ORIGINAL RESEARCH PAPER

Botany

EFFICACY OF TRADITIONAL FOOD ADDITIVE KOLAKHAR ON FUNGI

KEYWORDS:

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ABSTRACT

Kolakhar is a conspicuous traditional food additive employed by indigenous and local communities of Assam since time immemorial. This home-made palatable product finds its pertinence in cooking as well as other domiciliary purposes. Its profound alkaline attribute and phytochemical constituents contribute to its use for folk remedies as agent for antibacterial, anthelminthic, pediculicidal activity etc. Present work investigates its potential antifungal effect on three fungal species namely Aspergillus spp.,Cladosporium spp. and Rhizopus spp. Sporulation capacity of kolakhar exposed spores and fungal biomass production in presence of the same was analysed.

INTRODUCTION

Kolakhar is a popular ethnic herbal edible soda prepared ubiquitously and used extensively by the people of Assam. The name kolakhar has been derived from 'kol' means banana and 'khar' means alkali. This aqueous alkaline product (antacid) is a component of traditional Assamese cuisines. Besides employing as food additive in cooking purpose, it also finds its relevance in shampooing hairs, washing clothes in villages etc. The best quality Kolakhar is prepared by filtering water through the ashes of an endogenous wild heavily seeded banana plant of North East India, i.e., Musa balbisienaColla. Thisplant is regarded as an asset of household garden, as its leaves, pseudo stem, flowers, fruits as well as fruit peels are utilized by inhabitants of Assam (Borborah et al., 2016). Young pseudo stem of this plant is also cooked as vegetable and is a rich source of iron. The raw exudates of the pseudo stem are consumed to normalize digestive disorders, pinworm infection as well as sore throat (Kalita et al., 2004). The wild fruit also helps to mitigate nutrient loss in diarrhoea. Banana plant has been found to be endowed with insecticidal, anthelminthic, anti-ulcerogenic, anti-microbial, anti-venom, anti-allergic, anti-hyperlipidemia, antidiabetic, anti-oxidant as well as anticancer properties (Debabandya M, 2010; Hussain A, 2010; Jain P, 2011; Kadhirvel K, 2010). Traditionally, rhizome, pseudo stem or fruit peels of Musa balbisiana Colla are cut into small pieces and dried under sunlight over several weeks. After burning the dry material to ashes, kolakhar is prepared by water extraction followed by filtration. The filtrate can be stored for almost a year and used to give a characteristic taste to many native cuisines. Kolakhar is also used by farmers to kill leaches and forfend their attack while working in leech infected fields. It is also used as pesticides in different agricultural fields (Kalita et al., 2014). It also cures and prevents certain cattle diseases (Deka et al., 2007). Previous studies of this folk medicine have unveiled that Kolakhar is rich in alkali elements like potassium, sodium, calcium, carbonate, chloride conjointly other important elements like vanadium and zinc in significant amount which are perceived for its therapeutic properties.Because of the presence of vanadium, kolakhar can have propitious effect for heart patients (Mudiar et al., 2014).

Recently, diligence has been drawn by this accustomed home-made soda, for its potential pharmacological activities. It has been already found to possess analgesic activity, antibacterial, antioxidant as well as anti-lice activity (Kalita et al. 2014; Kalita P, 2015; Kalita et al., 2015). Inhibitory effect on -glucosidase and -amylase enzymes exhibited by kolakhar indicates that this can also be used as anti-diabetic agent (Kalita et al., 2015).

Fungi are heterotrophic organisms consist of yeasts, moulds and mushrooms. A variety of fungi are responsible for pathogenesis in plants as well as animals including humans. Some moulds releases myco-toxins that can result in poisoning of host, may lead to death. Phyto-pathogenic fungi can cause serious damage to fruit harvest and other crops. There are more than 300 fungal species are responsible for human infection. Aspergillus, Candida, Cladosporium are the most common fungal pathogens. The condition of infection worsens due to emergence of drug insensitive fungi making the treatment difficult. In developing countries like India, where antibiotics are used indiscriminately, emergence of drug resistant infection is quite common especially in immune-compromised individuals like transplant recipients, HIV/AIDS and cancer patients.

Hitherto, effect of kolakhar on fungi has not been demonstrated. This paper focuses on its affectivity against vegetative mycelia as well as spores of three fungal species namely, Aspergillus, Cladosporium and Rhizopus.

Cytotoxicity of kolakhar on spores of all three fungi was assessed and determined by observing CFU count on media after germinating pre-exposed spores. Effect of kolakhar incorporated media, on fungal biomass production was also scrutinized.

MATERIALS AND METHODS

Materials: Potato dextrose broth (PDB) and potato dextrose agar(PDA) were purchased from HiMedia Laboratories Pvt. Ltd, India.

Kolakhar preparation: Ash sample from dried Musa balbisiana Colla plant was collected from Guwahati, Assam for Kolakhar preparation. 25% ash slurry was filtered through a muslin cloth to make Kolakhar. The pH was checked and its absorption spectrum was analysed by UV-Vis spectroscopic analysis. This herbal soda was used to make PDB media for biomass production by replacing distilled water.

Culture preparation: Cultures of Aspergillus spp., Cladosporium spp., Rhizopus spp., were collected from Department of Biotechnology, University of Mumbai. All cultures were grown and maintained on PDA. The fungi were grown for a week for sporulation. Spores were gently dislodged in sterile phosphate buffered saline (PBS) for cytotoxicity study. During biomass production, all three cultures were grown on PDB for 48hrs for inoculum development.

Cytotoxicity study:Spores of all three fungi at standardized concentration were exposed to kolakhar for 4hrs at room temperature. Aliquots from each suspension was spread on sterile PDA plates and incubated for 48hrs. Germinated spores were calculated as CFU/mL. Sterile distilled water was used as control.

Biomass production: A specific volume of all three fungal inoculum was inoculated in control as well as kolakhar incorporated media. The cultures were grown for 48hrs under aerobic condition. 10mL of each culture was aliquot in pre-weighed centrifuge tubes and fungal biomass produced was estimated by discarding media after mycelia sedimentation followed by overnight drying of the tubes at 55°C.All experiments were performed in duplicates and the average result was calculated.

RESULT AND DISCUSSION

The pH of the prepared kolakhar was found to be pH12.3. Its alkali elemental compositions render this attribute. Analysis of UV-Vis spectrum of kolakhar was carried out and absorbance maxima was found to be at 324.8nm with an intensity of 3.83243.Effect of this antacid on three fungal species was evaluated by exposing spores to kolakhar for 4hrs. Amongst all three, Cladosporium spp. was found to highly sensitive towards the toxicity of this herbal soda killing 100% of the spores in comparison to control. This was followed by Aspergillus spp. killing 72% of the exposed spores. Rhizopus spp. exhibited least sensitivity with 21% spore killing. The effect was also perceptible during fungal biomass production by mycelia when fungi were cultured in media containing kolakhar. 63% inhibition in biomass compared to control was observable in Aspergillus spp., whereas, in Rhizopus spp. inhibition was up to 42%. On the other hand, presence of kolakhar drastically reduced Cladosporium spp. biomass up to 95%. Amongst all three fungi, Kolakhar exhibited highest efficacy towards Cladosporium spp., in both mycelia and spore form, although effect on others cannot be neglected.

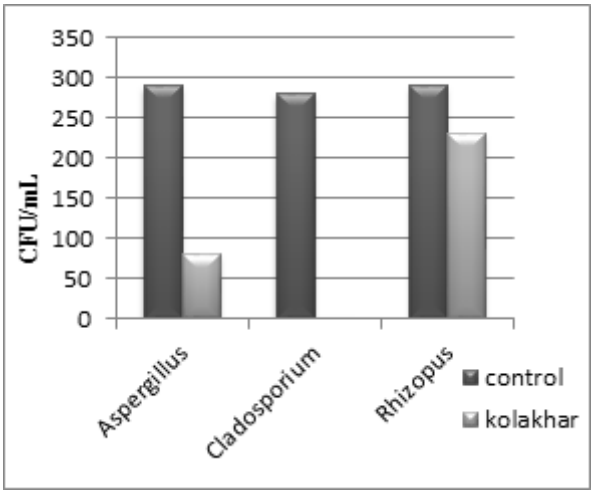

Fig1- Cytotoxicity study of spores

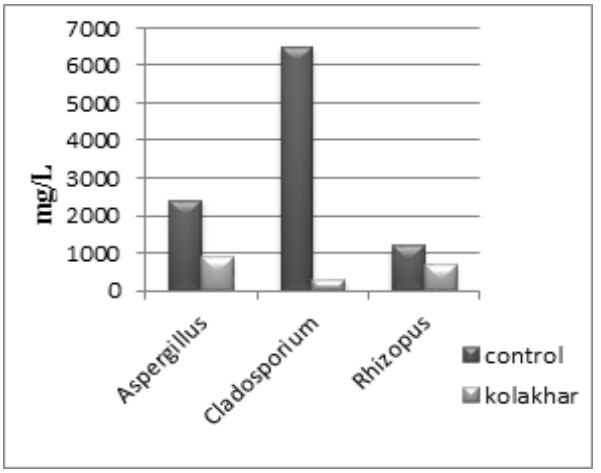

Fig 2- Effect on Fungal Biomass Production

Aspergillosis is a widespread disease in human. It also results in genesis of fruit rot disease in banana. Tomato leaf mould, fruit rot in olives is caused by Cldosporium spp. Rhizopus spp. is an opportunistic pathogen in diabetic patients. Since, antifungal drugs like amphotericin, azoles for human and chemicals like carbendazim, hexaconazole etc. for plants are always in accord with unavoidable side effects, hence, traditionally used products can be an interesting choice for treatment of mycosis both in human and in plants. As fungi require acidic condition to proliferate, treating those with highly alkaline kolakhar, inhibit its spreading and also destroy tough resistant spores. Inhibition in germination capacity assures controlled fungal dissemination. Controlled proliferation of germinated mycelial fungi is also attributed to its antacid property.

CONCLUSION:

Thorough analysis and identification of the bioactive component of kolakhar will surely pave a way to bring wisdom of the past to present for advanced and innovative drug formulation.

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REFERENCES:

1. . Borborah K, Borthakur SK & Tanti B, 2016. Musa balbisiena Colla-Taxonomy, Traditional knowledge and economic potentialities of the plant in Assam, India. Indian J Tradit knowle. 15 (1), 116-120.
2. . Kalita D & Deb B, 2004. Some folk medicines used by Sonowal Kachari Tribe of the Brahmaputra valley, Nat prod rad. 3(4), 240-246.
3. . Debabandya M, 2010. Banana and it's by product utilization: an overview. J. Sci. Ind. Res. 69, 323-329.
4. . Hussain A, 2010. In-vitro screening of the leaves of Musa paradisiaca for anthelminthic activity. J Anim Plant Sci. 20, 5-8.
5. . Jain P, 2011. Antibacterial and antioxidant activities of local seeded banana fruits. Afr. J. Pharm. Pharmacol. 5, 1398-1403.
6. . Kadhirvel K, 2010. Investigations on Anti-Diabetic Medicinal Plants Used by Tribal Inhabitants of Nalamankadai, Chitteri Reserve Forest. Dharmapuri. Ethnobotanical Leaflets. 14, 236-247.
7. . Kalita P & Kander CC, 2014. Kolakhar- a traditional herbal soda of Assam, J Adv Pharm Res Biosci. 2(5), 122-123.
8. . Deka DC & Talukdar NN, 2007. Chemical and spectroscopic investigation of kolakhar and its commercial importance. Indian J Tradit knowle. 6(1), 72-78.
9. . Mudiar RH, Kelkar V& Bhagwat A, 2014. Analysis of traditional food additive kolakhar for its physico-chemical parameters and antimicrobial activity. European Academic Research. 2(8), 10531-10536.
10. Kalita P, 2015. Phytochemical and In Vitro Antioxidant Activities of Kolakhar: A Locally Used Herbal Soda of Assam, India. Res. J. Pharm., Biol. Chem. Sci. 6(3), 1117-1122.
11. Kalita P, Kandarb CC, Deya BK & Sahariaha BJ, 2015. Anti lice activity of Kolakhar. International Journal of Institutional Pharmacy and Life Sciences. 5(4), 74-78.
12. Kalita P, Kandarb CC & Deya BK, 10-11 Oct 2015. Alpha-glucosidase and alpha-amylase enzymes inhibitory activity of kolakhar: a traditionally used local soda of Assam. Innopharm1,1st International Conference on Novel Frontiers in Pharmaceutical & Health Sciences. M. P. Council of Science and Technology, Vigyan Bhawan, Bhopal, MP, India.