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Elementary Chemical Profiling and Antifungal Properties of Cashew (*Anacardium occidentale* L.) Nuts

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Abstract: Cashew (*Anacardium occidentale* L.) has high demand in the world market due to wide range of applications. Cashew nut shell liquid (CNSL) has various range of biological properties. In the present study, an attempt was made to identify the phytochemicals present in the CNSL using solvent extract method. Antifungal activity of acetone, ethanol and ethyl acetate extracts on *A. flavus*, *A. fumigatus*, *A. niger*, *Curvalaria* sp and *Fusarium* sp. were studied. Distinct range of antifungal spectrum observed was compared with the phytochemicals present. It is concluded that ethanol extracts were found to have broad spectrum and higher percentage of antifungal activity.

Key words: *Anacardium occidentale* L. · CNSL · Phytochemicals · Antifungal activity

INTRODUCTION

Anacardium occidentale L. belonging to Anacardiaceae member, native of Brazil and have great economic and medicinal value. The commercial importance of cashew is due to its richness in nutrient that constitutes of 47% fat, 21% protein and 22% carbohydrate, vitamins and all essential aminoacids especially thiamine [1]. India is the second largest producer of cashew nuts that accounts of 25% world exports. The liquid obtained from the shell of the nut, CNSL have wide commercial applications [2-4], biological and medicinal properties. The biological properties of CNSL such as larvicidal [5], molluscicidal [6, 7]; antifungal and antibacterial [8, 9] were also reported. The medicinal properties of phytochemicals present in CNSL reported are cytotoxic activity against several tumor cell lines [10], anti-diabetic [11], anti-inflammatory and analgesic effects [12, 13].

The phytochemicals are the prime and bioactive compounds of plants that are responsible for the extended biological properties. Innovative approaches to explore the biological properties exhibited by phytochemical compounds present in the plant nut extracts would be helpful to reach the market and economy to a considerable extent. The present investigation to analyze the phytochemicals present in three different extracts viz., ethanol, ethyl acetate and acetone of cashew seed coats. The antifungal property of *A. occidentale* L. seed coats against five various fungal isolates have been worked out. From these studies, it would be able to relate that phytochemicals entails in the antifungal activity of CNSL.

MATERIALS AND METHODS

The nuts of *A. occidentale* were collected from cashew plantation areas of Thathanur, Ariyalur dt, Tamilnadu, India. The seed coats of *A. occidentale* L. were dried at room temperature, ground into powder and used for further analysis.

Analysis of Phytochemicals of *A. occidentale* L Nuts: The solvents chosen with different polarity to bring out all the phytochemicals present in the cashew seed coats. 30 g of seed coats powder was extracted with 250 ml of ethanol, acetone and ethyl acetate by continuous hot percolation (75°C) for 72 hrs. The solvent present in the extract were removed by distillation under reduced pressure to obtain a dark brown residue. The extractants were tested for the presence of elementary phytochemicals like alkaloids, carbohydrates, flavonoids, phenolics, steroids, triterpenoids, volatile oils and xanthoproteins following standard methods [14].

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Crude Cashew Nut Seed Coat Extract: 1gm of Cashew seed coat powder was extracted using three different solvents by hot percolation method. The extractant was centrifuged and the supernatants obtained were used for antifungal assay.

Antifungal Assay: Fungal cultures: Five human pathogenic fungi namely, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium* sp. and *Curvalaria* sp. were grown and maintained on potato dextrose agar medium (PDA).

***In vitro* Antifungal Activity Test:** *In vitro* antifungal activity test was carried out by disk diffusion agar plate assay [15]. PDA was poured into sterile petriplates and allowed to solidify; 3 days old fungal broth cultures serve as inoculum and spread over the agar surface. The extracts of 10 µl was added onto a sterile filter paper discs measuring 5 mm diameter and allowed only phytochemicals to be the causative agents for mycelial growth inhibition. The plates were incubated at 25°C for 3 days and the colony diameter in each plate was recorded. For control plate, 10 µl of Amphotericin B was added and each experiment was performed in three replicates.

Statistical Analysis: DMRT analysis was done to interpret the results with reference to [16].

RESULTS AND DISCUSSION

Analysis of Phytochemicals of *A. occidentale* L. Nuts: A variety of rich secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils are present in plants in general [17]. The ethanolic extracts of *A. occidentale* L. nuts result the presence of various phytochemicals compounds such as triterpenoids, phenolics and volatile oils. Ethyl acetate extracts exhibited a different combination of phytochemicals, phenolics, volatile oils, xanthoprotein and carbohydrates. Acetone found to be effective in dissolving the phytochemicals since many different compounds like triterpenoids, phenolics, volatile oils, flavonoids, xanthoprotein and carbohydrates (Table 1). However, Acetone, acted as good solvent for flavonoids extraction. The obtained results are in accordance with the reports of Tedong *et al.,* [18] that phytochemical analysis of *A. occidentale* L. revealed the presence of alkaloids, polyphenols and saponins.

Table 1: Chemical profiling of *Anacardium occidentale* L. nuts using different solvents

Phytochemicals	*Ethanol*	*Ethyl acetate*	Acetone
Alkaloids	-	-	-
Emodins	-	-	-
Flavonoids	-	-	+
Steroids	-	-	-
Triterpenoids	+	-	+
Anthracene glycosides	-	-	-
Phenolics	+	+	+
Volatile oils	+	+	+
Xanthoprotein	-	+	+
Carbohydrates	-	+	+

+-Present --Absent

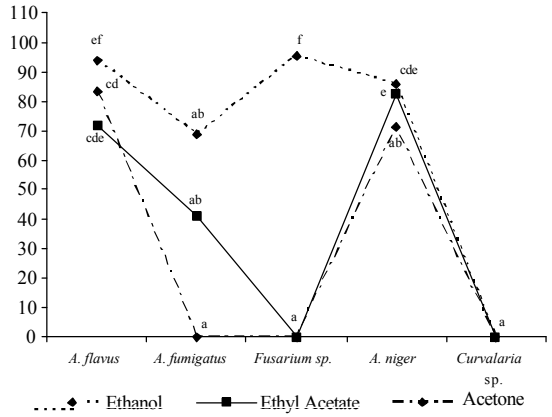

Fig. 1: Percentage of Fungal Inhibition of *A. occidentale* L. nut extracts

Antifungal Assay: The ethanolic extracts of *A. occidentale* L. nut extracts showed antifungal activity 4 out of 5 pathogenic isolates. The maximum percentage of inhibition was shown on *A. flavus* (94%) and *Fusarium* sp. (95.45%) and less against *A. fumigatus* (68.96%) (Fig. 1). *Curvalaria* sp. is highly resistant towards *A. occidentale* L. nut extracts and showed nil percentage of inhibition. The ethyl acetate extracts had less percentage of antifungal activity, inhibited 3 fungal isolates. *A. niger* (82.14%) was more sensitive compared to *A. flavus* (72%) and *A. fumigatus* (41.37%). No activity was seen in the isolates of *Fusarium* sp. and *Curvalaria* sp. As similar with the case of ethanol extract, ethyl acetate also showed minimum inhibitory action on *A. fumigatus*. *A. flavus* and *A. niger* were sensitive and *Fusarium* sp. and *Curvalaria* sp. exhibited resistance pattern. Incase of acetone extracts, *A. flavus* (83.3%) and *A. niger* (71.42%) were sensitive. Fungistatic effect of A. *occidentale* ranged from 95% to 41.37%. As well as

Fusarium sp. and *A. fumigatus* were shown resistance against both the extracts and acetone respectively. But *A. flavus* and *Fusarium* sp. were very sensitive to ethanol extracts and the percentage of inhibition is morethan 94%. There are several reports by early researchers on antifungal potential possessed by plant extracts and phytochemicals [19-22].

All the three extracts possess antifungal activity and effective against *A. niger* and *A. flavus*. *Curvalaria* sp. was resistant to inhibition. The antifungal activity is due to the bioactive components that may active against fungal isolates individually or in combination. Fungistatic effects by ethanol extracts against *A. niger*, *A. flavus*, *A. fumigatus* and *Fusarium* sp. are due to triterpenoids, phenolics and volatile oils. These compounds do not support *Curvalaria* sp. inhibition, the concentration of compounds present in the sample may not be sufficient for growth inhibition. Ethyl acetate extracts contains phenolics, volatile oils and xanthoprotein that are responsible for inhibition of *A. niger*, *A. flavus* and *A. fumigatus*. This mixture of phytochemicals had specific activity towards the genus *Aspergillus* sp. and not to *Curvalaria* sp. and *Fusarium* sp. Since phenolics are well known antifungal compounds present in plants [23]. These are the prime compounds that rapidly accumulate at the site of the infection in plants to resist the fungal or other pathogens [24]. The effect of volatiles on *F. oxysporum* was reported by Tariq and Magee [25].

By equating the antifungal activity by these two extracts, it is concluded that triterpenoids possess the capability to inhibit *Fusarium* sp. This is proved by Anke *et al*. [26] that broad spectrum antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus*, *Fusarium verticillioides*, *Trichophyton mentagrophytes* and *Microsporum gypseum* at concentration 10-50 mg/ml by benzoic acid esters of a tetracyclic triterpenoids. Antifungal activity of tetranotiterpenoids was stated by Govindachari *et al.* [27].

The acetone extracts of *A. occidentale* L. consists more number of phytochemicals than ethanol and ethyl acetate extracts but the antifungal spectrum is comparatively less than ethanol extracts. The inhibition zone was observed incase of *A. niger* and *A. flavus*. These two organisms were very sensitive since inhibited by all the three extracts. The common bioactive components responsible for inhibition were phenolics and volatile oils. *In vitro* studies on antifungal activity of acetone extracts of garlic had been reported previously [28]. The extract can able to inhibit mycelial growth and spore germination of fungus. The antifungal activity studied on *Aspergillus flavus, Aspergillus fumigatus*, *Fusarium oxysporum* and *Curvalaria lunata* and several other fungal species supported our investigation. Ezoubeiri *et al.* [29] stated that phenolic compounds are responsible for antifungal activity against *C. albicans* and the phenolics compounds extracted from *Pulicaria odora* L. The inhibitory action against *A. fumigatus* and *Fusarium* sp. was not found in acetone extract; the bioactive component responsible for the antifungal activity may be suppressed by the occurrence of an additional compound of flavonoids. The inference was controversial to the works of following researchers. Grayer and Harborne [30] surveyed antifungal phenolics compounds from higher plants and many among them are flavonoids. Some *in vitro* studies reveal a structure-activity relationship and fungal growth inhibiting effect with respect to the antifungal effect of flavonoids [31]. This may be due to the presence of synergistic activity of several other secondary metabolites.

Ethanol was able to dissolve only certain compounds but showed a broad spectrum of activity towards fungal isolates and higher percentage of activity was also observed. Ethyl acetate, a medium polar solvent dissolved phytocompounds higher than ethanol and the antifungal spectrum is also moderate. Incase of acetone extract, 7 different compounds occurred and had narrow spectrum of antifungal activity.

Geyid *et al.* [32] conducted chemical screening of 67 plant extracts showed the presence of several secondary metabolites, mainly, polyphenols, alkaloids, tannins sterols/terpenes, saponins and glycosides. Fungicidal and /or growth retardation activity of the plant extracts were reported. The strong antifungal properties of plant extracts are demonstrated due to the presence of secondary metabolites. The variations in the activities were due to difference in the concentrations of bioactive components in the sample or synergistic reaction of various phytochemicals in the extracts. This note is in accordance with the statement that the presence of certain compounds acts synergistically/additively when mixed with several proportions [33]. The broad and narrow spectrum activities of the plant extracts on the fungus are directly related to the nature and potential of the chemical compounds.

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