

The antibacterial activity of natural honey on local isolates of diarrhea-causing bacteria

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Abstract

This study was aimed to determine the antimicrobial effects of different types of natural honey on some diarrhea-causing bacteria these are *Escherichia coli*, and *Shigella flexneri* was investigated. The natural honey samples were effective in inhibiting the growth of investigated bacteria, with inhibition zone ranging from 24 to 32 mm, *Shigella flexneri* was the most effected bacteria, the differences among the test bacteria against three types honey were significant $P < 0.05$ for thyme honey benefit.

The antimicrobial effects of natural honey samples were compared with the antimicrobial effects of some antibiotics, the isolated bacteria were resistant to most of antibiotics except ciprofloxacin, gentamicin, chloramphenicol, and cefotaxime. The inhibition zone of ciprofloxacin treated bacteria was 30 mm, and of chloramphenicol treated bacteria was 25 mm, in comparison with inhibition zone (24-32mm) of honey samples, the effect of honey was similar to that of antibiotics effect. Therefore the natural honey showed high efficiency on bacterial growth inhibition, these results encourage the interest to prepare pharmacological formula from the natural honey

Introduction

Diarrhea and gastroenteritis (diarrhea with vomiting) are major cause of death and ill health in many developing countries, especially in areas of inadequate water supplies, sanitation and little or no health education. Loss of water and electrolytes from the body can lead to severe dehydration which can be fatal in young children, especially those already in poor health and malnourished. Diarrhea can be caused by organisms such as certain serotypes of *Escherichia coli*, *Shigella* spp. , and other organism such as *Salmonella* spp., *Campylobacter* spp., and *Yersinia enterocolitica* (Hudson *et al.*, 2000).

Therapy with bee products as honey is an old tradition and honey has had many therapeutic uses from ancient times to the present. It has been suggested that pure honey is bactericidal for many pathogenic organisms, including various gram negative and gram positive bacteria (Jeddar *et al.* 1985; Obi *et al.* 1994; Ceyhan and Ugur, 2001; Al-Jabri *et al.*, 2003). Other therapeutic effects of honey include its use in the treatment of fungal infections (Obaseiki-Ebor and Afonya, 1984), burns (Subrahmanyam, 1991), infantile gastroenteritis (Haffejee and Moosa, 1985) wounds and decubitus ulcers (Bergman *et al.* 1983).

Many research works have been made on the antimicrobial effect of honey on different bacterial isolated. Molan (1999) reported that honey is becoming accepted as a reputable and effective therapeutic agent by practitioners of convectional medicine and by the general public. This is because of good clinical results that are being obtained. Honey has been reported to be effective in the healing of infected postoperative wounds (Al-Waili and Saloom, 1999). It has also been reported to

inhibit the growth of many bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella dublin*, and *Sh. dysenteriae* (El-Sukhon *et al.*, 1994; Ceyhan and Ugur, 2001). It has also been reported to inhibit the growth of *Bacteroides* spp. (Elbugoury and Rasomy, 1993).

Materials and methods:

1. Honey samples:-

The honey samples used for this work were purchased from local market these are honey A (thyme honey), honey B (lemon honey), honey C (nabk honey).

2. Test bacteria:-

The test bacteria used in this study were collected from the Microbiology Department of Medicine College Kufa University isolated from fecal specimens from infants aged 2-14 months (watery and bloody diarrhea). These are diarrhea-causing were confirmed using standard bacteria *Escherichia coli* and *Shigella flexneri* bacteriological methods according to Collee *et al.*, (1996) and MacFaddin, (2000).

3. Nutrient agar plates were separately flooded with different test bacteria already in sterile nutrient broth by culturing at 37°C for 24hr. Muller-Hinton agar plates were drained and allowed to dry at 37°C for 30 min before wells of 6 mm in diameter were punched using cork borer at different sites on the plates, three types of honey samples these are honey A (thyme honey), honey B (lemon honey), honey C (nabk honey), were separately placed in the different punched wells and the plates were incubated at 37°C for 24 hr. (Egorove, (1985), the diameter of the zones of inhibition was measured and recorded.

Muller-Hinton agar plates which had been flooded separately with different test bacteria, were allowed to dry at 37°C for 30 min before placing conventional antibiotics disks shown in table (1) by the disk diffusion technique on it, the plates were incubated at 37°C for 24 hr. based on the method of Barry, (1976), the diameter of the zones of inhibition was measured and recorded according to the NCCLS, 2003.

Table (1): Antibiotic disks (Bioanalyse, Turkey)

No.	Antibiotic	Symbol	Concentration (µg) /disc
1	Chloramphenicol	C	30
2	Gentamicin	CN	10
3	Ciprofloxacin	CIP	5
4	Tetracycline	TE	30
5	Trimethoprim	TMP	5
6	Cefotaxime	CTX	30
7	Cefixime	CFM	5
8	Cephalexin	CL	30

Note: All antibiotic discs to be stored between 0-8 °C (for prolonged use store below -20 °C)

4. Statistical analysis

The data were analyzed statistically, using the least significance differences test (LSD), T test and analysis of variance (ANOVA) at the P value level of 0.05 (Daniel, 1999).

Results and discussion:-

The honey samples had antimicrobial activity against all the selected bacteria (table 2, 3, Fig 1, 2). The inhibitory effect was highest on *Sh. flexneri* (32 mm for honey A, 25 mm for honey B and 26 mm for honey C) and *E.coli* (24 mm, 20 mm and 17 mm respectively). The honey A (Thyme honey) was highest. The inhibitory effect than other the differences are highly statistically significant (P value is $p < 0.05$) and it persist among the three sources of the honey. For the selected antibiotics, all except

Cefotaxime, Chloramphenicol, Gentamicin and Ciprofloxacin not inhibited the growth of all the test bacteria.

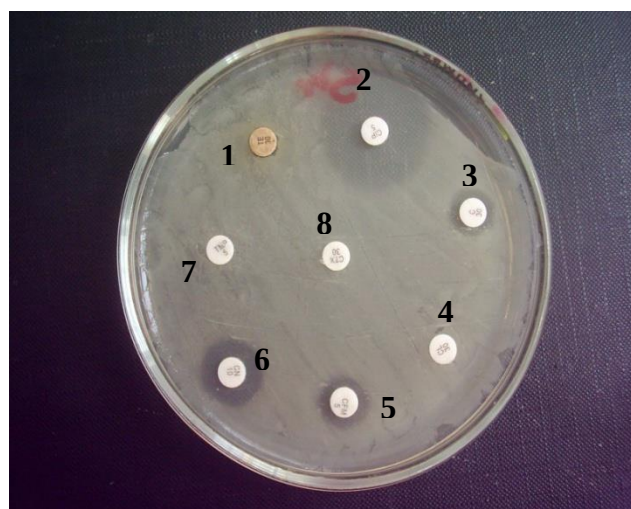
Table (2): Antimicrobial activity of natural honey samples on test bacteria

No	Honey type	<i>E.coli</i>	<i>Sh. flexneri</i>
1	Thyme honey	24	32
2	Lemon honey	21	25.3
3	Nabk honey	18.3	27
LSD		0.971	1.06
P valu < 0.05			

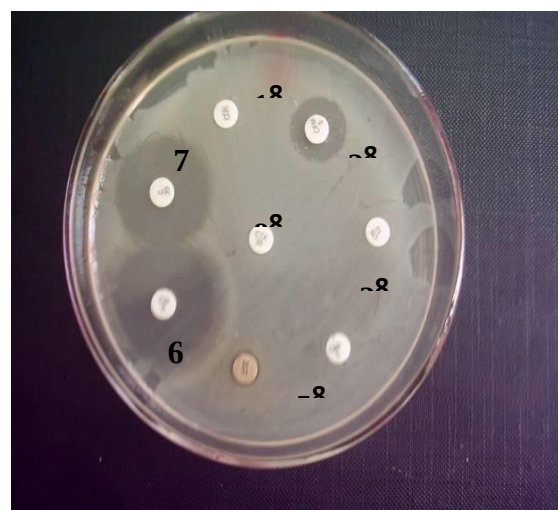
Table (3): Antimicrobial activity of conventional antibiotics on test bacteria

No.	Test bacteria	CL	CFM	CTX	C	CN	Cip	TE	Tmp
1	<i>E.coli</i>	R	15	R	25	R	30	R	R
2	<i>Sh. flexneri</i>	R	10	R	10	13	25	R	R

CL: Cephalixin, CFM: Cefixime, CTX: Cefotaxime, C: Chloramphenicol, CN: Gentamicin, CIP: Ciprofloxacin, TE: Tetracycline, TMP: Trimethoprim, R: resistance

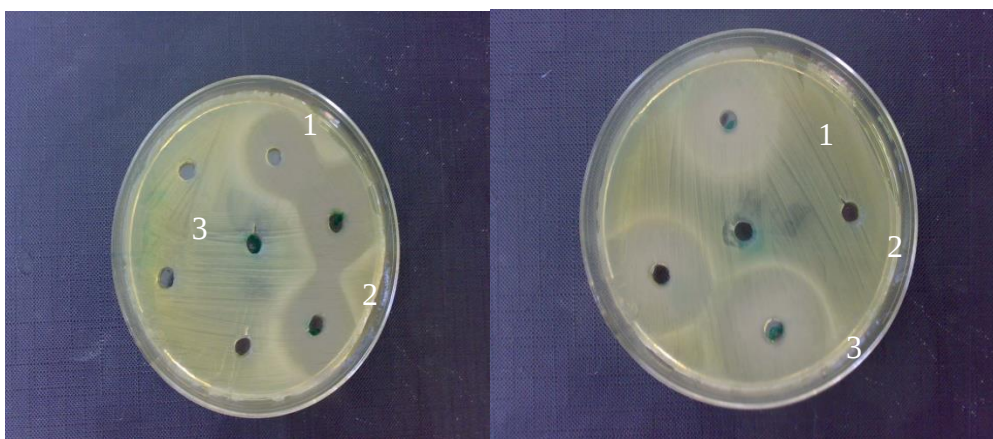


1: TE, 2: cip, 3: C, 4: CL, 5: CFM, 6: CN, 7: TMP, 8: CTX
Sh. flexneri



1: CL, 2: CFM, 3: CN, 4: TMP, 5: TE, 6: Cip, 7: C, 8: CTX
E. coli

Fig (1): Antimicrobial activity of conventional antibiotics on test bacteria



1: Honey A (Thyme honey), 2: Honey B (Lemon honey), 3: Honey C (Nabk honey)

Fig (2): Antibacterial activity of natural honey samples on test bacteria

The growing resistance of bacteria to convectional antimicrobial agents is a source of concern to clinical microbiologists all over the world. As a result efforts are being made to develop antimicrobial agents from local sources for better chemotherapeutic effects (Gills, 1992), from this study, all the test bacteria were inhibited by the three honey samples used.

The inhibition of *Sh. flexneri* by the three honey samples used was the same in some cases with that of standard antibiotics. For example, honey sample A gave zone of inhibition of 32 mm which was the same as that of ciprofloxacin, and honey sample B gave a zone of clearance of 25 mm which was a little higher than that of chloramphenicol (23 mm), these results suggest that the honey samples used contain bio-components whose antibacterial activities are highly comparable with that of these two antibiotics against *Sh. flexneri*. The inability of tetracycline to inhibit the growth of any these bacteria may be as a result of misuse and abuse of this drug (Mah and Memish, 2000). Tetracycline and other antibiotics are among the common antibiotics that have been greatly abused, this result agrees with the finding of Allen *et al.* (1992).

This work has been able to show that honey has antimicrobial activity against bacteria that can cause diarrhea especially the ones that are caused by *Sh. flexneri*, so honey can be used in treating diarrhea caused by these bacteria.

This antimicrobial effect of honey may be due to its physicochemical properties (osmotic effect and pH) or the presence of unknown substances with antibacterial activity (Jeddar *et al.*, 1985).

In conclusion, the good antibacterial effect, sterility, and no or minimal side effects of honey in comparison to many antibacterial drugs, makes honey an ideal antibacterial agent.

However, it is necessary that further investigations be undertaken to discover the mechanisms involved in the antibacterial activity of honey, and the possible ways of its clinical use

References

-Allen, K., L., Molan, P., C., and Reid, G., M. (1992). A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmacol. 43:817-22.

- Al-Jabri, A. A., Nzeako, B., Al-Mahrooqi, Z., Al-Naqdy, A. and Nsanze, H. (2003).** In vitro antibacterial activity of Omani and African honey. Br. J. Biomed. Sci. 60(1): 1-4.
- Al-Waili, N., S., and Saloom, K., Y. (1999).** Effect of topical honey on postoperative wound infection due to gram positive and gram negative bacteria following caesarean section and hysterectomies. Eur. J. Med. Res. 4:126-130.
- Barry, A.L. (1976).** The antibiotic susceptibility test: principles and practices. Lea and Febiger, Philadelphia.
- Bergman, A., Yanai, J., Weiss, J., Bell, D., and David, M. P. (1983).** Acceleration of wound healing by topical application of honey. Am. J. Surg. 145: 374-376.
- Billingham, J. D. (1981).** *Campylobacter enteritis* in the Gambia. Trans. Royal Soc. Trop. Med. Hyg. 75:5.
- Ceyhan, N. and Ugur, A. (2001).** Investigation of in vitro antimicrobial activity of honey. Rivista di Biologia. 94(2): 363-371.
- Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A. (1996).** "Maki and MacCartney practical medical microbiology". 14th ed. Churchill living St U.S.A.
- Daniel, W.W. (1999).** Biostatistics, a foundation for analysis in the Health Sciences, 7th edition. Wiley company.
- El-Sukhon, S., N., Abu-Harfeil, N., and Sallal, A. K. (1994).** Effect of honey on bacterial growth and spore germination. J. Food. Prot. 57:918-920.
- Elbagoury, E., and Rasomy, S. (1993).** Antibacterial action of Manuka honey on anaerobic bacteriodes. Egypt. Dent. J. 39: 381-386.
- Egorove, N. S. (1985).** Antibiotics ascientific approach. Mir Publishers, Moscow.
- Gills, L., S. (1992).** Ethno medical uses of plants in Nigeria. Ilupeju Press Ltd. Pp. 165-250.
- Haffejee, I. E. and Moosa, A. (1985).** Honey in the treatment of infantile gastroenteritis. B. M. J. 290: 1966-1967.
- Hudson, J. A.; Nicol, C.; Capill, J.; and Bennett, J. (2000).** Isolation of Shiga toxin-producing *Escherichia coli* (STEC) from foods using EHEC agars. Lett. Appl. Microbiol. 30: 109-113.
- Jeddar, A., Kharsany, A., Ramsaroop, U. G., Bhamjee, A., Haffejee, I. E. and Moosa, A. (1985).** The antibacterial action of honey. S. Afr. Med. J. 67: 257-258.
- MacFaddin, J.F. (2000).** Biochemical tests for identification of medical bacteria (3rd ed.), Lippincott Williams and Wilkins, U.S.A.
- Mah, M.W.; and Memish, Z.A. (2000).** "Antibiotic resistance". J. Saud. Med. 21(12):1125-1129.
- Molan, P., C. (1999).** The role of honey in the management of wounds. J. Wound Care. 8:423-426.
- National Commetti of Clinical Laboratory Standards (NCCLS). (2002).** The performance standards for antimicrobial susceptibility testing methods. (7th ed.) Vol. 22, No. 1, USA.
- Obaseiki-Ebor, E. E., and Afonya, T., C., A. (1984).** In vitro evaluation of the anticandidiasis activity of honey distillate compared with that of some antimycotic agents. J. Pharmacy and Pharmacol. 36:283-284.
- Obi, C. L., Ugoji, E. O., Edun, S. A., Lawal, S. F., Anyiwo, C. E. (1994).** The antibacterial effect of honey and diarrhea causing bacterial agents isolated in Lagos, Nigeria. Afr. J. Med. Sci. 23: 257-260.

-Subrahmanyam, M. (1991). Topical application of honey in treatment of burns. Br. J. Surg. 78:797-798.

الفعالية المضادة لعسل النحل الطبيعي وأهميته كمضاد حيوي لبعض أنواع البكتيريا المرضية المسببة للإسهال

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الخلاصة:

هدفت الدراسة معرفة التأثيرات المثبطة لأنواع مختلفة من العسل الطبيعي على بعض أنواع البكتيريا المسببة للإسهال. حيث درست الأنواع *Escherichia coli*, and *Shigella flexneri* أظهرت عينات العسل الطبيعي تأثيراً مثبطاً على نمو البكتيريا المدروسة وبنطاق تثبيط تراوح 32-24 ملم، كما كانت *Shigella flexneri* أكثر أنواع البكتيريا تأثراً، فكانت هناك فروقات معنوية بين بكتيريا الاختبار تجاه أنواع العسل الثلاثة $P < 0.05$ لصالح النوع الأول وهو عسل الزعتر .
التأثيرات المضادة للميكروبات لعينات العسل الطبيعي قورنت مع التأثيرات المثبطة لبعض المضادات الحيوية، كانت عزلات البكتيريا مقاومة لأغلب المضادات عدا Ciprofloxacin, Gentamicin, Chloramphenicol, and Cefotaxime نطاق التثبيط للمضاد Ciprofloxacin كان 30 ملم وللمضاد Chloramphenicol كان 25 ملم بالمقارنة مع نطاق تثبيط عينات العسل التي كانت (32-24) ملم ، تأثير العسل كان مشابهاً لقطر منطقة التثبيط للمضادات الحيوية، وبذلك فقد أظهر العسل كفاءة عالية على تثبيط نمو البكتيريا، هذه النتائج تشجع على زيادة الاهتمام بتحضير وصفات دوائية من العسل الطبيعي.