Mycopath (2012) 10(2): 91-95 **Research Article**

Comparative antifungal activities of processed and market *Aloe vera* gel

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Abstract

During present study, *in vitro* antifungal potential of three types of *Aloe vera* gel i.e. locally available, imported and finally processed gel that was substantially free of phenols and anthraquinones was checked against three pathogenic *Aspergillus* species namely *A. niger*, *A. parasiticus* and *A. flavus*. Growth test results of test fungi in term of dry biomass production revealed that local and imported gels have antifungal properties that become significant as the concentration of gel was increased from 5-20%. By contrast, processed gel failed to reduce the growth even at the highest concentration. Therefore, it is assumed that processed gel is least toxic as compared to local and imported *A. vera* gels and recommended its use for oral or medicinal purposes.

Keywords: *Aloe vera* gel, antifungal activity, *Aspergillus*, anthraquinones.

Introduction

Aloe vera, family Liliaceae, is presently cultivated on large scale due to increasing demand in industry (Newall *et al.*, 1996). *A. vera* products are widely used for pharmaceutical, nutraceuticals, cosmetic and food industries (Klein and Penneys, 1988). For sun burn cure, injuries and wounds healing, *A. vera* gel is regarded as most suitable herbal remedy in the United States (Foster, 1999; Eshun and He, 2004). Aloe juice stimulates the immune system of body, particularly recovers the damaged stomach lining (Davis, 1997). Therapeutic potential of *A. vera* is well reported along with antifungal, antibacterial, antiviral activity (Ferro *et al.*, 2003). Anthraquinones and dihydroxyanthraquinones are regarded as antimicrobial agent in *A. vera* gel (Wu *et al.*, 2006; Dabai *et al*., 2007). Aloe gel and leaves both are reported to inhibit growth of many fungi including *Fusarium oxysporum, Rhizoctonia* solani, *Collectotrichum coccodes*, *Candida albicans* and *Staphylococcus aureus* (Jasso *et al*., 2005; Agarry *et al*., 2005). Cock (2008) documented significant antifungal action of *A. vera* gel against *Aspergillus niger*. Arunkumar and Muthuselvam (2009) examined marked inhibition of *A. niger* and *A. flavus* due to acetone extract of *A. vera*. Sitara *et al.* (2011) found that *Aloe vera* gel significantly inhibited the activity of *A. niger*, *A. flavus*, *Alternaria alternata*, *Drechslera hawaiensis* and *Penicillum digitatum.* Antifungal activity of various *A. vera* extracts and some specific constituents, aloin and aloe-emodin was investigated against *Colletotrichum* species and *Fusarium solani* (Eugene *et al*., 2011).

Present investigations were planned to assess the comparison of antifungal activity of locally available, imported and finally processed gel against three pathogenic *Aspergillus* species namely *A. niger*, *A. parasiticus* and *A. flavus*.

Materials and Methods

Collection of Plant Material

Local and imported *A. vera* gels were purchased from local market of Lahore, Pakistan. Two-years mature sound, undamaged, fungus/rot free leaves of *A. vera* were collected in order to preserve active ingredients. To prevent contamination of the gel, the leaves were handled carefully and soaked in a food grade sanitizer.

Processing of *A. vera* Gel

The two end portions of leaves were cut off with a sharp knife to obtain the substantially anthraquinone free gel. The leaves were further sliced across the width and a transparent googey-gel was collected. The aloe gel fillets were crusher using a commercial high speed homogenized at room temperature. Cellulase enzyme was added at 50 °C and allowed to react for 20 minutes that did not induce the loss of biological activity of polysaccharide in the gel. Toxic compounds like anthraquinones and phenols were removed from the crude juice by means of adsorption chromatography and transparent gel was obtained after passing through the column. The un-

pasteurized *A. vera* gel was fortified with citric acid to improve the flavour and stabilize the gel. Like the process of other vegetable juice, pasteurization was done for 15 minutes at 45 °C under reduced pressure.

Antimicrobial activity

Three species of *Aspergillus* were obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore. Pakistan. The organisms were maintained on 2% malt extract agar slants (20 g malt extract, 20 g agar 1000 L^{-1}) and kept at refrigerated temperature.

The antifungal bioassays were carried out in malt extract (ME) broth. To avoid bacterial contamination antibacterial chloromycetin capsules at 1 100 mL^{-1} of medium was used. To 80 mL of ME, 20 mL each of 5, 10, 15 and 20% of *A. vera* gel was added. Control contained the same quantity of distilled water. Actively growing mycelial discs of *A. niger, A. flavus and A. parasiticus* were transferred to the flasks containing culture media aseptically, each treatment was in replicate of three. Flasks were incubated at 25 °C ± 2 for 10 days on an electric shaker. After 10 days, the mycelial biomass was filtered on a pre-weighted filter paper and oven dried at 60 °C for 24 hours and weighed.

Statistical Analysis

All the data was subjected to analysis of variance followed by mean separation through Duncan's Multiple Range (DMR) Test (Steel and Torrie, 1981) using computer software COSTAT.

Results and Discussion

A. vera gel is not only famous for its nutritive value but is significant due to its cosmetics and medicinal constituents. It is believed that Aloe gel has the ability to cure a range of illnesses from dermatitis to aids (Anonymous, 2008). Moreover antifungal activity of *A. vera* gel is also well established (Ali *et al*., 1999; Jasso *et al*., 2005; Bajwa *et al.,* 2007; Sitara *et al.,* 2011). In this present study, three different types of gels from *A. vera* plant were evaluated against three pathogenic species belonging to genus *Aspergillus* with the objective to explore eco-friendly and biodegradable alternatives of plant diseases control.

Antifungal activity of imported *A. vera* gel

In vitro analysis of imported *A. vera* gel depicted a general trend of decrease in biomass production with its increasing concentration from 5-20% (Fig. 1). Such results were comparable with the findings of Bajwa *et al.* (2001), where growth inhibition by aqueous extracts of Asteraceous allelopathic plants was studied against three *Aspergillus* species. The relative antifungal potential however, varied within different test species as well as the concentration of the imported gel used. Statistically non-significant inhibitory effect on *A. niger* and *A. flavus* biomass production was observed when grown in the presence of lowest concentration that became significant as the concentration of gel was increased. In case of *A. parasiticus,* although growth was inhibited significantly at all concentration as compared with the control, but inter-treatment antifungal potential was non-significant. Differential inhibitory response of fungal growth to the gel was due to species specific phytotoxins as demonstrated for other plant species (Braga *et al.,* 2007; Ahmad and Abdelgaliel, 2005).

Antifungal activity of local *A. vera* gel

The antifungal effect of locally available *A. vera* gel was not much different from that of imported gel of this plant (Fig. 2). Similar response of *A. parasiticus* and *A. flavus* (both members of aflatoxin producing group of genus *Aspergillus*) was observed. For both above mentioned fungal species, 5% concentration of local gel of *A. vera* was non toxic and was unable to reduce their growth significantly. This is probably due to nutritional compounds in the gel that masked the inhibitory effect at low concentration (Levin *et al.,* 1988). Higher the concentration of local gel more was the inhibitory effect as determined by fungal dry biomass production. Likewise, with the previous findings by Bajwa and coworkers (2007) gradual inhibition in the biomass *Alternaria alternata, A. citri, A. tenuissima* was increased as the concentration of un-processed *A. vera* gel was increased from 2-6%. In case of *A. niger,* all the concentrations of local gel of *A. vera* significantly retarded the fungal biomass production. Amongst these, 10% and 15% concentrations were the most effective in suppressing the biomass production. Toxicity of un-processed *A. vera* gel is thought to be associated with the presence of anthraquinones that would be probably higher in concentration as compared to local market and imported gel samples (Goodman *et al*., 1990).

Antifungal activity of processed *A. vera* gel

It was noticed that none of the concentration proved to be toxic or growth inhibitory for any of

the fungal strain tested (Fig. 3). Negligible and statistically non-significant fungal growth retardation was recorded for all treatments. Anthraquinones which are abundant in *A. vera* gel have antimicrobial properties that are why aloe-based drinks with lesser anthraquinones (i.e. 5 ppm) are considered safe for consumers (Mapp *et al*., 1970; Goodman *et al*., 1990; Fabio *et al*., 1995). The anthraquinones that come from the rind of the leaf (Reynolds *et al*., 1999) may cause harm to human health even when present in less than 50 ppm concentration (Madis *et al*., 1989). For processed gel, traditional hand-filleting method is used to avoid the contamination of such compounds. Further purification of processed gel is carried out by adsorption chromatography that removes aloin and anthraquinones (Ramachandra and Srinivas, 2008). During processing of *A. vera* gel phenols and anthraquinones are removed from the gel therefore processed gel possibly not have toxic effects on fungal growth.

Conclusion

It is concluded that local and imported gel exhibited more antifungal activity than processed gel. Further investigation is focused on quantitative analysis of anthraquinones components in all the gel samples.

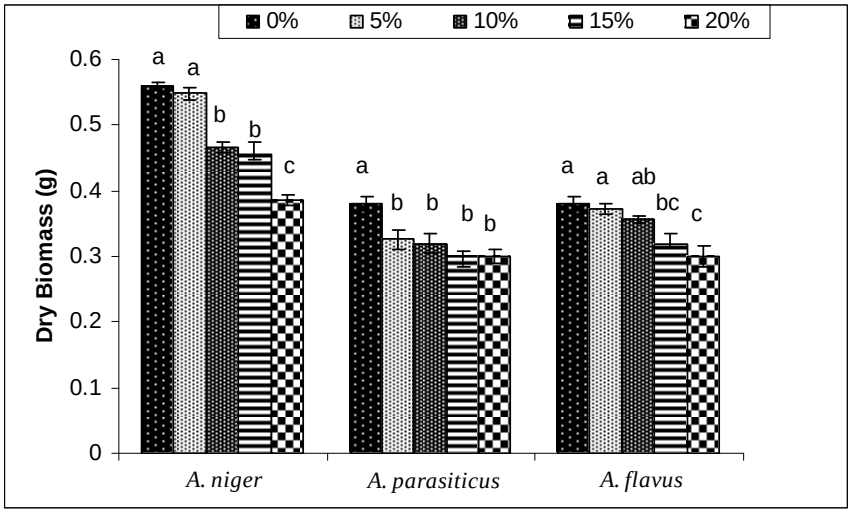

Fig. 1: Effect of different concentrations of imported *A. vera* gel on dry biomass production of *A. niger, A. parasiticus* and *A. flavus*. For each fungal species, values with different letters show significant difference at P≤0.05.Vertical bars indicate standard errors of means of three replicates. Values with different letters show significant difference ($P \leq 0.05$) as determined by DMR test.

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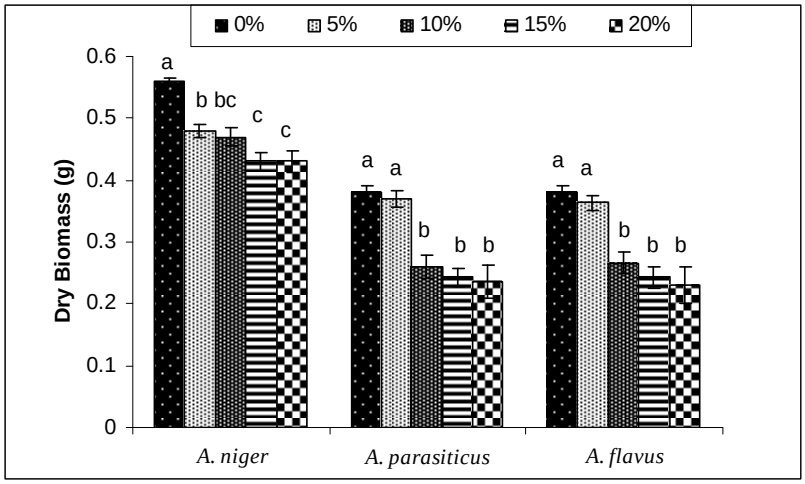

Fig. 2: Effect of different concentrations of local *A. vera* gel on dry biomass production of *A. niger, A. parasiticus* and *A. flavus.* For each fungal species, values with different letters show significant difference at P≤0.05.Vertical bars indicate standard errors of means of three replicates. Values with different letters show significant difference (P≤ 0.05) as determined by DMR test.

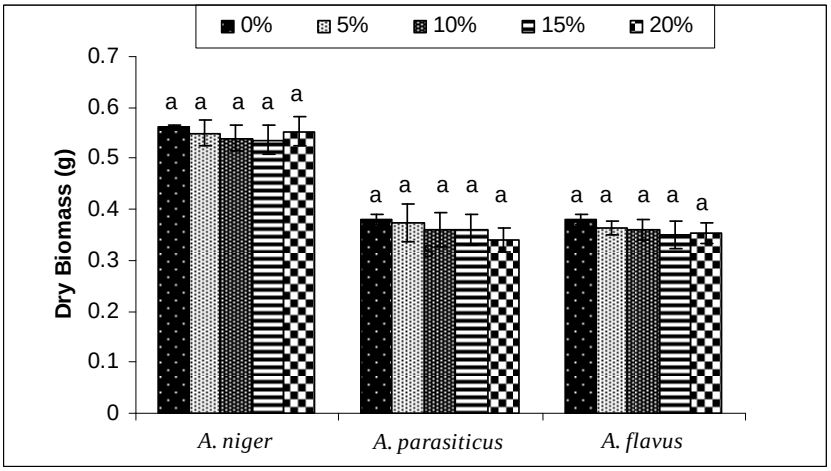

Fig. 3: Effect of different concentrations of processed gel of *A. vera* on dry biomass production of *A. niger, A. parasiticus* and *A. flavus*. For each fungal species, values with different letters show significant difference at P≤0.05.Vertical bars indicate standard errors of means of three replicates. Values with different letters show significant difference (P≤ 0.05) as determined by DMR test.

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