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Study of Honey as a Sweet Remedy against Fungal Supremacy

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Abstract: ***Different honey samples i.e. Dabur, Himalaya, Patanjali and Madhuban pure organic honey; available in local market examined to study their antifungal property against different fungal strains including Candida albicans, Candida tropicalis and Rhizopus oryzae with Agar well diffusion method. In the present study all honey samples showed inhibitory activity against Candida albicans, In case of Candida tropicalis Dabur and Madhuban honey showed antifungal property. Surprisingly all honey sample failed to resists growth of Rhizopus oryzae. Such difference in inhibitory action may be due to variation in antimicrobial components, source of honey or processing and packaging of honey. In case of Rhizopus oryzae there may need of further dilution of honey samples. Most importantly all honey samples are best as natural remedy against Candida albicans associated infections or diseases.***

Keywords: Honey, Candida albicans, Candida tropicalis, Rhizopus oryzae, Agar well diffusion method.

I. INTRODUCTION

Honey is a sweet food made by bees (including: Apis andreniformis, A. florea, A. dorsata, A. cerana, A. koschevnikovi, A. mellifera, A. nigrocincta) [1]. Honey exports from India grew 19% year-on-year in 2018-19 to $105 million. The U.S. is the largest importer of honey in the global market and also a top destination for Indian honey (https://economictimes.indiatimes.com/). Worldwide business with bee keeping and honey production including global giant manufactures mainly distributed in Brazil, India, Oceania, Europe and other developing countries. The Europe takes the market share of 39%, followed by North America with 31%. China's consumption market has a quicker growing speed of CAGR 16.4% (https://www.marketwatch.com/press-release/global-organic-honey-market-2019-to-2024-analysis-includes-key-developments-market-share-and-size-2019-05-21).

The Indian market have a lot of honey brands that sell honey, including key players at national as well as state level market including; Hitkari, Dabur, Beez, Apis Himalaya, Zandu pure honey, 24 Mantra honey, Patanjali and Madhuban pure organic honey etc. The raw honey proved most beneficial because of loads of nutrients and without added sweetness and preservative (https://www.grabon.in/indulge/health/best-pure-organic-honey-brands-india/). Honey is generally evaluated by a physicochemical analysis of its constituents which influence the storage quality, granulation, texture and flavour, nutritional and medicinal quality of honey. The International Honey Commission (IHC) has therefore proposed certain constituents as quality criteria for honey. These constituents include: moisture content, electrical conductivity, reducing sugars, sucrose content, minerals, free acidity etc. [2]. Besides the testing of these physicochemical parameter; no doubt they are concern with purity and durability of honey, the analysis of comparative medicinal properties of honey is also most important and fruitful. There are number of branded manufactures of honey standing with the promise of being pure, natural, affordable and health benefits aspects, and obviously they are leading at national and international levels. Beside this consumers getting confused regarding choosing honey for specific health benefits. As honey is prominent antimicrobial natural medicine, everyone should know about which honey is specific for particular antimicrobial activity as a basic home remedy.

According to the Ahamad and co-workers, 2017[3] fungal diseases exert burden on the healthcare of developing and underdeveloped regions. Antibiotics treatment failed against the fungal diseases, due to increased drug resistance to commercially available anti-fungal drugs. To overcome this there is a need to develop effective and cheap antimicrobials from natural sources. These facts led us to investigate antifungal activity of different honey samples against different fungal species. According to the World Health Organization (WHO) [4] statistics, about 80% of the people prefer natural product high potential and low toxicity [5]. Honey is one of the best natural products are mostly researched now a days [6]. According to Cruz and his co-workers (2019) [7] in ancient Egypt, beekeeping has been practiced for more than 4000 years, and honey has been used as a medicine in the treatment of wounds, ulcers, burns, abscesses, gastrointestinal diseases, inflammations, rigid joints, and even as a contraceptive method. Honey composed of complex supersaturated sugar with variable 181 substances [6]. Basically substances categorised in major compounds

such as the monosaccharaides (glucose and fructose) and the minor compounds including amino acids, enzymes, vitamins and minerals and polyphenols [8]. According to Anand and co-workers (2019) [9], the phenolic compounds in the nectar honey include free phenols (volatile compounds), phenolic acids, polyphenols (usually in the form of flavonoids), anthocyanins, procyanidins, and pigments.

According to various studies by Sikorski (2004) [10]; White and Doner (1980) [11] and Gheldof and colleagues (2002) [12], the honey is variable from colourless to dark brown; it can be liquid, viscous, or solid, with the variations in flavour, aroma, and composition as per the floral source in which the honeybee collects the nectar; and also with the influence honey environmental factors like temperature and humidity. Cruz in 2019 [7] stated that honey is great potent medicine with the antibacterial, antifungal anti-inflammatory, antioxidants, anticancer and antiviral properties, due to presence of high sugar, osmotic pressure and hydrogen peroxide in diluted honey. According to Madigan and co-workers (2010) [13] Mostly people relate the fungi with organic matter decomposition or superficial fungal infections, but fungi can cause various human diseases, from mild to firmly established systemic diseases. The incidence of Candida infections is increasing worldwide. Candida albicans is present in the normal human microbiota, causes a variety of diseases, such as vaginal, oral, and systemic infections, especially in immunosuppressed patients, as carriers of the HIV virus, these infections can be further aggravated by the increase in resistance levels of this fungus to the medicines [13] [14]. C. tropicalis one of the most common Non-Albicans-Candida species isolated from various clinical types of candidiasis. In India, C. tropicalis is the most common cause of health care associated candidemia [15]. According to literature survey Moghim and co-workers (2015) [16], Mucormycosis is an opportunistic fungal infection caused by fungi in the order of mucorales and can cause acute fungal infection. A Rhizopus oryzae is major cause of mucormycosis in humans. For present work the fungal strains used are Candida albicans, Candida tropicalis and Rhizopus oryzae tested against different honey sample available at local market and area nearby.

II. MATERIALS AND METHODS:

A. *Collection of Fungal Strains*

Candida albicans, Candida tropicalis and *Rhizopus oryzae* species were obtained from department of Microbiology, Kiran micro-path Diagnostics laboratory, Miraj.

B. *Collection of Honey Samples*

The honey samples were collected from local market. These honey samples were aseptically collected in sterile bottles and kept in a cool and dry place at room temperature. The purity of honey each sample was checked by streaking on blood Agar plates.

C. *Preparation of Inoculation Suspension*

The test organism or fungal strains were cultured aseptically on the surface of sabourauds dextrose agar (SDA) and incubate at 37ºc for 1-3 weeks. The 5 to 10 days old culture, which contained spores, was lightly scraped with sterile cotton swab. The well grown sporulation colonies were wasted with 5ml sterile distilled water. The mixture was filtered through sterile cotton wool to obtain the spore suspension. This procedure helps to remove the hyphae and debris. Sterile water was added to the suspension until it reached turbidity of 0.5 McFarland, which corresponded to $1\text{-}5\times10^{6}$ CFU/ ml. In case of Candida species, the test organisms were sub-cultured on SDA plates and incubated at 37ºc 24 hrs. The colonies were scrapped using sterile cotton swab and dipped into sterile tube containing 3 ml of distilled water to obtain 0.5McFarland turbidity. Antifungal activity was determined by using agar well diffusion method. A 100ml of the test inoculum was poured onto the SDA plate by using micropipette and spread it with sterile cotton swab. The plates were allowed to dry at 37ºc for 30 mins. After that four wells of 6mm in diameter were pierced using a sterile cork borer at 4 different sites of the plates. Then all wells were filled isolate 150µl of honey samples and allowed to stay for 15 mins for prediffusion to take place and it followed by incubation at 37ºc for 24 hrs. The diameter of zone of inhibition was recorded.

III. RESULTS AND DISCUSSION:

As per shown in table; the antifungal activity of four different honey samples viz., Dabur, Himalaya Patanjali and Madhuban honey were evaluated against pathogenic fungi (Candida albicans, Candida tropicalis and Rhizopus). The all types honey showed zone of inhibition or inhibitory activity against Candida albicans. Surprisingly the samples are unable resist Rhizopus oryzae infection. Here comparatively high inhibitory activity showed by Dabur and Madhuban honey against Candida tropicalis as compared to other honey samples.

Table: Antifungal activity of different honey sample against different fungal strains. Activity shown in the form of Zone of inhibition (in mm)

Fungal strains	Honey samples			
	Dabur	Himalaya	Patanjali	Madhuban
Candida albicans	17	15	11	12
Candida tropicalis	16	NZ	NZ	13
Rhizopus oryzae	NZ	NZ	NZ	NZ

Note: NZ: No inhibition zone

In recent years research has been increasing specially for new antifungal compound due to less efficiency and side effects and less drug resistant compounds. Now days many scientists focused on natural therapeutic products, in this regards honey stands as best option. Recent studies showed some honey have antifungal properties against Candida albicans, Candida glabrata, Candida dubliniensis, Rhodotorula, Aspergillus niger, Aspergillus flavus, Penicilliumchry sogenum, Microsporum gypseum, and Saccharomyces sp. Due to such protective characteristics, honey studied extensively as alternate medicine. [17] [14] [18] [19]. Irish and co-worker in 2006, [14] studied the clinical isolates of Candida albicans, Candida glabrata, and Candida dubliniensis were tested against four different honeys. Here floral honeys proved best against Candida albicans and Candida glabrata. According to Abdelmonem and collaegues in 2012, [20] showed that, honey as a prominent remedy against vulvo-vaginal candidiasis during pregnancy. Irish and co-workers in 2006, [14] also reported antifungal efficacy of various honeys against clinical isolates of C. albicans, Candida glabrata, and Candida dubliniensis. Khosravi and co-workers in 2008;[21] reported that honey has antifungal activity against Candida species such as Candida albicans, Candida parapsilosis, Candida tropicalis, Candida kefyr, Candida glabrata and Candida dubliniensis.

With this different honey samples studied to focus on major active ingredients and specific properties that resist fungal growth. Honey's nature is hygroscopic with a pH of 3.2-4.5, inhibits colonization and bacterial growth in tissues due to its acidic nature. Most microorganisms do not grow in pure honey because of its low water activity (a_w) of 0.6. [22]. Hydrogen peroxide, methylglyoxal, bee defensin, pH, osmotic effect as well as leptosin were known to be responsible for the antimicrobial effects of honey [22]. Many workers have studied the antimicrobial strength of the honey. They found that foremost components responsible of this action are hydrogen peroxide and gluconic acid which originate from the dissolution of sugar by honey's glucose oxidase [24] [25]. Action of honey is also linked with osmolarity, in fact, its high sugar contents, creates a high osmotic pressure unfavourable to bacterial growth and proliferation. White and his co-workers in 1963;[26] further Bang and colleagues in 2003 [27] studied that, Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [28]. The conventional treatment of fungal disease is limited, and part of the reason is due to the limited spectrum of the currently antifungal drugs, and the expensive treatment, particularly due to the need of prolonged therapy. In this regards honey proven best as natural remedy continuously.

Our results showed that undiluted honey was able to inhibit the growth of many species Candida albicans and trophicalis but failed to resist Rhizopus oryzae growth; there may be need of dilution honey samples. The results of present study are in accordance with many scientists. Including Irish et al, in 2006 [14] and Khosravi et al., 2008, [21] reported antifungal efficacy of various honeys against different Candida species. According to Moussa and colleagues (2012), [18] undiluted honey was able to inhibit the growth of many species of Rhodotorula sp. but there was no effect on C. albicans. Our results are in consistent with Moussa and colleagues (2012), [18] as here undiluted honey sample showing antifungal activity except in case of Rhizopus oryzae. Here there may be possibilities of presence of another factor in concentrated honey samples besides hydrogen peroxide that able resist candida growth.

From present study it is concluded that honey samples showed inhibitory activity of against Candida albicans and C. tropicalis. But all honey samples failed to inhibit Rhizopus oryzae growth. Such difference in inhibitory action may be due to variation in antimicrobial components, source of honey or may processing of honey. In case of glass-bottle packing, one does not have to worry about harmful substances from plastic leaching into the honey. In case of Candida tropicalis and Rhizopus oryzae; the Himalaya and Patanjali honey showed less antifungal activity it may be due plastic bottled packing. (https://www.grabon.in/indulge/health/best-pure-organic-honey-brands-india/). There is further need of quality assessment for the achievement of best antifungal honey. It is concluded that one can go for all the types of honey in case Candida albicans infection or associated symptoms as a basic and natural medicinal treatment or home remedies.

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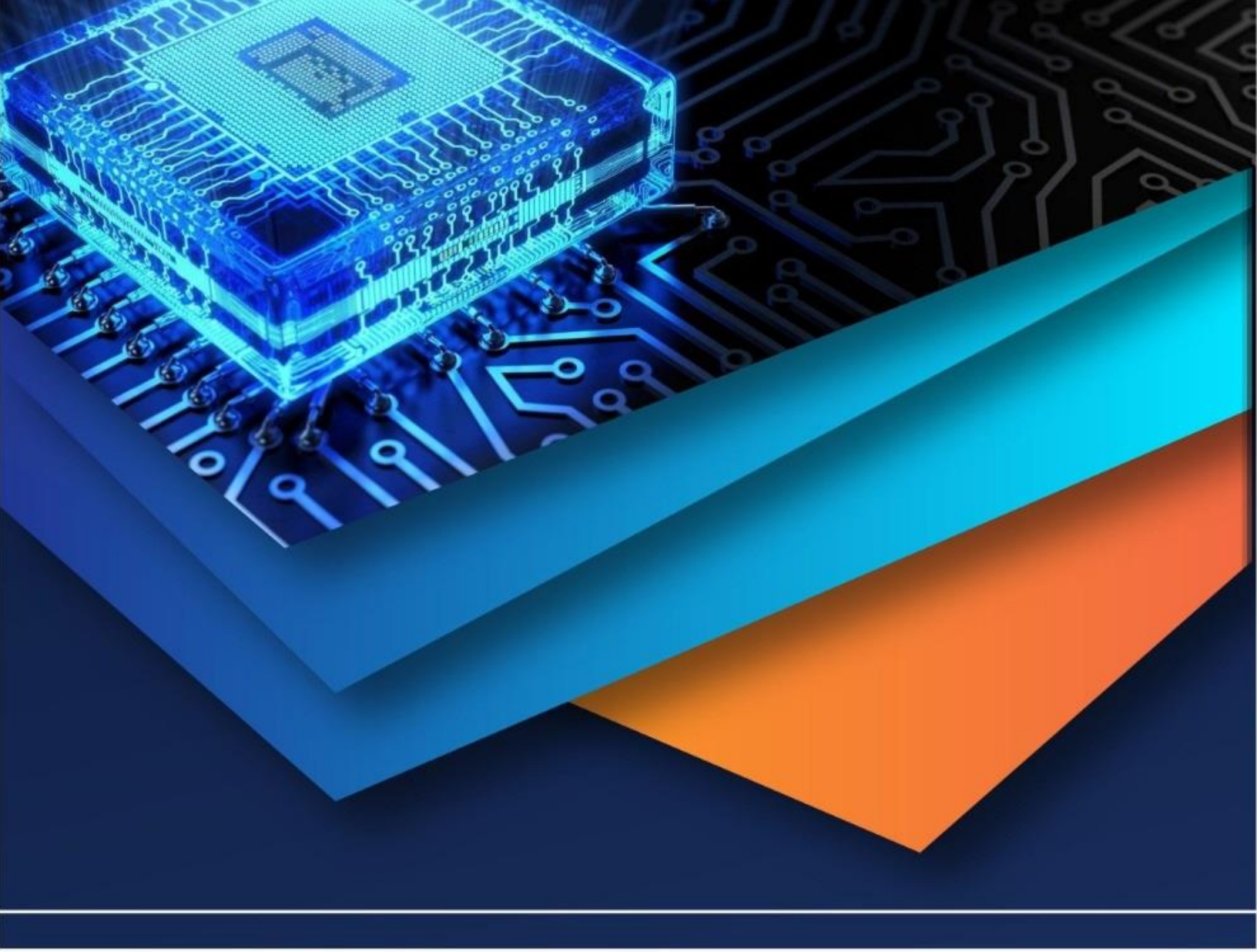

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