.amada* rhizome extracts growth inhibition was observed highest in chloroform extracts (43.94%) at 200 µg/ml doses (Fig 4).

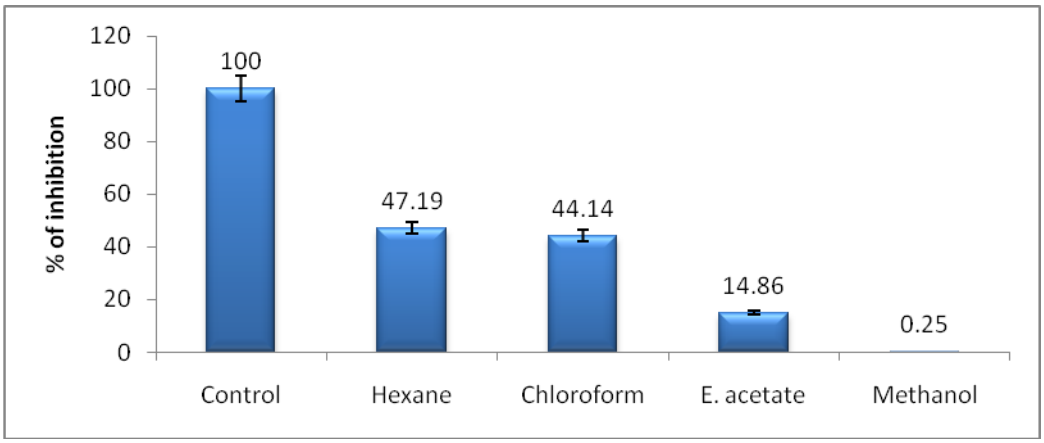

Fig3: Inhibition of different extracts of *C.longa* against *A.flavus* at 200µg/ml

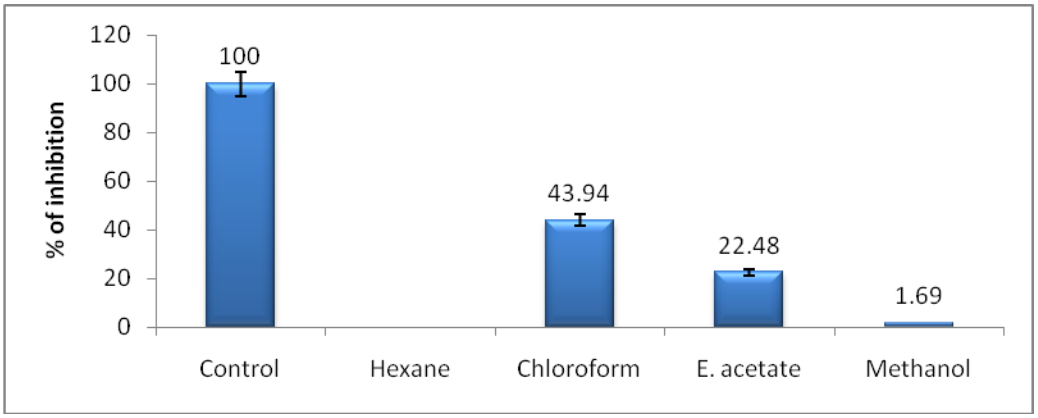

Fig4: Inhibition of different extracts of *C.amada* against *A.flavus* at 200µg/ml

Biomass inhibition assay: Weight of experimental Fungal mats obtained from the liquid broth medium were compared with the control samples to assess the extent of growth of fungus under treatment with solvent extracts. As can be observed in Table 2, Hexane extract of the *Curcuma longa* rhizome

extracts showed significant inhibition of fungal growth(76.12%) followed by Chloroform extract(60.89 percent, other extracts exhibited less than 50% inhibition of fungal growth in liquid medium. Results obtained are justified as *Curcuma longa* has oil extracts endowed with large number of active principles like curcumin, curcuminoides etc[11]. Whereas *Curcuma amada* is used mainly as pickles and possess a number of anti-inflammatory and antioxidant properties [12]

Table2: Biomass inhibition using Liquid broth method by C. longa and C.amada

Sample	C.longa (Mean ±SD)	C.amada (Mean ±SD)
Hexane	76.12±0.85**	25.88±3.47
Chloroform	60.89±5.14	49.41±4.92
Ethyl acetate	32.87±1.57	45.98±4.96
Methanol	15.22±4.48	NIL

**p value < 0.005

Overall study has provided lead in the form of chloroform extract of *Curcuma amada* which showed marked antifungal potential in all the methods like radical growth, biomass and agar diffusion method and hence needs further exploration.

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