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Original Research Article

Antibacterial Activity of Tea Tree (*Melaleuca alternifolia*) Oil against Methicillin Resistant *Staphylococcus aureus*

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A B S T R A C T

With the increasing failure of conventional medicines in treating various diseases as drug resistance among pathogens progresses phytomedicine are increasingly gaining popularity as alternative sources for their control. In view of this the purpose of the given study was to examine the effectiveness of Tea tree oil for the control of multidrug resistant bacteria. Agar well diffusion method and broth dilution methods were used to assess the antibacterial property. Tea tree oil was effective against all the strains of *Staphylococcus aureus* tested. The isolates were subjected to antibiotic sensitivity towards Ampicillin, Methicillin, Ciprofloxacin, Penicillin, Gentamycin, Vancomycin, Erythromycin, Oxacillin, Tetracycline, Co-trimoxazole. All the isolates were resistant against Methicilin and Oxacillin.MIC and MBC concentration was determined by the broth dilution method. All the test strains of *S. aureus* were inhibited by Tea tree oil in broth dilution method. While comparing the assessment of the inhibitory effect of Tea tree oil, agar well diffusion was effective as compared to broth dilution method. However, Tea tree oil was effective against the pathogenic strains tested. Tea tree oil represents a valuable weapon against MRSA. A broad spectrum activity of Tea tree oil could have future potential and strong antimicrobial agents and be used in therapeutic purpose as well as an alternative medicine. The present study demonstrates the potential of Tea tree oil for using in the treatment of MRSA infection and coming prospective of pharmaceuticals.

Keywords

Tea tree oil, *Staphylococcus aureus*, Antibiotic susceptibility test Antibacterial activity, Minimum inhibitory

Introduction

From ancient times there are varied methods of medicines in India like Aurveda, Homeopathy and Unani, which utilize plant materials for drug production. Currently, Aurveda is considered as a vital system of medicine and has governed worldwide recognition and also has non-toxic substances. Also, newly discovered non-antibiotic substances such as certain essential oils and their constituent chemicals Chavan et al (2006) have shown good fighting potential against drug resistant pathogens Cowan (1999) (Ahmad and Beg, 2001). Essential oils are aromatic oily liquids, which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots by steam distillation. Scientifically these oils have been proved highly potent antimicrobial agents in comparison to antibiotics. These plant essential oils are rich source of scents and used in food preservation and aromatherapy.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is recognized as a major nosocomial pathogen that has caused problem in hospital worldwide, with the UK having one of the highest rates of MRSA in Europe Johnson et al (2005) .Generally anterior nares, the groin, axilla and broken skin are the sites of MRSA colonization. It is generally in practice to decolonize the MRSA infection with topical antimicrobials.

Tea tree oil plant is a small tree in the Myrtaceae family, growing to a height of about (0.75m) with narrow, soft, alternate leaves and white to yellowish flowers the shape of bottle brushes. Its Latin name is *Melaleuca alternifolia.* This plant originated in Nambucca Valley, New South Wales, Australia. The indigenous Bundjalung people of Eastern Australia use "tea tree" as a traditional medicine by inhaling the oils from the crushed leaves to treat coughs and colds. They also sprinkle leaves on wound, after which a poultice is applied. The commercial tea tree oil industry was born after the medicinal property of oil was first reported by Penfold in 1920s. Tea tree oil is defined by International Standard Organization, Geneva, as oil of Melaleuca terpinen-4-ol type ISO (1996).

Tea tree oil has been scientifically investigated when applied topically for antiviral, antibacterial, antifungal and antiseptic qualities. It has been reported that the terpinen-4-ol is regarded as the main antimicrobial component constituting 80-90% of tea tree oil Southwell et al (1993).Other major component include p-cymene,linalool, α and γ –terpinen, α-terpinolene and together with cineole and terpinen-4-ol.Tea- tree oil has been considered as a potential agent for MRSA decolonization, as it has been shown to be an effective broad spectrum antimicrobial with good activity *in vitro* against a variety of bacteria including MRSA Hammer et al (1999).

Materials and Methods

Microorganism

S. aureus were derived from the stock cultures of the "Microbial culture collection bank" of Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad. *S. aureus* were cultured in nutrient broth at 37°C for 24 h. The test organisms were streaked on the nutrient agar slants and incubated overnight at 37± 1°C. Cultures were kept under refrigerated condition and were sub cultured every 15 days.

Procurement of tea tree oil

The essential oil of *Melaleuca alternifolia* required for the present study was purchased (local market).

Antibiotic susceptibility test of *Staphylococcus aureus*

All clinical isolates of *S.aureus* were subjected to *in-vitro* antibiotic susceptibility testing method on Nutrient agar, using fresh nutrient broth culture and antibiotic discs Bauer et al (1966). Briefly, the zone of inhibition around the disc were measured and interpreted as sensitive, moderately sensitive, and resistant using the interpretation chart supplied by the antibiotic disc manufacturer. The disc diffusion method was used to determine the susceptibility of all clinical isolates to Ampicillin, Ciprofloxacillin, Co-trimoxazole, Erythromycin, Gentamycin, Methicillin, Oxacillin, Penicillin, Tetracyclin, Vancomycin, according to Clinical and Laboratory Standards Institute (Wayne,2003) guidelines.

Antibacterial analysis of Tea tree oil against *Staphylococcus aureus*

The technique was used *in-vitro* Agar well diffusion method for measuring the antibacterial potential of essential oil (Fitzpatrick, 2010). To seed one ml of the clinical isolates in the Petri dishes containing previously melted nutrient agar antimicrobial agent was prepared in concentration of 1µl, 2µl, 3µl, 4µl, 5µl, 6µl and filled into wells aseptically. After the latency period at 37 ±1°C for 24-48 hours, the zone of inhibition surrounding the disks was measured.

Determination of the minimal inhibitory concentration (MIC)

The determination of MIC and MBC of the essential oil of *Melaleuca* oil on the test bacterial strain was carried out by using broth dilution method Carson et al (1995). 4.5ml nutrient broth was used which was supplemented with 0.5ml Dimethyl sulphoxide. Serial doubling dilutions of *Melaleuca alternifolia* oil was prepared in a test tube over the range of (5.12-0.005%v/v). Active cell suspension of (0.5ml) of test organism and media was placed into oil test tube. Media, treatment and organism control were prepared. All the test tubes were incubated at 37°C for 24-48 hrs.

To confirm MICs and to establish MBCs, (10µl) of broth was removed from test tube and their plating was performed. After incubation, plates were observed for growth of organism.

The MIC of low concentration resulted in the maintenance of reduction of inoculums while the MBC was the point where 99.9% of the inoculums were killed. Each trial was triplicated.

Statistical analysis

The data recorded during the course of investigation were analyzed statistically using ANOVA (one way classification) The calculated value was determined at 5% level of significance for appropriate degree of freedom (Panse and Sukhatme,1967).One way ANOVA clarified the pattern of activity of different strains.

Results and Discussion

Of all the clinical isolates of *S.aureus* tested, 50% were found to be multi-drug resistant (MDR). Ciprofloxacillin, Cotrimoxazole, Erythromycin, Gentamycin, Tetracyclin and vancomycin were shown to be the most effective antibiotics against all the *S.aureus* isolates inhibiting the bacterial growth to the highest extent. The least effective antibiotic was Methicillin. 90% resistance of isolates was detected against the drug Methicillin followed by Penicillin (80%) and Oxacillin (50%). (Table-1)

In similar studies a higher percentage sensitivity for vancomycin (90-100%) was reported Prakash et al (2007),Sanjana et al (2010),Kaleem et al (2010) . Other antibiotics like Ciprofloxacin and Erythromycin, exhibited a sensitivity range of 62.0 to 82.0 percent Nwanze et al (2007),Mamhood et al (2010) . The present investigation showed a higher percentage sensitivity for Vancomycin (100%) Ciprofloxacin and Erythromycin.

Various literatures have also reported all resistant strains of MRSA resistant to Penicillin (100%) with most MRSA strain showing multidrug resistance (Khadri and Alzohairy,2010) Somanathan et al (2010) .However in contrasted by Farzana et al (2011) reported the sensitivity of the bacterium against Ampicillin and Co-

trimoxazole. According to (Hoekstra and Paulton,2002) antimicrobial resistance was commonly found for Penicillin G, Lincomycin, Tetracycline and Trimethoprimsulphamethoxazole in *Staphylococcus aureus*.

Prolonged and extensive use of antibiotics in the medical centers and Hospitals leads MRSA to build resistance towards them. The resistance to antibiotics and multiple-drug resistance in MRSA are due to the presence of plasmid DNA and several drug resistant genes in a single plasmid, each with its own resistance marker.

The tea tree oil showed highest inhibitory activity against test organism. Strain number 9 with an inhibition zone of 30.00mm and least activity was observed for strain number 10 giving an inhibition zone of 16.00mm .The Strain numbers 2, 4, and 5 gave an inhibition zone of size of 24.00mm The strain numbers 1, 3, and 7, showed inhibition zone is size of 18.00mm. The strain number 6 gave an inhibition zone of 20.00mm.The maximum effect was at 6µl concentration and minimum effect was at 1µl concentration of tea tree oil (Table-2 and Fig-1).

In accordance with the present investigation various literature have shown the antibacterial potential of *Melaleuca alternifolia* (tea tree) oil and members of the *Myrtaceae* family, against strains of *Staphylococcus aureus* especially MRSA, exhibiting zones of inhibitions ranging from 10-45mm (Carson and Riley,1994) Bosnic et al (2006) Park et al (2007) Chao et al (2008).

However, Dalirsani et al (2011) showed a small zone of inhibition (0.60mm) was produced by tea tree extract against *Streptococcus mutans*.

The inhibition zone among different observation may differ due to many factors. First, the composition of plant oils is known to vary according to local climate and environmental conditions Janseen et al (1987) Sivropoulou et al (1995). .Furthermore, some oils with the same common name may be derived from different plant species Windholz et al (1983) (Reynolds,1996).The method used to assess antimicrobial activity and the choice of test organisms, varied between different studies Morris et al (1979) (Smith palmer, 1998).The usefulness of Agar well diffusion method is limited to the generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils prevents the uniform diffusion of these substances through the agar medium Ross et al (1980). Hence these variations in the antibacterial activity were observed.

The antimicrobial activity of an essential oil is linked to its chemical composition. The functional group of some compounds found in most plant materials are alcohol, phenols, terpenes and ketones associated for its antimicrobial characteristics.

The essential oil kills bacteria by damaging the cell membrane's structure, inhibiting membrane function. When tea tree oil acts on microbial membrane; it alters their permeability and affects the membrane's ability to osmoregulate the cell adequately. Due to this it inhibits respiration and genetic material being lost from the cell. The highest antimicrobial activity is due to presence of catechins and polyphones which damages bacterial cell membrane. The interaction between two components of tea tree oil 1, 8-cineole and terpinene is chiefly responsible for the antibacterial action of *Melaleuca alternifolia***.** 1, 8-cineole exhibits little antimicrobial activity inherently, however, it has been shown to enhance the lethal action

of terpinene. It is hypothesized that 18-cineole helps permeabilize bacterial membranes, allowing more active terpinen to enter and kill the bacterial cell Carson et al (2002) (Gibbons, 2004).

The minimum inhibitory concentration and minimum bactericidal concentration of tea tree oil was (0.04%v/v) and (0.08%v/v) against strain numbers 1, 3, 5, and 8 of *S. aureus*. The minimum inhibitory concentration and minimum bactericidal concentration of tea tree oil was (0.02%v/v) and (0.04%v/v) against strain numbers 2, 6, 7, and 10.The MIC and MBC of tea tree oil was (0.04%v/v) against strain numbers 4 and 9.

Nelson1(997) reported the MIC value of (0.25% v/v) and MBC (0.5%v/v) of tea tree oil obtained against MRSA strain. Loughlin et al (2008) in their study showed a higher MIC (2%v/v) and MBC (4%v/v) of tea tree oil against 30 isolates of *S.aureus*. Harkenthal et al (1999) demonstrated the MIC and MBC value of tea tree oil to be (0.12-0.5%v/v) against various methicillin resistant strains of seven species of *Staphylococcus*, e.g. *S.aureus, S.epidermidis, S.haemolytics*.

(Mann and Markham ,1998) reported MIC value of (0.04%v/v) of tea tree oil against *S.aureus* which was in agreement to the given investigation.

Emulsifying agent such as Tween -20,80, and dimethylsulphoxide (DMSO) may cause changes in the physiocochemical properties of the test system, resulting in either enhancement or reduction of the antimicrobial activity. Lipophilic molecules, including the components of tea tree oil, may become solubilized with in the n micelle formed by non-ionic surfactants, such as Tween 20 and Tween 80, and are thus partitioned out of the aqueous phase of the suspension resulting in varying values of MICs (Beylier ,1979) (Walsh and Longstaff,1987) Patkar et al(1993) (Dean and Ritchie,1987) (Scortichini and Rossi, 1991).

Ability to compare data from different studies is limited due to differences in test methodologies and in the criteria selected for the determination of the end point. In testing of essential oils, broth dilution micro methods employ an indicator to determine cell viability, as the turbidity of oil-water emulsions interferes with the determination of end points Villar et al (1986) Chand et al (1994). A number of factors need to be considered; Culture condition, e.g. the type and volume of broth, the concentration and age of inoculums, the temperature and time of incubation are among the most important factors, which should precisely be stated in reports. Additionally, a variety of other factors, such as the solvent or dispersing agent, may also influence the result (Kalemba and Kunicka, 2003).

In conclusion, the present study confirmed antimicrobial properties of tea tree oil that showed a significant growth inhibition for *S.aureus* tested. The encouraging result indicate that the tea tree oil might be exploited as natural antibiotic for the topical treatment of several infectious diseases caused by *S.aureus* and could be useful in understanding the relations between traditional cures and use of current medicines.

Isolation and characterization of potential drug molecules and their commercialization for future clinical application is further required. More clinical and pharmacological studies need to be undertaken to establish dose pattern of tea tree oil. It is worthwhile to develop new techniques and the

guidelines for standardization of techniques implied for plant's essential oil analysis so that inter-study outcome can be safely measured. There is a need to investigate oil of *Melaleuca* species from different geographical locations for the most active ingredients responsible for their antibacterial activity**.**

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References

Ahmad, I., A.Z. Beg. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi, drug resistant human pathogens. J of ethno Pharmacal, 74: 113-23.

Bauer, A. W., Kirby, W.M.M., M. D., Sherris, J. C., and Turck. M. 1966. Antibiotic Susceptibility testing by a Standarized single disk method. Am .J .Clin. pothole .45:493-496.

Beylier, M. F. 1979. Bacteriostatic activity of some Australian essential oils.Perfumer a, 33nd Flavorist.4: 23-25.

Bosnic, T., Softic, D. and Vasic, J. G. 2006. Antimicrobial activity of some essential oils and major constituents of essential oils. Acta Medica Academica.35: 19-22.

Carson, C.F., Hammer, K.A., and Riley, T.V. 1995. Broth micro-dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). Microbios.82:181-185.

Carson, C. F., Mee, B. J. and Riley, T. V. 2002. Mechanism of action of *Melaleuca alternifolia* (Tea tree) oil on *Staphylococcus aureus* determined by time-kill, lyses, leakage, and salt tolerance assays and electron microscopy. Antimicrob.Agents Chemother . 46: 1914-1920**.**

Carson, C.F. and Riley, T.V. 1994. Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia*. Lett. Appl. Microbiol .19:24-25.

Chand, S. Lusunzi, I., Veal, A.D., Williams, R. L. and Karuso, P. 1994.Rapid screening of the antimicrobial activity of extracts and natural products. J. Antibiotics .47:1295-1304.

Chavan, M.J., D.B. Shinde and S.A. Nirmal. 2006. Major volatile constituents of Annona squamosa Linn. Bark. Nat. Prod. Res., 20: 754-757.

Chao, S. Young, G. Oberg, C and Nakaoka, K. 2008. Inhibition of methicillin – resistant *Staphylococcus aureus* (MRSA) by essential oils. Flavour Fragr. J. 23:444- 449.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol., 12: 564-582.

Dalirsani, Z., Aghazadeh, M., Adibpour, M., Amirchaghmaghi, M., Pakfetrat, A., Mosannen, M., Mehdipour, P., and zenooz, T. 2011. In vitro comparison of the antimicrobial activity of ten herbal extracts against *Streptococcus mutans* with chlorhexidine. J. Appl. Sci .11 (5):878-882.

Deans, S.G., and Ritchie, G. 1987. Antibacterial properties of plant essential oils. Int .J. Food Microbiol. 5:165-180.

Farzana, k., Hameed. A., Waqas. K. M., Murtaza. Najam –us - saqib. Q.,

Waseem. A., Asad. B. H. H. M and Hussain. I. 2011. Bactericidal activity of various brands of cephradine against *staphylococcus aureus.* Int. J. Phys. Sci.6 (6):1501-1507.

Fitzpatrick .M. 2010. Antimicrobial action of tea tree oil (*Melaleuca alternifolia*) on five common bacteria. J. Occup. Med and Toxicol.1-10.

Gibbons, S., 2004. Anti-Staphylococcal plant natural products. Nat. Prod. Rep.21:263-277.

Hammer, K.A., Carson, C.F. and Riley, T.V. 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl .Microbiol .86:985-990.

Harkenthal, M., Reichling, J., Geiss, H.K. and Saller.R.1999. Comparative study on the in vitro antibacterial activity of Australian tea tree oil, niaouli oil, manuka oil, kanuka oil, and eucalyptus oil.Pharmazie.54(6):460-463.

Hoekstra, K.A., and Paulton, R.J.L. 2002. Clinical prevalence and antimicrobial susceptibility of *Staphylococcus aureus* and *Staph.intermedius* in dogs. J. Appl .Microbiol. 93:406-413.

International Organization for Standardization 1996. ISO 4730 Oil of Melaleuca,terpinen-4-ol type (tea tree oil).Switzerland: International Organization for Standardization.

Janssen, A.M., Scheffer, J.J.C and Svendsen, A.B. 1987. Antimicrobial activity of essential oils: a 1976-86 literature review. Aspects of the test methods.Planta Medico. 53:395-398.

Johnson, PDR; Martin, R; Burrell, LJ; Grabsch, EA; Kirsa, SW; O'Keeffe, J; Mayall, BC; Edmonds, D; Barr, W; Bolger, C; Naidoo, H; Grayson, ML.2005. Efficacy of an alcohol/chlorhexidine hand hygiene program in a hospital with high rates of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Med J Australia, 183 (10): 509-514.

Kalemba, D., andKunicka, A. 2003. Antibacterial and antifungal properties of essential oils.Bentham Science Publishers Ltd.10:813-829.

Kaleem, F., Usman, J., Hassan, A., Omair, M., Khalid, A. and Uddin, A. R. 2010. Sentivity pattern of Methicillin resistant *Staphylococcus aureus* isolated from patients admitted in a tertiary care hospital of Pakistan. Iranian .J. Microbiol .2 (3):141-143.

Khadri, H., and Alzohairy, M. 2010. Prevalence and antibiotic susceptibility pattern of methicillin-resistant and coagulase-negative Staphylococci in a tertiary care hospital in india. Int. J. Med. Med. Sci. 2 (4):116-120.

Loughlin, R., Gilmore, F.B, Carron, Mc. and Tunney, M.M. 2008.Comparison of the cidal activity of tea tree oil and terpinen-4-ol against clinical bacterial skin isolates and human fibroblast cell. Lett. Appl. Microbiol.46:428-433.

Mamhood, K., Tahir, T.Jameel, T. and Aslam, H.F. 2010. Incidence of methicilin-resistant *Staphylococcus aureus* (MRSA) causing nosocomial infection in a tertiary care hospital. J. Antimicrob. Chemother.16 (2):91-96.

Mann, M.C. and Markham L.J. 1998. A new method for determining the minimum inhibitory concentration of essential oils. J. Appl. Microbiol.84:538-544.

Morris, J.A., Khettry, A., and Seitz, E.W. 1979. Antimicrobial activity aroma chemicals and essential oils. J. Am. Oil Chem. Soc. 56:595-603.

Nelson, R.R.S. 1997. In vitro activities of five plant essential oils against Methicillin –resistant *Staphylococcus aureus* and Vancomycin – resistant *Enterococcus faecium*. J. Antimicrob. Chemother.40:305-306.

Nwanze, P.I., Nwaru, L.M. Oranusi, S., Dimkpa, U., Okwu, M.U., Babatunde, B.B., Anake, T.A., Jatto, W. and Asagwara, C.E. 2007. Urinary tract infection in Okada village Prevalence and antimicrobial susceptibility pattern. Sci. Res. Essays. 2 (4):112-116.

Panse, V.G and Sukhatme, P.V. 1967. Statistical methods for Agricultural workers. ICAR publication, New Delhi.36:225-238.

Park, H., Jang, C.H., Cho, Y.B. and Choi, C.H. 2007. Antibacterial effect of Tea tree oil Methicillin-resistant *Staphylococcus aureus* Biofilm Formation of the Tympanostomy tube: An In vitro study. Int. J of Exp and Clin Pathophysio and drug research .21: 1027-1030.

Patkar, K.L., Usha, C.M., Shetty, H.S., Paster, N. and Lacey, J. 1993. Effects of spice essential oils on growth and aflatoxin B, production by *Aspergillus flavus*. Lett . Appl. Microbiol .17:49-51.

Prakash, M., Rajasekar, K., and Karmegam, N. 2007. Prevalence of Methicillin Resistant *Staphylococcus aureus* in Clinical samples Collected from Kanchipuram Town, Tamil Nadu, and South India. J. Appl. Sci. Res .3 (12):1705-1709.

Reynolds, J.E.F. 1996. Martindale the extra pharmacopoeia 31 1stedn. London: Royal pharmaceutical Society of Great Britain. Int. J. Pharm.142 (2):257-258.

Ross, S.A., EI-Keltawi, N.E. and Megalla, S.E. 1980. Antimicrobial activity of some Egyptian aromatic plants. FIT, 51:201-205.

Sanjana, K.R., Shah, R., Chaudhary, N. and Singh, I.Y. 2010. Prevalence and antimicrobial susceptibility pattern of methicillin- resistant *staphylococcus aureus* (MRSA) in CMS- teaching hospital: a preliminary report. J.Collge. Med. Sci-Nepal .6 (1):1-6.

Scortichini, M. and Rossi, M.P.1991. Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burril) Winslow et al. J.Appl .Bacterio .71:109-112.

Sivropoulou, A., Kokkini, S., Lanaras, T., and Arsenakis, M. 1995. Antimicrobial activity of mint essential oils. J. Agri. Food Chem.43: 2384-2388.

Smith-Palmer, A., Stewart, J., and Fyfe, L., 1998. Antimicrobial properties of plant essential oils and essences against five important food-born Pathogens. Lett. in Appl. Microbiol**.** 26:118-122.

Somanathan, M. D., Dawande, Y. A. and Kawale, M. 2010. Antimicrobial activity of clinical and community isolates of *Staphylococcus aureus*. Asiatic J. Biotech Res.01:14-19.

Southwell, H.A., Markherm, J.and Leach, D. 1993. The search for optimally bioactive Australian Tea tree oil. Acta Horti .334:256-265.

Villar, A., Recio, M.C., Rios, J.L. and Zafra-polo, M.C. 1986.Antimicrobial activity of essential oils from *Sideritis* species. Pharmazie.41:298-299.

Walsh, L. J. and Longstaff, J. 1987. The antimicrobial effects of an essential oil on selected oral pathogens.J.Periodontol.8: 11-15.

Wayne, P. A. 2003. Clinical Laboratory Standards Institute, Disk Diffusion supplement tables CLSI Document MIW-513(M2) pp: 1-25.

Windholz, M., Budavari, S. and Blumeric, R.F. 1983. Th merck index, an Encyclopedia of biological 10thedn. Rahway, NJ: Merck and yourself, R.T. and Twail G.G. (1980) Antivolatile oils. Die Pharmazie. 35: 698-701.