Antibacterial activity of tea tree oil against clinical isolates of Escherichia coli

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Abstract:

Introduction: Complementary and alternative medicines such as tea tree oil have become increasingly popular in recent years. This essential oil has been used for several decades in European countries but have now become increasingly popular in a wide array of products. The primary uses of tea tree oil is mainly because of the antibacterial and antiseptic properties of the oil itself. Antibacterial activity of tea tree oil on clinical isolates of Escherichia coli has been reviewed in this study.
Aim: To determine the antibacterial activity of tea tree oil against clinical isolates of E. coli. **Materials and methods:** A total of 20 non repetitive urinary isolates of Escherichia coli were collected followed by qualitative method of Antibiotic Susceptibility testing.
Results: We have observed that, clinical isolates of E. coli were inhibited from 0.03-0.25% of tea tree oil. The MIC of tea tree oil was appeared to be 0.03% for E. coli
Conclusion: Tea tree is found to have antibacterial activity against clinical isolates of E. coli. However, the studies on toxic and irritant properties of essential oils are imperative, especially when considering any new products for human administration. This can be used as alternative and complementary antibacterial agents for controlling the infections.

Introduction:

Escherichia coli, one of the most common and frequent etiological agents of urinary tract infection (UTI) and is also responsible for wide array of other infections such as bacteremia, pneumonia, soft-tissue infection and neonatal meningitis. Multiple antibiotic resistance exhibited by such E. coli strains is a major concern, as they are showing resistance even to the carbapenems, which are considered to be the last resort of antibiotics for such stubborn E. coli infections. Tea Tree Oil (TTO) or melaleuca oil is known since long for many of its medicinal uses and also for other uses [1]. It is an essential oil obtained from the leaves and terminal branches of M. alternifolia through steam distillation method. It is available all over the world and is used for its antimicrobial properties [2]. It should be handled with care as it is toxic on oral consumption and for injections but can be used for its topical application use [3] studies have shown that TTO is not genotoxic in vitro in mammalian cells [4]. It is incorporated as an active substance in many topical applications used for treatment of cutaneous infections for controlling dandruff, lice, herpes, acne and other skin infections [5]. After a study on Tea tree oil samples [6] about 100 compounds were identified in tea tree oil. Antibacterial activity of Tea tree oil has been noted and has been reported to be broad-spectrum inhibiting bacteria. The minimum inhibitory concentration (MIC) for tea tree oil for most of the susceptible bacteria has been reported ranging from 0.003% for Prevotella intermedia [7,8] and maximum greater than 8% (v/v) for Enterococcus faecalis strains of bacteria [9]. A recent study on ATCC reference and clinical strains [10] of different bacteria namely, Pseudomonas aeruginosa , Staphylococcus aureus, E. faecalis, Salmonella Enteritidis, S. Typhimurium and escherichia coli. Tea tree oil is bacteriostatic in low concentration but bactericidal at higher concentrations. Though exact mechanism of action is yet to be understood it is hypothesized and proved to some extent [11,12] that TTO act through increasing permeability of liposomal systems causing lysis and the loss of membrane integrity and manifested by the leakage of ions and the inhibition of respiration and ultimately death of the bacterium [13].In early studies on the

antimicrobial activity of TTO, it was hypothesized to be more active against antibiotic-resistant bacteria [14] thus attracted considerable interest. Thereafter, several studies were conducted but only on a limited number of strains specifically using mupirocin resistant and methicillin-resistant strains of *Staphylococcus aureus* (MRSA) and of other bacteria [15,16,17]. Most of the studies concluded an insignificant difference in TTO sensitivity of antibiotic-resistant and sensitive strains. Resistance to TTO in clinical isolates has not yet been reported and resistance of bacteria to conventional antibiotics has not been correlated with susceptibility to TTO, suggesting that cross resistance does not occur. Tea tree oil is seen as important alternative as a topical antimicrobial for antibiotics. Search for "alternatives to antibiotics" is identified as one of the most important goals to combat the emerging antibiotic resistant pathogens [18]. It is often claimed that TTO can replace antibiotics at least for topical applications [19]. The present study aimed to test the sensitivity of variety and number of the bacterial strain, isolated from clinical cases with the potential to cause a wound or cutaneous infections, to TTO and simultaneously to common use topical antimicrobials. Data, interpreted and was analyzed for contact and association between Tea tree oil sensitivity and antimicrobial sensitivity and multi-drug resistance in different bacteria different origins. Thus the aim of the study was to determine the antibacterial activity of tea tree oil against clinical isolates of E. coli.

Materials and methods:

Bacterial isolates: A total of 20 non repetitive urinary isolates of Escherichia coli were collected from Saveetha Medical College and Hospitals, Chennai. They were processed for standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy broth stock and were stored at 4 ºC until further use. Antibiotic susceptibility testing was done.

Antibiotic susceptibility testing: Antibiotics/antimicrobial agents are the major drugs of choice of the physician's desk to treat the host of various infections. This prescribing pattern may be one of the reasons for the development of resistance for the antibiotics [20]. Therefore, Antibiotics susceptibility testing plays an important role to check the effectiveness and efficacy of a drug against a bacterial strain or several bacterial strains and select the best drug that acts against the strains. The main objectives of the testing are to find out drug resistance in common microorganisms and the susceptibility to drug of choice for a particular infectious organism or bacterium can be found out and can be assured. Mechanism of antimicrobial resistance: There are number of ways by which microorganisms are resistant to antimicrobial agents. These includes: Bacteria produce enzymes which can destroy the antimicrobial agents before it reaches the targets e.g. Beta lactamase enzyme hydrolyses beta lactam drugs which develop resistance to it. Impermeable cell for antimicrobial drugs e.g. Gram negative bacteria may become resistant to Beta lactam antibiotics by developing a permeability barrier. Mutation develops macrolide resistance. Bacterial efflux pump that expels antimicrobial drugs from cell before they can reach their targets. Specific Metabolic pathways in the bacteria are genetically altered so that antibacterial agents cannot exert an effect [21,22]. Antimicrobial susceptibility testing has several important purposes which can include a) A laboratory test which can determine how effective antibiotic therapy is against a bacterial infection or infections b) Antibiotic Susceptibility testing can control the use of antibiotics in clinical practice. c) Antibiotic susceptibility testing will help the clinicians in choosing the choice of drugs for the treatment of infection or infections d) Antibiotic Susceptibility Testing can help the local sequence of antibiotic prescriptions. e) Antibiotic Susceptibility Testing can be used to reveal the changing trends in local isolates.
Methods of Antimicrobial susceptibility testing:

There are several methods of antibiotic susceptibility testing. The method followed in this study was the qualitative method of Antibiotic Susceptibility testing.
Qualitative Method: This method is used for testing of isolates from healthy patients with an intact immune defense system, in less serious infections such as Urinary Tract Infection. There are two qualitative methods.
Disk diffusion test: The disk diffusion sensitivity test also known as the Kirby Bauer disk diffusion method. It is a simple and practical test which uses the antibiotic- impregnated wafers or disks to test whether particular bacteria is susceptible to specific antibiotic or not [23,24] The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petridish Mueller Hinton agar. The antibiotic disks were placed on top of the previously inoculated Mueller Hinton agar medium surface with the help of sterile instruments like forceps. Each disc must press down on one another to ensure complete contact with agar surface. The plates were incubated for 18–24 h at 35-37 degree C temperature in bacteriological incubator before an interpretation and arrival of the result. The antibiotic is found to diffuse from the disc into the agar in decreasing amounts, the further it is away from the disk. This states that the antibiotic that is furthest away from the disks has a better permeability in the disks. If the organisms were killed or inhibited by the concentration of the antibiotic, there will be no growth in the immediate area around the disks will be represented as the zone of growth inhibition. The diameter of the zone of inhibition is directly proportional to the sensibility of the isolate and to the diffusion rate of antibiotics through the agar medium. A zone of inhibition was measured in millimeters by either measuring the Radius: Measure half the distance of the zone and then multiply by 2. This method was used when part of the zone is not clear or has grown into another zone. Diameter: Measure the entire length of the zone and subtract the disk diameter . The standard disk size was found to be about 5 to 6 millimeters. The result of the test can be interpreted by using the criteria published by Clinical and Laboratory Standard Institute (CLSA formerly the National Committee for the Clinical Laboratory Standard or NCCLS) [25]. The results of the disk diffusion test are "qualitative" and will be reported out as: Susceptible: 'The term "susceptible" represent that isolates are inhibited by the usually recommended dosage of an antimicrobial agents. However, this term doesn't fully assure clinical success in all cases. Predicting clinical outcome based on susceptibility testing and the use of drugs shown to be in the susceptible category is very imprecise. This imprecision is due to the effect of host responses, site of infection, toxin production by bacteria that is independent of antimicrobial susceptibility, the

presence of biofilm, drug pharmacodynamics and other factors. As an outcome, there can be two categories, namely Intermediate and resistant categories.

A. Intermediate: 'The "intermediate" category includes isolates with antimicrobial MICs that approach usually attainable blood and tissue levels and for which response rates might be lower than for other susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated, quinolones or beta lactams in the urine or when a higher than normal dosage of a drug can be used (e.g. Beta lactams).

B. Resistant: 'The category indicates that the isolates are not inhibited or stopped by the usually achievable concentrations of the antibiotics with normal dosage schedules, which demonstrate an existence of the specific microbial resistance mechanisms (e.g. Beta lactamase). The merits of the disk diffusion methods are simplicity in test, most patient cost friendly, a wide variety of disks can be chosen during disk selection, and the result can be easily interpreted by the clinicians. However, the demerits of this can include manual test, lack of automation and all slow growing bacteria cannot be accurately tested by this method. The limitation of this testing show that the microbiologist and clinician both should be cautious and should not forget that the response therapy in vivo may not always reflect the result of testing the sensitivity of patient's pathogen in vitro. A study conducted by Rakesh Kumar to study antimicrobial sensitivity pattern of Escherichia coli from urine samples of UTI patients used the resistant test method [26].

Well diffusion method: In this agar well diffusion method, a suitable agar medium was prepared, once the agar is solidified the medium was fully inoculated and was swabbed with bacterial suspension of approximately 1-2 X 108 CFU/mL using a cotton swab. The wells were prepared by punching with a six millimeters diameter standard sterile cork borer made up of stainless steel . These wells were filled up with 25 – 50 µL of the antimicrobial solution/s to be tested. Well diffusion test has been used for susceptibility testing of antifungals like fluconazole, itraconazole. The plates were incubated at 35 ± 2°C for 18 – 24 h. The antimicrobial activity is calculated in millimeter by using the expression: ZOI = Total Diameter of growth inhibited zone minus diameter of the well, where, ZOI is Zone of inhibition. The factors which may affect the result of AST included Density of an inoculum, Disk application time, Temperature of incubation, Potency of drug, inappropriate storage conditions, pH the agar medium, Moisture on the surface of the medium and effects of Thymidine or Thymine containing agar medium [20]

Results:

Sample wise distribution of clinical isolates of E.coli:

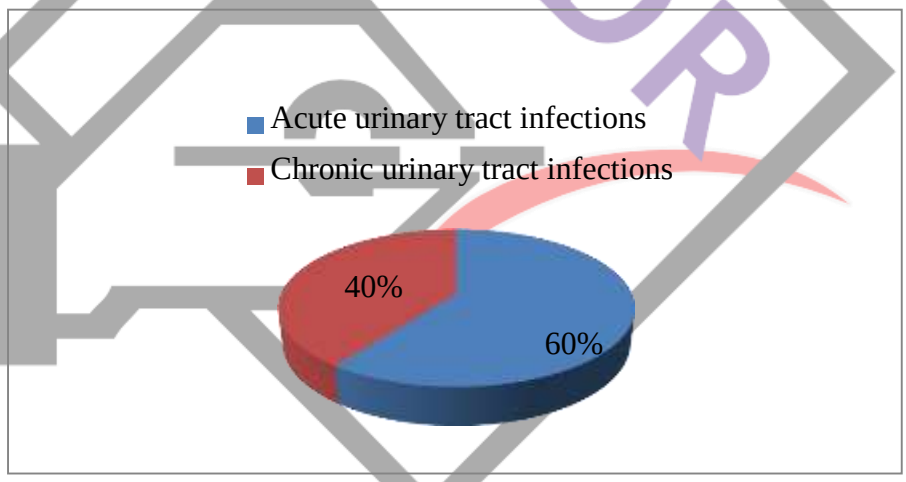

Figure 1: Sample wise distribution of urinary isolates of *E.coli*

Antibiotic susceptibility testing:

Antibiotics	Sensitivity (20) (%)	Intermediate (20) (%)	Resistant (20) (%)
Ampicillin	5	0	95
Amoxicillin	5	0	95
Ceftazidime	10	10	80
Cefotaxime	5	5	90
Amikacin	70	10	20
Gentamicin	45	20	35
Norfloxacin	15	15	70
Ciprofloxacin	20	5	75
Imipenem	70	0	30

Table 1: Antibiotic sensitivity pattern of E.coli

We have observed that, clinical isolates of E. coli were inhibited from 0.03-0.25% of tea tree oil. The MIC of tea tree oil was appeared to be 0.03% for E. coli.

Dilutions of Tea tree oil	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
No. of organisms	4 (20)	2 (10)	6 (30)	8 (40)	0	0	0

Table 2: MIC of Tea tree oil

Discussion:

Antibiotic susceptibility test fixed to routinely used antibiotics such as ampicillin, amoxicillin, norfloxacin, ceftazimide, cefotaxime, ciprofloxacin and gentamicin, imipenem by the Kirby Bauer disc diffusion method. Complementary and alternative medicines such as tea tree oil have become increasingly popular in recent decades. This essential oil has been used for almost 100 years in Australia but is now available worldwide both as neat oil and as an active component in an array of products. Tea tree oil have been historically used for their antiseptic and anti- inflammatory actions. Food processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of food borne illness outbreaks caused by some pathogens. The increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another concern. Hence, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. Plant essential oils are a potentially useful source of antimicrobial compounds. Although numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food borne pathogens a review of the earlier literature reveals that the results reported for these different studies are difficult to compare, presumably because of the different test methods, bacterial strains, and sources of antimicrobial samples used.
Detection of antibacterial activity of tea tree oil against clinical isolates of E. coli:

Anti-bacterial activity of tea tree oil was tested against E. coli isolates by minimum inhibitory concentration method.
Mueller Hinton broth was supplemented with 0.002% (V/V) tween 80 (HiMedia, Mumbai) to enhance the dispersion of the essential oil. Agar dilution method was performed to attain the different concentrations of essential oil such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% in Mueller Hinton Agar (MHA). Media containing various concentrations of essential oil were poured over the sterile petridishes and allowed to dry. Media without essential oil was served as control plate.
Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37ºC for overnight. The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC. The lowest concentration of the essential oil, here tea tree oil that completely inhibited the growth of the isolates of Escherichia coli was found to be about 0.03 % in this study. This is found to be a much lower MIC when compared to the MIC achieved by other plant essential oils in a similar study which covers a whole spectrum of antibiotics. The MIC achieved in this study conducted in Australia was found to be about 0.23%.[21]. Another study performed confirms that many essential oil plant extracts possess in-vitro antibacterial and antifungal properties. The MIC as per this study was found to be roughly the same as in our study.[22] Another study, aimed at studying the membrane integrity of e coli bacteria when exposed to several different antibiotics upon tea tree oil, found that e coli lost its surface cell membrane integrity when it was exposed to around 0.5 % MIC of the antibiotic. [23]

Conclusion:

Tea tree is found to have antibacterial activity against clinical isolates of E. coli. Nevertheless, the studies on toxic and irritant properties of essential oils are imperative, especially when considering any new products for human administration. This can be used as alternative and complementary antibacterial agents for controlling the infections.

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