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Research Article

QUALITATIVE PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF *CARICA PAPAYA* LEAF EXTRACT AGAINST HUMAN AND PLANT PATHOGENIC FUNGI

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ABSTRACT

Plants have been explored extensively all over the globe in quest of a novel bioactive compound that could a good therapeutic candidate treating infectious diseases especially against drug resistant microbes. Qualitative phytochemical analyses of *Carica papaya* leaf extract reveal that except steroids and tannins all the possible phytochemical constituents including carbohydrates, proteins, anthraquinones, flavonoids, saponins, cardiac glycosides and alkaloids were present. Two ways of *Carica papaya* leaf extract preparations i.e crushed and boiled were tested for their antifungal activity against 6 saprophytic fungi *Penicillium* sp, *Aspergilus flavus, Aspergillus niger, Fusarium* sp, *Rhizopus* and *Helminthosporum*, 5 dermatophytic fungi *Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans* and 6 yeasts including *Candida albicans*, *Candida albicans ATCC 0383, Saccharomyces cerevisiae*, *Candida galbrata, Candida tropicalis, Candida kruzei.* The activity was found against majority of fungi but was much better in case of crushed leaf extract.

Keywords: saprophytic fungi, yeasts.

INTRODUCTION

Bioactive compounds derived from natural sources could be a potential candidate as antifungal agent, especially in the current scenario in which human and plant fungal pathogens have adopted resistance against antifungal antibiotics. In humans and animals, skin, hair, nail and other tissues are infected by several organisms[1]; mainly dermatophytes and cause seriously suffer with dermatomycosis[2]. *Carica papaya* (Caricaceae) is believed to probably originate from Southern Mexico and Costa Rica and then plantation crop was started in all almost all regions of tropical and subtropical[3]. *Carica papaya* is widely grown now and used in different parts of the world not only for food but also for ornamental purpose[4]. It has also been utilized in traditional medicine for providing relief in various ailments[5]. In Pakistan, it is also widely cultivated in Punjab and Sindh[6]. The burden of Infectious diseases is a big challenge and nuisance to human health and responsible for almost 50,000 deaths on daily basis[7-8]. Plants have now scientifically proven as effective, cheaper alternative sources and have very least side effects than commercially available synthetic drugs[9-10]. The aim of this study was to assess the antifungal activity of *Carica papaya* two different preparations of leaf extracts against pathogenic human and plant fungi.

MATERIALS AND METHOD

Collection and Preparation of Extracts

The fresh leaves from Papaya tree were plucked from the road side gently from a cosmopolitan city –Karachi-Pakistan in a summer season in 2012. These fresh leaves were thoroughly washed with tap water and later rinsed with sterile distilled water. The extracts were prepared by two treatments.

a) In one treatment, 5 % leaf extract was prepared by boiling leaves for 15 minutes by constant stirring[11].
b) In second treatment, the leaves were pulverized, using sterile laboratory mortar and pestle, to get a thick paste, later suspended in 100 ml of sterilized water[12].

Both the extracts were stored in air tight glass containers sealed further with parafilm protected from sunlight till further work.

Sterility Testing of Extracts

The sterility testing of extract was done by passing extract through Millipore filter (0.22 micron meter). Later, inoculate 2 ml of sterile extract into 10 ml of sterile nutrient and Sabourd dextrose broth. Incubation was done at 37^0c for 24 h. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation[13].

Screening of Antifungal Activity

The test organisms for this study were members of the 6 saprophytic fungi *Penicillium* sp, *Aspergilus flavus, Aspergillus niger, Fusarium* sp, *Rhizopus* and *Helminthosporum*, 5 dermatophytic *Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans* and 6 yeasts including *Candida albicans*, *Candida albicans ATCC 0383, Saccharomyces cerevisiae*, *Candida galbrata, Candida tropicalis, Candida kruzei.* The fungal isolates were obtained from the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan. All the fungal isolates were checked for purity and maintained on Sabourd Dextrose agar (SDA) at 4^0c in the refrigerator until required for use. Antifungal activity papaya leaf extract was tested using agar-well method. Autoclaved distilled water was used for the preparation of fungal spore suspension and transferred aseptically into each SDA plates[14]. All plates were incubated at 28 $\pm$ 2°C for 24-48 h

and after incubation diameter of zone of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory Concentration (MIC) of leaf extracts was found out by Micro broth dilution method using 96-well microtitre plate[11]. Two fold serial dilutions of extracts was made in 100 µl broth and subsequently 10 µl of two hour refreshed culture matched with 0.5 Mac Farland index was added to each well. One well served as antifungal agent control while other served as culture control. Microtitre plate was incubated for 24 h at 37 ºc. The MIC was read as the well showing no visible growth.

Qualitative Phytochemical Screening

The Chemical tests for the qualitative analysis of *Carica papaya* on dried finely ground powder extract specimens using the standard methodology with slight modification[15-16].

Table 1: Qualitative Phytochemical Analyses of *Carica papaya* Leaf Extract

Tests	Presence / absence
Flavanoids	+
Alkaloids	+
Tannins	-
Saponins	+
Cardiac glycosides	+
Anthroquinones	+
Steriods	-
Carbohydrates	+
Proteins	+

Table 2: *In vitro* Antifungal Activity of *Carica papaya* Leaf Extracts against Human and Plant Pathogenic Fungi by Agar Well Method

Types of treatments / Name of tested Fungi	Papaya leaf extract (crushed) mm	Papaya leaf extract (Boiled) mm
Candida albicans	20	13
Candida albicans ATCC 0383	19	12
Saccharomyces cerevisiae	-	-
Candida galbrata	19	15
Candida tropicalis	21	15
Candida kruzei	20	16
Microsporum canis	20	19
Microsporum gypseum	-	-
Trichophyton rubrum	-	-
Trichophyton mentagrophytes	18	16
Trichophyton tonsurans	-	-
Aspergillus flavus	19	15
Aspergillus niger	17	16
Fusarium sp.	-	-
Penicillium sp	19	15
Rhizopus	20	17
Helminthosporum	-	-

*For the sake of comparison of results of test drug, negative control was distilled water and positive control was an antifungal drug i.e Griseofulvin was used.

Table 3: Minimum Inhibitory Concentration (MIC) of *Carica papaya* Leaf Extracts Against Human and Plant Pathogenic Fungi by Microbroth Dilution Method

Name of tested fungi	Crude extract (mg / ml)	Aqueous extract (mg / ml)
Candida albicans	140	120
Candida albicans ATCC 0383	160	140
Saccharomyces cerevisiae	-	-
Candida galbrata	180	200
Candida tropicalis	140	140
Candida kruzei	120	80
Microsporum canis	260	320
Microsporum gypseum	-	-
Trichophyton rubrum	-	-
Trichophyton mentagrophytes	180	240
Trichophyton tonsurans	-	-
Aspergillus flavus	120	160
Aspergillus niger	160	220
Fusarium sp.	-	-
Penicillium sp	80	40
Rhizopus	120	160
Helminthosporum	-	-

*For the sake of comparison of results of test drug, negative control was distilled water and positive control was an antifungal drug i.e Griseofulvin was used.

RESULTS AND DISCUSSION

In Pakistan, there are approximately 6000 species of flowering plants and 700 of them have medicinal properties[17-18]. Pakistan is rich with variety of vegetation that are used by local communities for the sake of treatment and prevention of diseases[19]. Today, all over the world, there is vast interest in exploiting the potential of drugs from plant origin[20]. According to our reserach findings regarding the qualitative anlysis of *Carica papaya* leaf extract, except steroids and tannins all the possible phytochemical constituents were present. The extract possessed carbohydrates, proteins, anthraquinones, flavonoids, saponins and alkaloids as mentioned in Table 1. These findings were in agreement of similar nature of study conducted in past in which researchers

did not find tannins rather other substances were present like saponins, cardiac glycosides and alkaloids[21]. Moreover, flavonoids are very important constituent of natural product and have got apart antioxidant activity and also an ability to combat tumor growth[22]. In current study, the antifungal ctivity was obatined against majority of clinical and plant pathogenic fungi. Interestingly, the activity was achieved in both natures of extract preparations. Moreover, the activity was noted slighly better in crushed nature of extract than boiled one. Among 6 saprophytic fungi activity was observed in only 4 saprophytic fungi namely; *Penicillium* sp, *Aspergillus flavus, Aspergillus niger, Rhizopus* in the range of 15-20 mm zone of inhibition. In case of 5 dermatophytic fungi, activity was observed in only 2 fungi namely; *Microsporum canis* and *Trichophyton mentagrophytes i*n the range of 16-20 mm. While; in yeast results, among 6 yeasts tested, activity was observed in all Candida species including *Candida albicans*, *Candida albicans ATCC 0383,Candida galbrata, Candida tropicalis, Candida kruzei* in the range of 12-20 mm as mentioned in Table 2. Minimum inhibitory concentration (MIC) in 4 saprophytic fungi were found 4 saprophytic fungi namely; *Penicillium* sp, *Aspergillus flavus, Aspergillus niger, Rhizopus* in the range of 120-160 mg / ml. MIC of *Microsporum canis* and *Trichophyton mentagrophytes, dermatophytic fungi* were in the range of 180-320 mg / ml. While; in yeasts, MIC were found in *Candida albicans*, *Candida albicans ATCC 0383, Candida galbrata, Candida tropicalis, Candida kruzei* in the range of 80-200 mg / ml as indicated in Table 3. All the antifungal results were compared with Greseiofulvin antifungal drug which gave around > 20 mm zone of inhibition in almost all fungi tested. Phytochemical analyses indicate that the extracts contain alkaloids, tannins, saponins, flavaonids, glycosides, phenols,vitamins, minerals, proteins and enzymes[15,23-24]. Some past studies also report that *Carica papaya* roots has been suggested as a purgative and its various parts are also being used to treat a number of infections like gastroenteritis, uretritis, otitis media, typhoid fever and some wound infections[23,25]. The plant is also considered to be a potent amoebicide[26]. Papaya leaf extract has also been found to have anti- malarial effect[27]. The leaves of many plants possess tannins in an abundant quantity and may have biocide activity[28]. As in one of the studies, the tannins in the leaf extracts of *Terminatia citrine* cause inhibition of fungal cell wall formation, thus leading to death of the organisms[29]. The leaf extract in one of the study showed excellent antifungal potential fungal phytopathogens like *Rhizopus stolonifer, Fusarium* spp. and *Colletotrichum gloeosporioides* (Pedro Cha´vez-Quintal *et al*, 2011) The MIC_{50} for the leaf extract was 0.625 mg ml^{-1} for *Fusarium* spp. and > 10 mg ml^{-1} for *Colletotrichum gloeosporioides*, both equal to approximately 20 % mycelial growth inhibition. In one of the study, antifungal activity (*Carica papaya*) against *Fusarium* sp and *Candida albicans* was found good suggesting it can be used as therapeutic agent. Similarly, in other study, paw-paw (*Carica papaya*) on major seed-borne fungi: *Aspergillus niger, Aspergillus flavus, Botryodiplodia theobromae* and *Fusarium moniliforme* of African yam bean (*Sphenostylis stenocarpa*) seeds and on seed germination and seedling emergence were studied *in vitro* and *in vivo*[30].

CONCLUSION

The inhibitory effect of aqueous and crude extracts of *Carica papaya* on some human and plant pathogenic fungi indicate its therapeutic potential as antifungal agents and no doubt could be an ideal candidate to combat with the current global challenges of antifungal antibiotics agents. Further, it is infact preliminary screening; however, further studies with proper scientific knowledge and documentation should be carried out to explore other areas to really make it a successful therapeutic candidate in future.

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