INTRODUCTION

Honey has been used as a medicine since ancient times in many cultures. Aristotle (ca. 350 BC) referred to the use of honey in medicine (Smith and Ross, 1910). Since early times, common uses of honey included treatment of ulcers, bedsores and infections resulting from burns and wounds (Goulart, 1979; Armon, 1980). Honey has also been found to be effective against organisms isolated from urinary tract infections (Ibrahim, 1981) and in the treatment of infantile gastro-enteritis (Haffejee and Moosa, 1985).

The first study on the antimicrobial effects of honey was reported by Van Ketel in 1892 and is mentioned by Dustmann (1979). Since then, various other studies have been published on this subject (Allen et al., 1991; Basson et al., 1994; Dustmann, 1979; Ibrahim, 1981; Molan, 1992; Revathy et al., 1980). A great deal of work has been done on the use of honey in the treatment of various infections and other medical conditions.
done in an attempt to identify the antimicrobial agents in honey and the range of organisms susceptible to this antimicrobial action. The word "inhibine" was introduced to describe these antimicrobial agents and this term is still used today (Dold et al., 1937). One review article lists 64 different bacterial and 13 fungal species on which antimicrobial action has been tested (Molan, 1992).

*Staphylococcus aureus* is an organism that has been included in most comparative studies and has been found to be one of the most sensitive to the antibacterial action of honey. This is of medical importance, since *S. aureus* has become a major cause of wound infections and septicaemia in hospitals (Efem, 1988).

In patients with impaired immunity, neonates or the elderly, *C. albicans* can cause oral candidiasis, also known as thrush. A survey of the literature shows that very little work has been done on the antimicrobial effects of honey in the oral environment. Basson et al. (1994) found that bacterial growth of seven species in the oral *Streptococcus* group was uninhibited by bluegum honey (*Eucalyptus cladocalyx*) at concentrations below 21% (v/v). However, the growth of *Streptococcus anginosus* and *Streptococcus oralis* were inhibited at 17% and 12% respectively.

Sela et al. (1998) found, in an in vivo study, that the initial microhardness of the surface of human enamel decreased significantly after consumption of a teaspoonful of honey in subjects with a normal salivary flow, while the microhardness did not change in patients with a dry mouth condition. Grobler et al. (1994) showed through scanning electron microscopy and microhardness tests that their honey sample did not dissolve human enamel in vitro, despite the low pH value (4.2) of the sample. This result could only be partially attributed to the calcium, phosphorus and fluoride levels in honey; the elements normally associated with the solubility of enamel and dentine. It is well known that enamel dissolves easily in a solution with such a low pH value (Grobler, Jenkins and Kotze, 1985; Grobler et al., 1989).

The major antimicrobial factors in honey may be summarised as follows:

**Osmotic effect.** Eighty two percent (w/w) of honey is a mixture of various carbohydrates, such as glucose, fructose, maltose and sucrose, resulting in a very low water content (Root, 1983; National Honey Board, 1988). This condition is inhibitory to the growth of bacteria, since water is removed from the bacterial cell. Fungi are generally much more tolerant than bacteria to the high osmotic effect and do not cause spoilage of honey when the water content is below 17% (Molan, 1992).

**Acidity.** The pH of honey is between 3.2 and 4.5, mainly due to the presence of gluconic acid (White, 1975). This is considerably lower than the optimum pH of 7.2 to 7.4 required for the growth of most bacteria.

**Hydrogen peroxide (H₂O₂).** This is sometimes found in diluted honey and is a product of the oxidation of glucose by the enzyme glucose oxidase. This enzyme is inactivated by exposure to sunlight (White et al., 1963). The addition of catalase to honey results in the breakdown of H₂O₂.

**Other.** This includes plant-derived substances termed flavonoids (Havsteen, 1983), the most important member being pinocembrin (Molan, 1992). Lysozymes and volatile substances found in plants may also be included here.

The objective of this study was to examine the antifungal action of single samples of honey against *C. albicans*, a common inhabitant in the oral cavity of normal healthy humans.

2. MATERIALS AND METHODS

2.1. Honey samples

A single randomly-selected sample of 3 different types of honey, commonly found
in the Western Cape region of South Africa, were collected from hives by apiarists. After removal of the honey from the combs, the 3 samples were delivered to the authors. The honey was stored, in the dark and at room temperature, in 500 ml plastic jars until required for use. No artificial additives or diluents were added to the honey and they were not subjected to any excessive heating. Honey types were identified and considered to be monofloral in two cases by the apiarists themselves. They were from the floral types bluegum (Eucalyptus cladocalyx) and wasbessie (Myrica cordifolia). Fynbos is a mixture of many heather shrubs, but mainly Erica species.

All samples were examined for sterility and irradiated by gamma rays at a dosage of 20 kilograys for 10 hours (Molan and Allen, 1996). Because honey is very viscous, it was more convenient and practical to prepare dilutions by weight and not by volume. All honey dilutions referred to in this study are on a weight by weight (w/w) basis. The specific gravity of the bluegum, fynbos and wasbessie honey was investigated and found to be 1.455, 1.469 and 1.440 respectively.

All honey samples were weighed in 100 ml plastic containers before varying amounts of sterile water and 25 ml of double strength brain heart infusion broth (BHI broth code CM225, Oxoid, Basingstoke, England) were added. The final volume was 50 ml and dilutions ranged from 0 to 25% (w/w). The initial dilution contained no honey, but a small amount of glucose (0.2 mg per 100 ml) was present in the BHI broth. This amount of glucose, considered to be minimal, is therefore also present in all the subsequent dilutions.

2.2. Control

A hypertonic solution with a carbohydrate composition similar to natural honey (National Honey Board, 1988; Anderson et al., 1973) was made up under sterile conditions. The various sugars were D-glucose (31% w/w), D-fructose (38.5% w/w), maltose (7.2% w/w) and sucrose (1.5% w/w). When tested, this proved to be sterile and was therefore not subjected to gamma irradiation. This highly viscous solution was kept refrigerated at 4 °C when not in use. In this study, the control solutions were diluted and treated as described for the honey samples.

2.3. Inoculum

A C. albicans reference strain 3118 from the National Collection of Pathogenic Fungi (NCPF) at the Central Public Health Laboratory (London, England) was used in this study. An overnight culture in BHI broth was diluted until an optical density (OD) of 0.1 was obtained in a Beckman model DU 64 spectrophotometer, set at a wavelength of 500 nanometres (Beckman Instruments, Fullerton, USA). This corresponded to a growth of $1.7 \times 10^6$ colony forming units (cfu) per ml of broth.

One half (0.5) millilitre of this inoculum was added to 50 ml of each honey and control dilution and mixed thoroughly. A 1 ml aliquot was taken from each dilution and kept refrigerated at 4 °C until further use. These aliquots would serve as blank reference standards when setting the spectrophotometer for reading optical densities at a later stage. All control and honey dilutions were performed in duplicate. The raw undiluted honey was tested for the presence of hydrogen peroxide by means of a commercial test strip (Peroxid-Test, Merck, Darmstadt, Germany).

The inoculated honey and control dilutions were placed in a shaking water bath (Haake model SWB 20, Haake Industries, Berlin, Germany), with the temperature set at 37 °C and the shaker at 60 revolutions per minute. After 24 hours of incubation, 1 ml of each dilution was taken and the OD was read in the spectrophotometer, using the unincubated aliquots as a zero baseline reference standard. The OD of each honey
and control solution was recorded on a graph (Fig. 1), while the variance of each set of readings was calculated.

3. RESULTS

Figure 1 illustrates the stimulation and inhibition on the growth of *C. albicans* in the 3 different honey samples tested, after 24 hours of incubation. The x-axis represents the various honey concentrations ranging from 0 to 25% (w/w), while the y-axis shows the resultant optical density (OD) of each concentration tested. Stimulation in the growth of *C. albicans* was considered to have taken place when the OD showed an increase from one concentration to the next. Any reduction in the OD from one concentration to the next was considered to be partial inhibition, while any OD less than that produced in the initial concentration of 0% was deemed inhibition. Total inhibition takes place at that concentration where the OD produced is equal, or less, than that of the unincubated sample.

In 8 of the 11 dilutions, the sugar control produced an OD higher than that found in the 3 honey samples tested. A distinct peak occurred in the curve of the sugar control at the concentration of 2.5%. The dilutions between 2.5% and 15% showed some partial inhibition in the growth of *C. albicans*.

In the 3 honey samples tested a distinct peak, similar to the one observed in the sugar control, can be seen at 2.5% and 5%. Honey concentrations tested above 5% resulted in partial inhibition on the growth of *C. albicans*. In the 3 honey samples, the greatest partial inhibition on the growth was observed in the wasbessie honey sample, followed by the bluegum and fynbos honey samples respectively. Wasbessie honey, at a concentration of 20% and higher, produced an OD lower than that observed in the initial dilution of 0% and was therefore the only honey type to produce an inhibitory effect on the growth of *C. albicans*. At 25%, the highest concentration tested, the degree of inhibition found in the wasbessie honey was 29.4%. None of the samples tested could produce total inhibition on the growth of *C. albicans*. No hydrogen peroxide was found in any of the 3 undiluted honey samples.

The variance, or degree of error, was calculated for the 4 sets of OD readings and found to be as follows: control = 0.043; fynbos honey = 0.046; bluegum honey = 0.034 and wasbessie honey = 0.053. Curves of the OD readings from the various types of honey were also compared statistically with the

![Figure 1. The antifungal activity of different types of South African honey against C. albicans.](image-url)
control by means of the Wilcoxon signed rank sum test. Two of the honey types tested were found to differ significantly from the control; they were bluegum honey \((p = 0.011)\) and wasbessie honey \((p = 0.008)\). The difference between the fynbos honey and the control was found to be statistically insignificant \((p = 0.441)\). Amongst honey types, there was also a significant difference between the wasbessie and bluegum honey \((p = 0.008)\) and wasbessie and fynbos honey \((p = 0.008)\). The difference between fynbos and bluegum was less, but still significant \((p = 0.015)\).

4. DISCUSSION

The resultant OD curve produced by the control solution reached a distinct peak at the concentration of 2.5%, where the maximum growth was achieved. Some partial inhibition on the growth of \(C. albicans\) was observed in the control solution and is best demonstrated in the concentrations higher than 17.5%, after which the OD gradually decreases. The most likely explanation is that the stimulatory effect of the increasing amounts of additional carbohydrates ceases to have any effect on the growth of \(C. albicans\) beyond a concentration of 17.5% and is overtaken by the inhibitory factors present in the hypertonic solution. It is the hypertonic conditions that cause plasmolysis of the microbial cell, growth inhibition and death (Jay, 1970). For this reason, high sugar concentrations are used as a preservative in the manufacture of fruit preserves, candies and condensed milk.

By examining the curves of the 3 honey samples in Figure 1, a distinct peak can be seen at the concentrations of 2.5% and 5%. The additional carbohydrates, as in the sugar control, stimulated the growth of the \(C. albicans\) up to an optimal point. Above this concentration, the inhibitory factors present in the honey caused a partial inhibition on the growth of \(C. albicans\). This is evident by the gradual decline in the OD curve. In the fynbos and wasbessie honey, this occurred above a concentration of 12.5%, while bluegum honey showed a gradual decline in the OD above the concentration of 15%.

While it must be borne in mind that only a single sample from 3 different types of honey were tested and could be described as unrepresentative, a clear pattern does emerge. After the initial peak in the curve of the control and the 3 honey samples, there is a gradual decrease in the OD to the highest concentration tested. The wasbessie honey sample proved to have the greatest inhibitory effect on the growth of \(C. albicans\). This is also the only honey sample to produce an OD lower than that of the initial concentration of 0%, which contained no honey. In terms of our definition of inhibition, wasbessie honey at a concentration of 20% or higher was thus the only sample in our study to have shown inhibition; this was calculated to be 29.4%. The partial inhibitory effect on the growth of \(C. albicans\) produced by fynbos honey was greater than that demonstrated by the control, while bluegum honey’s inhibitory effect was less than that found in the wasbessie honey. Total inhibition was deemed to have taken place when the OD in any concentration was equal or lower, after incubation, than that recorded in the unincubated sample. None of the samples tested in this study displayed total inhibition.

Molan and Allen (1996) studied the possible effects of gamma irradiation on the antibacterial activity of 5 different types of New Zealand honeys against \(Staphylococcus aureus\). After gamma irradiation of the samples at 25 and 50 kilograys, no significant difference could be found in the antibacterial activity of the honey tested. Since the honey samples in the present study were also irradiated, it was accepted that the antibacterial activity of the South African honeys remained unchanged.

One may conclude that the non-carbohydrate factors present in the 3 honey
samples (such as plant-derived flavonoids, pH and buffering capacity) produced additional inhibition when compared with the control. The initial and rapid rise in the OD curve from the concentration of 0% to 5% may be attributed to the additional nutrients available in the honey, such as carbohydrates, minerals and vitamins. Above the concentration of 5%, the inhibitory factors in the honey become dominant and results in the gradual lowering of the OD curve. This means that the growth benefits of the additional nutrients were masked by the inhibitory factors of honey at higher concentrations.

The various degrees of inhibition demonstrated in this study is most likely due to factors present in the different plant species from which the honey is collected, since the same bee species (Apis mellifera) was responsible for all the nectar collection. It could not be attributed to the water collected by the bees, since Du Toit et al. (1995) concluded that the elemental composition of water did not contribute substantially towards the levels of calcium, phosphorous or fluoride of honey. In a study by Allen et al. (1991) in which the antibacterial activity of honey from 26 different floral sources was tested on S. aureus, it was found that the difference between floral sources were high.

It is still unknown what the effect of honey would be on the many other microbial species found in the human oral cavity. Marsh and Martin (1998) listed more than 80 such microbes.

C. albicans is mainly associated with oral candidiasis; in patients with impaired immunity, neonates or the elderly. Other clinical conditions associated with C. albicans are vaginitis, otitis externa and chronic skin and mucous membrane infections (Shanson, 1989).

In a review by Molan on the antimicrobial activity of honey (1992), the findings of several workers are discussed. Cavanagh, Beazley and Ostapowicz (1970) found that a 100% (v/v) concentration of honey had a complete fungicidal effect on C. albicans, while a 50% concentration was necessary to bring about the same action in the species of C. stellatoidea, C. reukaufii and C. tropicalis. However, only a 10% (v/v) honey concentration was necessary for a fungicidal effect against C. pseudotropicalis. In our study, we did not test whether the inhibitory effect was fungicidal and is therefore unable to make comparisons to these findings.

In the study of Dolezal, Dolezal and Medrela-Kuder (1988), it was found that a low honey concentration of 1.6% (v/v) could bring about complete inhibition of C. albicans. This was inconsistent with our findings and those of Revathy and Banerji (1980), who found that a 100% (v/v) honey concentration caused complete inhibition. In our study, it was found that the wasbessie honey became inhibitory for C. albicans at a concentration of 20% (w/w) or higher.

It may be deduced from the literature and the results of this study that various types of honey, at different concentrations, have different antimicrobial effects on various microbial species. Molan (1992) also reported that the antimicrobial activity of honey, within the same floral source, may be significantly different. After testing the 3 single samples of South African honeys, the conclusion is made that wasbessie honey was the only type that could produce inhibition on the growth of Candida albicans. At 25% (w/w), the highest concentration tested, this inhibition was found to be 29.4%. Bluegum and fynbos honey could only produce a partial inhibition on the growth of Candida albicans; the weakest antifungal action was seen in the fynbos honey.

Résumé – Action antifongique de trois miels d’Afrique du Sud vis-à-vis de Candida albicans. On sait peu de chose concernant l’action du miel sur la microflore orale. Les données connues laissent penser que
l’action antibactérienne du miel est largement due aux effets osmotiques de la teneur hypertonique en sucres, au faible pH du miel et à la présence occasionnelle de péroxyde d’hydrogène.


Les échantillons de miel ont été prélevés dans des ruches de la région de Western Cape en Afrique du Sud par des apiculteurs et envoyés au laboratoire. Tous les échantillons ont été irradiés aux rayons γ à la dose de 20 kilogrammies pendant 10 h afin de les rendre stériles. Dans cette étude les dilutions ont été calculées sur une base poids par poids (w/w). Le poids spécifique a été déterminé pour chacun des miels : 1,455, 1,469 et 1,44 pour les miels d’eucalyptus, de fynbos et de *M. cordifolia* respectivement. Les diverses dilutions de miel, de 0 à 25 % (w/w) ont été préparées dans 50 ml d’un bouillon infusé cervelle-cœur. Ce bouillon a été inoculé avec 0,5 ml d’une culture de nuit de *C. albicans* (souche de référence 3 118 de la collection nationale de champignons pathogènes (NCPF) du « Central Public Health Laboratory » à Londres, Angleterre. Une solution hypertonique (78 % w/w) avec une teneur en sucres semblable à celle du miel a servi de témoin. Les suspensions inoculées ont été incubées à 37 °C dans bain marie agité durant 24 h à 60 tours/minute. Des échantillons de 1 ml ont été prélevés et la densité optique (DO) mesurée à l’aide d’un spectrophotomètre réglé à la longueur d’ondes de 500 nanomètres. On a considéré qu’il y avait stimulation de la croissance lorsque la DO diminuait et inhibition totale si la DO de l’échantillon incubé était égale ou inférieure à celle trouvée avant l’incubation.

On a observé une stimulation de la croissance de *C. albicans* et divers degrés d’inhibition pour le témoin sucre et les trois échantillons de miel. La plus forte inhibition de croissance de *C. albicans* a été obtenue avec le miel de *M. cordifolia*, suivi par le miel d’eucalyptus et le miel de fynbos (Fig. 1). La courbe de DO pour le témoin sucre, les miels de fynbos et de *M. cordifolia* ont atteint un pic à la dilution de 2,5 %.

Des concentrations plus fortes ont abouti à une certaine inhibition de la croissance de *C. albicans*. Le miel d’eucalyptus a provoqué une croissance maximale à la concentration de 5 %, après quoi une diminution graduelle de la DO a été observée. Le miel de *M. cordifolia* a provoqué une croissance maximale à la concentration de 2,5 %. À la concentration de 20 % et plus, il était le seul miel à inhiber la croissance par rapport à la concentration 0 %. Les autres miels n’ont provoqué qu’une inhibition partielle.


Eine Stimulierung des Wachstums wurde angenommen, wenn eine erhöhte optische Dichte (OD) von einer Konzentrationsstufe zur nächsten gemessen wurde. Eine Teilhemmung fand statt, wenn die OD abnahm. Wir waren der Ansicht, dass eine vollständige Hemmung stattfand, wenn die OD der inkubierten Probe gleich oder geringer war als ohne Inkubation.


Bei 2,5 % Honig von *M. cordifolia* erreichte der *C. albicans* seine maximale Wachstumsrate. Ab 20 % und höher erwies sich der Honig von *M. cordifolia* als einziger Honig, der eine Hemmung des Pilzwachstums im Vergleich zu den Lösungen ohne Honig bewirkte. Die anderen Honige erzeugten nur eine Teilhemmung.

**REFERENCES**


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