JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2012. Vol. **6**(3), p. 1363-1367

In vitro Effect of Honey on *Aspergillus* Species Isolated from Different Clinical Infections

Patil Rohini Pradeep[1] and Deshpande Sunita Dhananjay[2]

[1]Department of Microbiology, RKT College, Ulhasnagar, Dist Thane - 421 203, India.
[2]Department of Microbiology, TNMC & BYLNair Hospital, Mumbai Central, Mumbai - 400 008, India.

(Received: 09 December 2011; accepted: 12 January 2012)

Honey is the natural sweet substance produced by honeybees. In India more than 300 varieties of honey are available but very little data regarding their antifungal properties and activity is known. Hence, *in vitro* antifungal activity of Agmark standard Indian Jambhul honey was tested to check its sensitivity against clinical isolates of *Aspergillus* species by using Agar well diffusion method and Agar dilution method. It was further compared with *in vitro* sensitivity of conventional antifungal agents. *In vitro* antifungal susceptibility test revealed MIC for various *Aspergillus* species in the range of >32-2.17μg/ml, >32-13.07μg/ml and 0.5μg/ml for AmphotericinB, Itraconazole and Voriconazole respectively. *In vitro* activity of Jambhul honey towards all *Aspergillus* species was in the range of 30-40% (v/v). Jambhul honey was found to be effective even against Amphotericin B & Itraconazole resistant *Aspergillus* species. Study suggests, honey is one of the inexpensive, easily available natural products with no side effects. It is effective even against resistant *Aspergillus* species.

Key words: Indian Jambhul Honey, MIC.

Honey is the first sweetening agent known to man with an interesting history. Honey is known to be effective against aerobic and anaerobic Gram positive and Gram negative bacteria[1]. Antifungal activity of honey has been reported against some yeast, *Aspergillus*, *Penicillium* species and all the common dermatophytes[1-3]. Honey is also known to have antiprotozoal and antiviral activity[4-5]. The antibacterial and antifungal activity of honey is due to its high molarity, low pH, hydrogen peroxide content, various enzymes and inhibines.[6]

Aspergillus is one of the medically & commercially important fungi. More than 60 *Aspergillus* species are known to cause infection in human & animals through production of mycotoxin, induction of allergenic responses and localized or systemic infection[7]. Wide ranges of antifungal agents are used in treatment against *Aspergillus* infections. Overuse and abuse of antifungal agents result in emergence of resistant strains in the organisms. Resistance to these agents actually limits the choice of these agents in treatment of fungal infection[8-9].

The prevalence of resistant organisms justifies the need in reevaluation of ancient remedies and natural products in therapeutics.

* To whom all correspondence should be addressed.
Mob.: +91- 9820298560
E-mail: rohini_106@yahoo.com

MATERIALS AND METHODS

Total 590 various suspected clinical samples were studied for their fungal etiology in Mycology Section of Microbiology Dept, Tertiary Care Center, Mumbai 08.

Each clinical sample was processed for fungal study using standard methods of microscopy & culture. Identification and speciation was confirmed by microscopic and cultural characteristics[10].

In vitro antifungal susceptibility test was carried out as per CLSI guide lines using Amphotericin B, Itraconazole, and Voriconazole[11]. and MIC was determined. Agmark standard Indian Jambhul honey was procured before heat processing from Phondaghat collection centre, Mumbai. Sterility testing was done by performing "Test of sterility" by direct Inoculation method as stated in Indian Pharmacopoeia[12]. If found non sterile then, it was sterilized by gamma irradiation at 25 kGy at the ISOMED unit of BARC- Mumbai[13].

In vitro activity of honey was checked by carrying out 'Agar diffusion method'& MIC was estimated for each clinical isolate of *Aspergillus* by 'Agar dilution method'**.** [14]

RESULTS

From 590 clinical samples, 90 showed growth of *Aspergillus* species on Sabouraud's dextrose agar as shown in Table 1. Maximum culture

Table 1. Culture positivity in total clinical samples and sample-wise distribution of different *Aspergillus* species

N=590,	N1=90									
sample	Corneal	Hair	Biopsy	Ear	Blood	Pus	Tissue	Skin	Nail	Nasal
samples received N= 590 →	42	5	4	11	58	25	24	25	186	210
positive samples N1= 90 (15.25)→	01 (2.38)	01 (20.0)	02 (50.0)	02 (18.18)	03 (5.17)	05 (20.0)	05 (20.83)	12 (48.0)	26 (13.97)	33 (15.71)
Asp. Spp↓										
A. niger 27 (30.0)	-	-	01	-	02	-	02	05	10	07
A.flavus 39 (43.33)	01	-	-	02	01	03	01	04	06	21
A. fumigatus 10 (11.11)	-	01	-	-	-	-	-	-	06	03
A. nidulans 09 (10.0)	-	-	01	-	-	02	01	02	01	02
A. terreus 03 (3.33)	-	-	-	-	-	-	-	-	03	-
A.ustus 01 (1.11)	-	-	-	-	-	-	-	01	-	-
A flavipus 01 (1.11)	-	-	-	-	-	-	01	-	-	-

N= Total samples, N1= Culture isolates, ( ) = %

positivity was observed in samples of biopsy (50.0%) followed by skin (48.0%), tissue (20.83%), pus and hair (20.0% each), ear (18.18%), nasal (15.17), nail (13.97%), corneal (13.80%) and blood (5.17%). All the isolates were identified on the basis of microscopic morphology and culture characteristics. The most common species isolated was *A. flavus* (43.33%) followed by *A.niger* (30%), *A. fumigatus* (11.11%) *A. nidulans* (10.0%), *A. terreus* (3.33%), *A. ustus* (1.11%) and *A. flavipus*(1.11%)as shown in Table 1.

Antifungal susceptibility test showed 12.22% and 25.55% of *Aspergillus* isolates resistant to Amphotericin B and Itraconazole respectively but none was resistant to Voriconazole as given in Table 2.

Table 2. *In vitro* sensitivity pattern of antifungal agents and Indian Jambhul honey for *Aspergillus* isolates

Antifungal agent→	Amphotericin -B		Itraconazole		Voriconazole		Indian Jambhul Honey*	
Aspergillus spp↓	S	R	S	R	S	R	S	R
A.niger 27 (30.0)	22	05	18	09	27	00	27	00
A.flavus 39 (43.33)	34	05	27	12	39	00	39	00
A.fumigatus 10 (11.11)	09	01	08	02	10	00	10	00
A.nidulans 10 (11.11)	09	00	09	00	09	00	09	00
A.terreus 03 (3.33)	03	00	03	00	03	00	03	00
A.ustus 01 (1.11)	01	00	01	00	01	00	01	00
A.flavipus 01 (1.11)	01	00	01	00	01	00	01	00
Total 90 (100)	79 (87.78)	11 (12.22)	67 (74.45)	23 (25.55)	90 (100)	00 (00)	90 (100)	00 (00)

( ) = %

·Indian Jambhul honey was used in the concentration range of 5%-50% (v/v) for its MIC estimation.

·Amphotericin –B , Itraconazole, Voriconazole were used in the concentration range of 64mcg/ml to 0.0625 mcg/ml

In vitro antifungal activity of Jambhul honey by 'Agar diffusion method' did not show zone of inhibition against any of the *Aspergillus* isolates. In present study MIC of Indian Jambhul honey was determined by 'Agar dilution method' and all *Aspergillus* isolates were completely inhibited as given in Table 2. The MIC of Indian Jambhul honey was observed in the range of 30 – 40% (v/v).

DISCUSSION

Out of 90 *Aspergillus* spp. *A. fiavus* (43.33%)was the main isolate from nasal polyp and sinus aspirate, where as ,*A. niger* (30%) from nail samples. Xess *et al* [15] in 2004 studied species prevalence of *Aspergillus* isolates in various clinical samples at All India Institute of Medical Sciences, New Delhi and observed similar results.

They reported *A.flavus* (46.93%) as the most common isolate, followed by *A.fumigatus* (37.72%) and *A.niger* (15.35%). Also *A.flavus* was predominantly isolated from nasal polyps and *A.niger* from nail specimens. Higher prevalence of *A. flavus* in fungal sinusitis was also reported by Agarwal *et al*[16] in 2005 and Saravanan *et al* [17] in 2006, Chatterjee *et al* [18] in 2009.

Resistance was more for Itraconazole followed by AmphotericinB. High MIC value of AmphotericinB and Itraconazole was mainly observed for *A. niger, A. flavus* and *A.fumigatus*. In 2008 Li *et al* [19] also isolated *A. flavus* resistant to several antifungal agents like Fluconazole (32 µg/mL), 5-Flucytosine (>64 µg/mL), Miconazole and Nystatin (4 µg/mL each), and Amphotericin B (≥16 µg µg).

Elevated MIC of Itraconazole (>16micro/ml) for three isolates of *A. fumigatus* was also reported by Denning *et al* [20] in 1997. Similarly in 1999 Dannaoui *et al* [21] reported MIC of Amphotericin B, Itraconazole for 230 isolates of *Aspergillus* species. According to their results MIC of >16mg/ml for Itraconazole was reported for four isolates of *A. fumigatus* and one of *A. nidulans.* They also reported significantly less susceptibility of *A. flavus, A. nidulans* and *A. terreus* to AmphotericinB than *A. fumigatus* and *A. niger.* Susceptibility of *Aspergillus* to Voriconazole with MIC ≤2 µg/ml was reported by Jesús Guinea *et al* in 2008**.**[22]

In vitro activity of Indian Jambhul honey by Agar diffusion method was not observed and it may be due to activity of honey, low and below the level of detection against *Aspergillus* species. Similar results have been reported by Allen *et al* in 1991.[23] In their survey of 345 samples of some New Zealand honeys, large number of samples (36%) had activity near or below the level of detection in an agar diffusion assay**.**

Muli *et al* in 2008[24] also tested antifungal activity of honey of stingless bee by disk diffusion method and no inhibition was observed for *A, niger* and *C. albicans.*

MIC of Indian Jambhul honey by 'Agar dilution method' was in the range of 30-40% (v/v). There is paucity of reports in literature on antifungal activity and MIC of honey for *Aspergillus* species**.** In 2007 Boukraa *et al* [25] reported MIC of two varieties of honey against *A.niger* as 51% and 59%. In 2008 during conference on honey in Malaysia Boukraa *et al* [26] again reported MIC of five different varieties of honey in the range of 46% – 50% (v/v) against *A.niger.* This MIC range of honey against *Aspergillus* species was much higher than MIC range of Indian Jambhul honey obtained in the present study.

There are no published reports regarding antifungal activity of Indian honey hence, it is pioneer study of this type.

CONCLUSION

1. In vitro Jambhul honey was effective against Aspergillus species resistant to conventional antifungal agents like Amphotericin B and Itraconazole and its effectiveness was comparable with Voriconszole.
2. In view of cost effectiveness in comparison with Voriconazole, Indian Jambhul honey can be considered as potential alternative to routine therapeutics after further in vitro and in vivo evaluation and clinical trials.

A comparative MIC value of Indian Jambhul honey confirms its effectiveness over honeys studied by other workers and reported in literature.

ACKNOWLEDGMENTS

We thankfully appreciate the moral support of Dean, TNMC & BYL Ch Hospital, Mumbai .Also Head & Professor, Dept of Microbiology, TNMedical College, Mumbai.

REFERENCES

1. Molan P C. The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee World*, 1992; **73**(1): 5-28.
2. *Brady NF et al.* The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Journal of Pharmaceutical Sciences*, 1996; **2**: 1-3.
3. Radwan SS, EI-Essawy AA, Sarhan MM. Experimental evidence for the occurrence in honey of specific substances active against microorganisms. *Zentralbl Mikrobiol*., 1984; **139**(4): 249-55.

4. Zeina B. Zohra BI, al-assad S The effects of honey on Leishmania parasites: an *in vitro* study *Trop Doct*. 1997; **27**(1): 36-8.
5. Al-Wailli NS. Topical honey application vs. acyclovir for the treatment of recurrent herpes simplex lesions. *Medical Science Monitor.*, 2004;**10**: 8, MT94–98.
6. Bergman A, Yanai J, Weiss J, Bell D, David MP. Acceleration of wound healing by topical application of honey. *The American Journal of Surgery*. 1983; **145**: 374-376.
7. *Joan W. Bennett. An Overview of the Genus* Aspergillus.*In: Masayuki Machida and Katsuya Gomi editors.* Aspergillus*: Molecular Biology and Genomics Published:* 2010 ISBN: 978-1-904455-53-0
8. Bavdekar SB. Antibiotic resistance: Unless we act soon! *J Postgrad Med*. 2003; **49**: 107-108.
9. Jarvice WR. Preventing the emergence of multidrug resistant microorganisms through antimicrobial use control: the complexity of the problem. *Infect Control Hosp Epidemiol* 1996; **17**: 490-5.
10. Jagdishchandra. Text Book of Medical Mycology, 3rd ed. Mehta Publishers: New Delhi.
11. CLSI guidelines, Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. 2002 M38-A.
12. Test for sterility, Govt. of India, Ministry of Health and Family Welfare, *Indian Pharmacopoeia*, 2007; Ch. 2.2.11, **2**: 52.
13. Molan PC, Allen KL. The effect of gamma irradiation on the antibacterial activity of honey. *J Pharm Pharmacol*, 1996; **48**: 1206-1209.
14. CLSI guidelines, Performance Standards for antimicrobial susceptibility testing 16th informational supplement, M2-A9 & M07-A7 Jan 2006; 26(3).
15. Xess I, Mohanty S, Jain N, Banerjee U. Prevalence of *Aspergillus* species in clinical samples isolated in an Indian tertiary care hospital .*Indian Journal of Medical Sciences.*, 2004; **58**(12): 513-519.
16. Agarwal S, Kanga A, Sharma V, Sharma DR, Sharma ML. Invasive Aspergillosis involving multiple paranasal sinuses- A case report. *Indian Journal of Medical Microbiology.*, 2005; **23**(3): 195-197
17. Saravanan K, Panda NK, Chakrabarti A, Das A, Bapuraj RJ.Allergic Fungal Rhinosinusitis:An Attempt to Resolve the Diagnostic Dilemma. *Arch Otolaryngol Head Neck Surg,* 2006; **132**: 173-178.
18. Chatterjee SS, Chakrabarti A. Epidemiology and medical mycology of fungal rhinosinusitis. Otorhinolaryngology Clinics: *An International Journal* ., 2009; **1**(1): 1-13.
19. Li DM, Xiu DR, Li RY, Samson RA, de Hoog GS, Wang DL. *Aspergillus flavus* myositis in a patient after liver transplantation. *Clin Transplant.*, 2008; **22**: 508-511.
20. Denning, D. W., Venkateswarlu, K., Oakley, K. L., Anderson, M. J., Manning, N. J., Stevens, D. A. *et al.*. Itraconazole resistance in *Aspergillus fumigatus*. *Antimicrobial Agents and Chemotherapy.*, 1997; **41**: 1364-8.
21. Dannaoui E, Persat F, Monier MF, Borel E, Piens MA, Picot S. In-vitro susceptibility of *Aspergillus* spp. isolates to amphotericin B and itraconazole. *Journal of Antimicrobial Chemotherapy.*, 1999; **44**: 553-555.
22. Guinea J, Recio S, Peláez T, Torres-Narbona M, Bouza E. Clinical Isolates of *Aspergillus* Species Remain Fully Susceptible to Voriconazole in the Post-Voriconazole Era. *Antimicrob Agents Chemother*. 2008; **52**(9): 3444–3446.
23. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. *J Pharm Pharmacol*., 1991; **43**(12): 817-22.
24. Muli EM, Maingi JM, Macharia J. Antimicrobial properties of propolis and honey from the Kenyan Stingless bee, Dactylurina schimidti. APIACTA43. 2008; 49-61.
25. *Boukraâ L, Bouchegrane S., Additive action of honey and starch against* Candida albicans *and* Aspergillus niger*, Iberoam Micol.* 2007; **24**(4):309-11.
26. Boukraâ L, Benbarek H, Ahmed M. Synergistic action of starch and honey against *Aspergillus niger* in correlation with Diastase Number. *Mycoses*., 2008; **51**(6): 520-522.