INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 23, No. 6, p. 215-223, 2023

RESEARCH PAPER OPEN ACCESS

Production of herbal fermented honey using isolated *Candida sp.* and analysis of antimicrobial and antioxidant activity

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Key words: Honey, *Candida albicans,* Fermentation, Antimicrobial, Antioxidant activity

http://dx.doi.org/10.12692/ijb/23.6.215-223 Article published on December 12, 2023

Abstract

Honey use and production has a long and varied history. In many cultures, honey has associations that go beyond its use as a food. It is frequently used as a talisman and symbol of sweetness. For more than 5000 years, Indians have been continually using it, today there are lots of practitioners, and there are Academies founded all over the world. People trust it and ayurveda remains one of the most natural ways of health care. In the present study was carried production of herbal fermented honey using isolated *Candida sp.* and analysis of antimicrobial and antioxidant activity. The spoiled fruit samples were collected from Market at Pudukkottai district and isolate the *Candida sp*. The isolated *Candida sp.* was conformation by 18 S rRNA sequencing and fermentation was performed using isolated *Candida sp.* with garlic in carboy. Analysis of antimicrobial and antioxidant activity such as superoxide anion radical scavenging activity and reducing power activity was using herbal fermented honey. Fermented honey active compounds were analyzed by FT-IR. HiCrome Candida Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of Candida species namely *C. albicans, C. krusei* and *C. glabrata* on the basis of coloration and colony morphology. Based on the molecular characteristics and sequence alignments the isolated strain was conformed as *C. albicans*. FT-IR spectrum confirmed the presence of Alcohols, Phenols, Alkanes, Primary amines, Aromatics, Aliphatic amines and Nitro compounds.

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Introduction

Honey is a sweet, viscous food substance produced by bees and some related insects. The variety of honey produced by honey bees is the best-known, due to its worldwide commercial production and human consumption (Crane, 1990). People trust it and ayurveda remains one of the most natural ways of health care. The honey that is prescribed by ayurvedic practitioners is a cultured or purified honey, called Samskritha Madhu, made by most of the authentic ayurvedic manufacturing units, following the ayurvedic scriptures (Conboy *et al.,* 2009). Possibly the world's oldest fermented beverage dating to 9,000 years ago, mead (honey wine) is the alcoholic product made by adding yeast to the honey–water must, followed by weeks or months of fermentation. Cell immobilization methods, however, proved effective for enhancing mead quality (Subramanian *et al.,* 2007). Components of honey under preliminary research for their potential antibacterial properties include methyglyoxal, hydrogen peroxide and royalisin (O'Meara *et al.,* 2014).

For chronic and acute coughs, a Cochrane review found no strong evidence for or against the use of honey (Eekhof *et al.,* 2012). Honey is recommended by one Canadian physician for children over the age of one for the treatment of coughs, as it is deemed as effective as dextromethorphan and more effective than diphenhydramine (Bardy *et al.,* 2008). The present work has been undertaken with production of herbal fermented honey using isolated *Candida sp*. and analysis of antimicrobial and antioxidant activity.

Materials and methods

In this study various spoiled fruit samples were collected from market at Thanjavur district. The samples were collected in sterile, dry, wide necked, leak proof container. *Candida sp* were isolated from the collected sample using Sabouraud Dextrose Agar and HiCrome Candida Differential Agar by streak plate method. After the streaking the plates were incubated at 37˚C for 24-48 hrs. After incubation cultural characteristics and colony morphology were observed. Identification of isolated *Candida sp*. based on Gram staining and Germ tube test (Cappuccino and Sherman, 1999). Molecular characterization of isolated *Candida* sp. by 18 S rRNA sequencing (Edwards *et al.,* 1989).

The natural honey was collected from village area in Thanjavur Dt. of Tamil Nadu, India in Jan, 2022. The collected natural honey was warmed by water bath for 28°C for easy to pour and it doesn't overheat. The warmed natural honey used for further study. The equipment (scrub and carboy) clean must informant because minimize the risk of spoilage. The equipment was clean with hot water and continually washes with sodium percarbonate solution. The carefully inspect all equipment for cleanliness and store it on a clean surface.

The warmed honey was mixed with 8 g acid blend, 7 g yeast nutrient, 5 g DAP and 250 g clean garlic. All the material was stirred until dissolved. The nutrient honey was transfer to already clean carboy and then added isolated *Candida sp*. Within 24-48 hours the batch should start bubbling, showing that the fermentation has started. After complete fermentation the clean upper layer product was collected and analysis for further study.

The fermented herbal honey was analyzed for antioxidant activity. Measurement of superoxide anion radicals scavenging activity of plant extract was based on the method described by Nishimiki *et al.* (1989). The reducing power of the fermented herbal honey was determined according to the method of Oyaizu (1972).

The pathogenic microbial strains such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, C. albicans, C. krusei* and *C. glabrata* pure cultures were inoculated into the broth respectively. The inoculated broths were incubated at 37°C for 24 hrs. Disc diffusion method (Bauer *et al.*, 1966) was adopted for evaluation of antimicrobial activity of fermented honey extract.

The fermented honey samples physicochemical parameters such as moisture contents, pH, protein, carbohydrate and micronutrients calcium, magnesium, phosphorus, potassium, sodium, copper, iron, manganese and zinc) were analysed (Sadasivam and Manickam, 2004).

The fermented honey extract was examined under FT-IR spectrophotometer analysis, the extract were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation (Dhivya and Kalaichelvi, 2017). The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar, (1984).

Results and discussion

Candida Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C. albicans, C. krusei* and *C. glabrata* on the basis of colouration and colony morphology. On this medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory. On Hicrome *Candida* differential agar, *Candida albicans* produced Light green colonies, *C. krusei* dull blue to purple colonies that diffused into the surrounding agar with pale pink edge *C. glabrata* developed white large glossy pale pink colonies. Germ tube production was observed in case of *Candida* strains only (Table 1). Yeast has been isolated from variety of natural sources like leaves, flowers, fruits etc (Davenport *et al.,* 1980; Spencer and Spencer, 1997; Tournas, 2005; Li *et al.,* 2008). In the present study sabouraud dextrose agar plates were observed three different *Candida* colonies were noted from waste fruits.

With this in view, it was logical to assume that yeasts of the genera *Rhodotorula, Cryptococcus, Sporobolomyces*, *Saccharomyces, Candida, Pichia*, etc, isolated from fresh and rotten fruits (Rao *et al.,* 2007; Bhadra *et al.,* 2008) could produce ethanol.

The dominant C1 *Candida albicans* were used for molecular characterization and fermentation study. The purified C1 *Candida* sp fungal genomic DNA were separated by agarose gel electrophoresis. The separated fungal genomic DNA was used for further study. After separation, the result of DNA fragments are visible as clearly defined bands. The DNA standard should be separated to a degree that allows for the useful determination of the sizes of sample bands. In this study, DNA fragments ranging from 2361 bp to 2027 bp were separated on a 1.5% agarose gel. Extracted genomic DNA containing 18S rRNA from each isolate was amplified for gene sequencing using PCR. Partial sequencing of the genomic DNA of the test isolates C1 *Candida* sp revealed that the 18S rRNA portion contained base pairs range 592 for *Candida albicans* (Fig. 1).

Honey is a well-known natural antioxidant produced by honey bees. Honey contains phytochemical components like phenolic acid, flavonoids, vitamins, enzymes and some amount of mineral content particularly copper and iron (Erlund, 2004). The warmed honey was mixed with nutrients and 250 g clean garlic. The nutrient honey was transfer to carboy and then added isolated C1 *Candida sp*. Within 24-48 hours the batch should start bubbling, showing that the fermentation has started. After complete fermentation the clean upper layer product was collected and analysis for further study.

The present study, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Fig. 2 shows the percent inhibition of superoxide radical generation by 100 ml/ml of fermented herbal honey.

Table 1. Isolation and identification of *Candida* sp.

Organism	Morphology on SDA	Morphology on Hicrome *Candida* differential agar	Germ tube test
Candida albicans (C1)	White to cream colored smooth soft	Apple green colonies	Positive
Candida krusei (C2)	White to cream colored colonies	Dull blue to purple colour that diffused in to surrounding agar with pale pink edge	Positive
Candida glabrata (C3)	White to cream colored colonies	Small pink to purple colonies	Positive

Table 2. Assay of antimicrobial activity of fermented herbal honey

Microbes	Zone of inhibition (mm in diameter) (M±SA) n=3		
	Control	Standard*	Fermented Honey
Escherichia coli	-	20	10±0.80
Klebsiella pneumoniae	-	18	08±0.40
Staphylococcus aureus	-	16	07±0.20
Candida albicans	-	20	22±1.40
Candida krusei	-	22	22±0.90
Candida glabrata	-	18	10±0.40

Values are expressed Mean ± Standard deviation; Nitrofurantoin for Bacteria; Amphotericin b for Fungi.

Table 3. Physico-chemical analysis of fermented herbal honey

S. No.	Micronutrients	Fermented herbal honey (mg/ml)
1	Moisture contents	78.20±1.05
2	Protein	48.18±1.56
3	Carbohydrate	41.30±0.07
4	Calcium	4.90±0.72
5	Magnesium	0.58±0.02
6	Phosphorus	11.34±0.88
7	Potassium	6.10±0.42
8	Sodium	4.85±0.18
9	Copper	0.10±0.05
10	Iron	2.80±0.14
11	Manganese	0.64±0.02
12	Zinc	2.20±0.86

Values are expressed Mean ± Standard deviation; n = 3

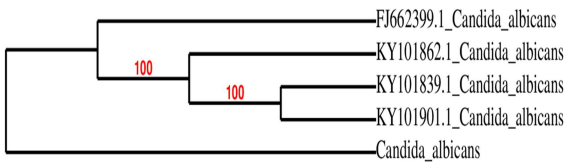

Fig. 1. Phylogenetic tree of bacterial isolates based on partial 18s rRNA sequence

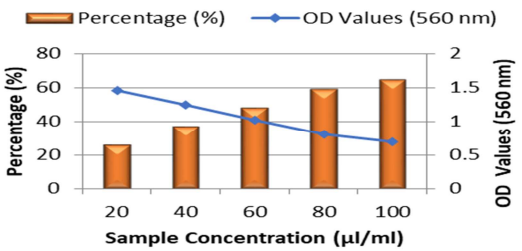

Fig. 2. Superoxide anion radical scavenging activity of fermented herbal honey

The fermented herbal honey has strong superoxide radical scavenging activity was found as 65.06%. Superoxide radical scavenging activity of those samples followed the order: 100 > 80 > 60> 40> 20> (Fig. 2). Superoxide radicals were generated in PMS-NADH system by oxidation of NADH and it was assayed by assessing NBT reduction forming blue formazan (Kanatt *et al.,* 2007). The fermented honey exhibited a dose response inhibition of superoxide anion radicals. The percentage inhibition of superoxide generation was found to be increasing with increase in concentration and maximum activity (65.06 %) was seen at a concentration of 100 µg/ml. Several studies have proven that, presence of polyphenols like phenolic acid, flavonoids and proanthocyanidin have a beneficial effect in scavenging harmful reactive oxygen species (Sakihama *et al.,* 2002).

Table 4. Analysis of FT-IR spectrum of fermented herbal honey

SN	Frequency	Bonds	Vibration
1	3169.69 cm^{-1}	N-H	Primary and secondaryamines and amides (stretch)
2	3009.20cm^{-1}	C-H	Alkenes(stretch) (out of plane band)
3	2972.19cm^{-1}	C-H	Alkanes (stretch)
4	2897.96cm^{-1} 2827.64cm^{-1} 2717.96cm^{-1}	C-H	Aldehyde
5	2616.93cm^{-1} 2528.81cm^{-1}	O-H	Alcohols
6	2269.28cm^{-1}	X=C=Y	Alkenes, ketenes, isocyanates, isothiocyanates
7	2212.69cm^{-1} 2123.39cm^{-1}	CΞC	Alkyne
8	1611.26cm^{-1}	C=C	Aromatic
9	1522.47cm^{-1}	N-H	(bend)
10	1444.71cm^{-1} 1412.49cm^{-1} 1333.01cm^{-1}	$-CH_3$	(bend)
11	1131.68cm^{-1} 1112.08cm^{-1} 1033.34cm^{-1}	C-O	Alcohols, ethers, ester, carboxylic acid, anhydrides
12	910.49cm^{-1} 892.86cm^{-1}	C-H	Phenols

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}) (Fig. 3). The results of this research showed that the reducing power of the fermented herbal honey OD values was increased and percentage was degreased. The reaction meaning that during the fractionation process an increase in the antioxidant activity occurred. The reducing power of honey is due to the presence of reductants in the solution which exhibits antioxidant activity **t**hrough breaking the free radical chain by donating a hydrogen atom (Xing *et al.,* 2005). The Fe^{3+}/ferricyanide complex get reduced to ferrous form signifying the antioxidant capacity of the sample. The reducing power of honey increases as concentration increases. Hence, the antioxidant action of honey on free radical induced erythrocyte hemolysis inhibition was proved. Based on all these results, we conclude that fermented honey exhibited satisfactory antioxidant properties and that it helps in preventing DNA and erythrocytes from oxidative damage.

The fermented herbal honey was analyzed antimicrobial activity against pathogenic microbes such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, C. albicans, C. krusei* and *C. glabrata*. Antimicrobial potential of fermented herbal honey was assessed in terms of zone of inhibition of microbial growth (Table 2). *C. albicans* and *C. krusei* was more sensitive for fermented herbal honey compare than other microbes. Antibacterial activity of honey may be because of the ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature (pH being 3.2-4.5), hydrogen peroxide concentration and its phytochemical nature, i.e. its content of tetracycline derivatives, peroxides, amylase, fatty acids, phenols, ascorbic acid, flavonides, streptomycin, sulfathiazole, terpens, benzyl alcohol and benzoic acids (Molan, 1992). In the present study *C. albicans* and *C. krusei* was more sensitive for fermented herbal honey compare than other microbes.

Similar studies have been reported in the form of antibacterial activity of honey against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, P. mirabilis, Streptococcus pyogenus, S. flexneri* and *Salmonella typhi* (Nzeako and Hamdi, 2000). The *in vitro* antimicrobial activity of honey was also reported by Radwan *et al.* (1984) who observed that honey stopped the growth of *Salmonella* and *Escherichia coli*.

The fermented herbal honey samples physic chemical parameters such as moisture contents, pH, protein, carbohydrate and micronutrients were analysed (Table 3).

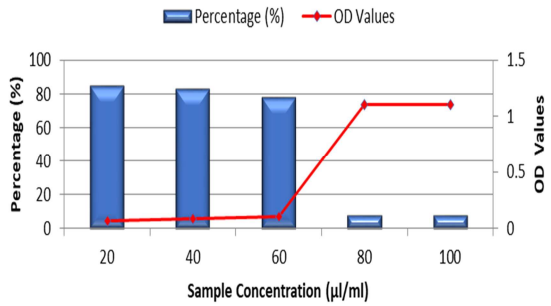

Fig. 3. Reducing power activity of fermented herbal honey

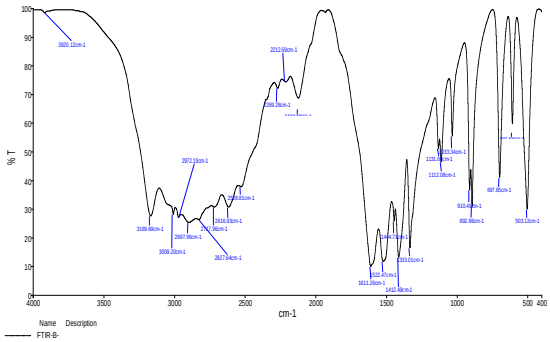

Fig. 4. Analysis of FT-IR spectrum of fermented herbal honey

The data showed that great variation in mineral levels was observed. The fermented herbal honey accumulated higher concentrations of Phosphorus, Potassium, Sodium and Calcium. On the other hand, lower levels of Magnesium, Copper, Iron and Manganese were recorded in edible. Legally, the honey must not exceed 20% moisture (Doner *et al.,* 2000). Above this threshold, honey will ferment quickly or will crystallize poorly. The moisture level of honey is measurable with a refractometer. In the present study fermented herbal honey have 78.20±1.05% of moisture. Honey is composed of about 80% carbohydrates. Among the carbohydrates, we mainly find fructose and glucose which are monosaccharides. Small amounts of polysaccharides such as sucrose, melezitose, and other sugars are also present. In total, over 25 different polysaccharides are reported in honey. The sugar spectrum varies according to the kind of honey: the content of glucose and fructose is specific to the kind.

Honey proteins consist mainly of enzymes from the secretions of bees. The amino acids present in honey come on the one hand from the nectar flow and on the other hand from bee secretions. Proline is the main amino acid from the bees. Honey contains various minerals and vitamins. Honeydew honeys are richer in minerals than nectar honey. Potassium is the main mineral present. Mineral content in blossom honeys is between 0.1 - 0.3% and can reach 1% in honeydew honeys.

The fermented herbal honey chemical constituents were analysed by FT-IR spectroscopic method. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum confirmed the presence of Alcohols, Phenols, Alkanes, Primary amines, Aromatics, Aliphatic amines and Nitro compounds (Table 4). It can be observed bands at 3169.69 cm^{-1}, related to primary and secondary amines (Kamble and Gaikwad, 2016). Bands approximately in the same range of wavelength in different alkenes samples were also identified, e.g. a band at 3009.20 and 2972.19 cm^{-1}, related to stretching (ν) vibration of hydroxyl groups and a band at 3433 cm^{-1}, related to OH wagging (OH of phenolic compounds). Bands at 2616.93 cm^{-1} and at 2528.81 cm^{-1} could be attributed to ethanol. The band at 2130 cm^{-1} could not be identified. A band was found at 1611.26 cm^{-1}. This band could be due to stretching vibration of C=C groups, due to aromatic ring deformations, due to flavonoids and amino acids: stretching vibration of C=O and of C=C, asymmetric bending vibration of N-H, due to C=O stretching vibration of caffeic acid and its derivatives and due to stretching vibration of C=O of lipids and flavonoids (Fig. 4).

Honey, consumed by humans since ancient times, is one of the few products which have conserved its natural character. It is this quality that is searched by most of the health conscious consumers. Honey had been used in almost all civilizations for centuries as a traditional medicine. Moreover, its production does not require special equipment since it is a natural food produced by honeybees and therefore man is involved mostly for harvesting. Although the high osmotic pressure and low pH of honey may not be

favourable for the growth and viability of probiotic lactic acid bacteria, it is seen that honey has good probiotic properties which enhance the viability of probiotics. Hence, honey could be a food of choice for the development of non-dairy probiotic products and thus effectively convey the probiotic lactic acid bacteria to the large intestine where they exert their health benefits. Researchers are working on the production of honey based probiotic products.

Conclusion

The spoiled fruit samples were collected from market and from the samples *Candida* species were isolated using Sabouraud dextrose agar and HiCrome Candida Differential Agar by streak plate method. The molecular characteristics and sequence alignments the isolated strains C1 were conformed as *Candida albicans* (C1). The warmed honey was mixed with nutrients and 250 g clean garlic. The nutrient honey was transfer to carboy and then added isolated C1 *Candida sp*. Within 24-48 hours the batch should start bubbling, showing that the fermentation has started. After complete fermentation the clean upper layer product was collected and analysis for further study. The fermented herbal honey was analyzed antioxidant and antimicrobial activity against pathogenic microbes such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, C. albicans, C. krusei* and *C. glabrata*. The fermented honey exhibited satisfactory antioxidant properties and that it helps in preventing DNA and erythrocytes from oxidative damage. The *C. albicans* and *C. krusei* was more sensitive for fermented herbal honey compare than other microbes. The fermented herbal honey accumulated higher concentrations of Phosphorus, Potassium, Sodium and Calcium. On the other hand, lower levels of Magnesium, Copper, Iron and Manganese were recorded in edible. The FTIR spectrum confirmed the presence of Alcohols, Phenols, Alkanes, Primary amines, Aromatics, Aliphatic amines and Nitro compounds. The fermented honey is a stable natural food having many beneficial effects on health and has prebiotic oligosaccharides that enhance the viability of probiotic lactic acid bacteria. Hence, it could be used as an exceptional food matrix for making honey based symbiotic formulations.

References

Bardy J, Slevin NJ, Mais KL, Molassiotis A. 2008. A systematic review of honey uses and its potential value within oncology care. Journal of clinical nursing **17(19)**, 2604–2623. https://doi.org/10.1111/j.1365-2702.2008.02304.x

Bauer AW, Kirby MM, Sherris JC, Truck M. 1996. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology **45**, 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bhadra B, Rao SR, Singh PK, Sarkar PK, Shivaji S. 2008. Yeasts and yeast-like fungi associated with the bark of trees: Diversity of yeasts and identification of yeasts producing extracellular endo-xylanases. Current Microbiology **56**, 489-494. https://doi.org/ 10.1007/s00284-008-9108-x

Cappuccino GG, Sherman N. 1999. Microbiology a Laboratory Manual Rockland Aommunity College, Suffern New York

Conboy L, Edshteyn I, Garivaltis H. 2009. Ayurveda and Panchakarma measuring the effects of a holistic health intervention. Scientific World Journal **9**, 272–280. https://doi.org/10.1100/tsw.2009.35

Crane E. 1990. Honey from honeybees and other insects. Ethology Ecology and Evolution **3(1)**, 100–105. https://doi.org/10.1080/03949370.1991.10721919

Davenport RK, Mossel DA, Skinner FD, Passinfre SM. 1980. Experience with some methods for the enumeration and identification of yeasts occurring in foods. In Biology and Activities of Yeasts, Academic Press London

Dhivya SM. Kalaichelvi K. 2017. UV-Visible Spectroscopic and FTIR Analysis of *Sarcostemma brevistigma*, Wight. and Arn. International Journal of herbal medicine **9(3)**, 46-49. https://doi.org/10.22159/ijcpr.2017.v9i3.18890

Doner LW, Honey Caballero B, Trugo L. Finglas PM. (Eds). 2000. Encyclopedia of Food Sciences and Nutrition, Oxford: Academic Press, India

Edwards U, Rogall T, Blocker H, Emde M, Bottger EC. 1989. Isolation and direct complete nucleotide determination of entire genes: Characterization of a gene coding for16S ribosomal RNA. Nucleic Acids Research **17**, 7843–7853. https://doi.org/10.1093/nar/17.19.7843

Eekhof JA, Van Wijk B, Knuistingh Neven A, van der Wouden JC. 2012. Interventions for ingrowing toenails. Cochrane Database of systematic reviews **4(4)**, 1-78. https://doi.org/ 10.1002/14651858.CD001541.pub3

Erlund. 2004. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability and epidemiology. Nutrition Research **24(10)**, 851–874. https://doi.org/10.1016/j.nutres.2004.07.005

Kamble V, Gaikwad N. 2016. Fourier transform infra-red spectroscopy spectroscopic studies in *Embelia ribes* Burm.F. A vulnerable medicinal plant. Asian Journal of Pharmaceutical and Clinical Research **9(3)**, 41-47.

Kanatt SR, Chander R, Sharma A. 2007. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chemistry **100**, 451-458. https://doi.org/10.1016/j.foodchem.2005.09.066

Li H, Veenendaal E, Shukor NA, Cobbinah JR, Leifert C. 2008. Yeast populations on the tropical timber tree species *Milicia excels*. Letters in Applied Microbiology **21**, 322-326. https://doi.org/10.1111/j.1472-765X.1995.tb01070.x

Molan PC. 1992. The antibacterial activity of honey. Bee world **73(1)**, 5-28. https://doi.org/10.1080/0005772X.1992.11099109

Nishimiki M, Appaji N, Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications **46(2)**, 849- 854. https://doi.org/10.1016/s0006-291x(72)80218-3

Nzeako BC, Hamdi J. 2000. Antimicrobial Potential of Honey on Some Microbial Isolates. Medical Sciences **2**, 75-79.

O'Meara S, Al-Kurdi D, Ologun Y, Ovington LG, Martyn St James M, Richardson R. 2014. Antibiotics and antiseptics for venous leg ulcers. Cochrane Database of Systematic Reviews **1(1)**, 1-137. https://doi.org/10.1002/14651858.CD003557.pub5

Oyaizu M. 1986. Studies on products of browning reaction: Antioxidative activity of product of browning reaction prepared from glucosamine. Japan Journal of Nutrition and dietetics **44**, 307-315. http://dx.doi.org/10.5264/eiyogakuzashi.44.307

Radwan SA, El-Essawy M, Sarhan MM. 1984. Experimental evidence for the occurrence in honey of specific substances active against microorganisms. Zentral Mikrobiologie **39**, 249-255.

Rao SR, Bhadra B, Kumar NN, Shivaji S. 2007. *Candida hyderabadensis* sp. nov., a novel ascomycetous yeast isolated from wine grapes. FEMS Yeast Research **7**, 489-493. https://doi.org/10.1111/j.1567-1364.2006.00206.x

Sadasivam and Manickam A. 2004. Biochemical Methods, 2nd Edition, New Age International (P) Limited Publishers, New Delhi, India.

Sakihama YM, Cohen F, Grace SC, Yamasaki H. 2002. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxicology **177**, 67-80. https://doi.org/10.1016/s0300-483x(02)00196-8

Spencer JFT, Spencer M. 1997. Yeasts in Natural and Artificial Habitats. Springer-Verlag, Heidelberg, Berlin.

Subramanian R, Hebbar H, Umesh, Rastogi NK. 2007. Processing of Honey: A Review. International Journal of Food Properties **10**, 127–143.

Tournas VH. 2005. Moulds and yeasts in fresh and minimally processed vegetable and sprouts. International Journal of Food Microbiology **99(1)**, 71-77.
https://doi.org/10.1016/j.ijfoodmicro.2004.08.009

Xing R, Yu H, Liu S, Zhang W, Zhang Q, Li Z, Li P. 2005. Antioxidant activity of differently regioselective chitosan sulfates *In Vitro*. Bioorganic and Medicinal Chemistry **13**, 1387-1392.
https://doi.org/10.1016/j.bmc.2004.11.002

Zar JH. 1984. In: Biostatistical Analysis, Englewood Cliffs, N.J.: Prenticehall, Inc, India.