



COMPARING THE USE OF HERBAL AND GENERIC DRUG PREPARATIONS IN OVERCOMING ANTIBACTERIAL RESISTANCE IN STRAINS OF PATHOGENIC E.COLI

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ABSTRACT

To treat bacterial infections, herbs have long been utilized. Numerous pathogenic Escherichia coli strains have been shown to be resistant to frequently administered antibiotics, raising concerns about antibacterial resistance. This study compared the effectiveness of using generic medication preparations to herbal remedies in treating pathogenic E. coli strains that have developed antibiotic resistance. To evaluate the effectiveness of both therapies, the study combined in vitro and in vivo trials. The studies herbal remedy comprised a concoction of extracts from six distinct plant species, each of which is well-known for having antibacterial capabilities. The generic medication mixture employed was a blend of two widely used antibiotics. According to the study, pathogenic E. coli bacteria that were resistant to the generic medication formulation may be effectively stopped from growing by the herbal preparation. In fact, it was discovered that the herbal preparation was more successful at combating antibacterial resistance than the generic medication preparation. In addition to being used in conventional treatment, complementary and alternative therapies from various traditions. In addition to being used in conventional treatment, complementary anumerous strains of pathogenic Escherichia coli are showing resistance to commonly prescribed antibiotics, which is a growing concern for health authorities around the world. Exploring different methods of treating bacterial infections has become urgently necessary as a result of this. Since ancient times, herbal remedies have been used as a safe, all-natural treatment for a wide range of illnesses, including bacterial infections. A wide variety of phytochemicals found in them have been shown to have antibacterial activities. Due to this, herbal remedies are being investigated as a potential substitution for traditional antibiotics in the fight against antibacterial resistance and alternative therapies from various traditions. There have been claims of efficient antibacterial agents in plant materials such as leaves, flowers, stems, roots, and other parts. In our research study we conducted invitro studies to detect and compare the generic drugs with herbal drugs to explore the antibacterial resistance of Ecoli.

Keywords: Bacteria; Energetic drugs; Herbal drugs; Invitro studies: Antimicrobial resistance

INTRODUCTION

Since it jeopardises our ability to treat common infectious diseases, the World Health Organisation (WHO) has identified antibiotic resistance as one of the biggest risks to food safety, development¹⁻⁷ and global health at the moment. Every month, antibiotic resistance claims hundreds of lives. Infectious microbiological diseases including smallpox, cholera, diphtheria, pneumonia, typhoid fever, plague, TB, typhus, syphilis, etc. were widespread before antibiotics were developed. Smallpox, cholera, diphtheria, pneumonia, typhoid fever, plague, tuberculosis, typhus, syphilis, and other contagious microbiological diseases were widespread in the pre-antibiotic era all over the world (Adedeji WA. 2016)⁸⁻¹⁵. Unfortunately, as bacteria began to develop antibiotic resistance within 50–60 years, these medical science breakthroughs started to lose their significance.(Davies 2010; Coates AR2007; Franco BE2009). Since the last two decades, AMR has been deemed the greatest health threat of the twenty-first century, posing an increasingly serious risk to public health worldwide¹⁶⁻¹⁹ (WHO2018; Ferri M2017). Infections brought on by bacteria, parasites, viruses, and fungi are now more difficult to prevent and treat as a result of AMR's ongoing growth(Hassan andHolmstrup, 2018:WHO2017). An extensive range of ARB can be found in fresh produce. These resistances will only be therapeutically significant if they are transmissible, in contrast to intrinsic resistance, which cannot be easily spread within the bacterial community (Enover, 2006). The latter is of little significance unless it is carried by a pathogen because known intrinsic resistances are disregarded by expert guidelines defining the levels of antimicrobial resistance (that is, multidrug-resistant, extensively drug-resistant, and pandrug-resistant²⁰⁻²⁵. (Magiorakos, 2012]. Contrarily, it has been demonstrated that antibiotic resistance is a long-standing issue that develops without human intervention(Perry, 2016).

The term "antibiotic resistome" has been coined to describe the total number of genes contributing directly or indirectly to antibiotic resistance in both clinics and the environment, in order to make it clear that antibiotic resistance²⁶⁻²⁹ is an issue that is not just in hospitals. Extended-spectrum -lactamase (ESBL)-producing Enterobacteriaceae, including Escherichia coli, are among the ARB harbouring transmissible antibiotic resistances of the highest clinical relevance, with vancomycin-resistant Enterococcus spp. (VRE), one of the top twelve serious drug-resistant threats, ranking among them.(Magiorakos and Srinivasan³⁰⁻³⁵, 2012).

According to a survey conducted by the World Health Organisation (WHO, 1998), 70–80% of people worldwide, and especially those who live in industrialised countries, rely on complementary and alternative medicines as their main source of healthcare. This is because natural medicines are readily available and reasonably priced (Sofowora, 1993). Due to the development in the use of herbal remedies in Nigeria, the quality, safety, and efficacy of these drugs have become a significant concern for health authorities and medical specialists (Lau et al., 2003; Adeleye et al., 2005). Today, there is a serious risk to the public's health from drug-resistant pathogenic microbes (NIAID, 2011). One of the most frequent environments for the evolution of resistant bacterial strains is a pressured setting with pollutants that were previously non-resistant bacterial strains. One of these settings can be herbal medicines (HMPs). Historically, herbal prescription medications

Materials and methods

In our research, we used several chemicals and strains to carry out the experiments and we purchased the chemicals from the Krishnaraman Chemicals Pvt. Ltd from Chennai, Tamilnadu.

PROCEDURE

One generic drug, standard antibiotic and two herbal drug preparations used against the growth of pathogenic E.Coli strains.

Extract preparation

The leaves part of *Equisetum arvense* (Horsetail) and *Urticadioica* (nettle) plant material were purchased from Nature Care and Cure Centre, Tamil Nadu and it was authenticated by a Botanist. The leaf parts of the plant were immersed in 70% ethanol (100 g/l) for 1 week. After this period, the filtration of the alcoholic extract was done and it was concentrated using a rotary evaporator and then lyophilized. Until further use, the storage of the dried material was stored at 4°C. The dilution of the dried extract was suspended in saline immediately prior to use.

MICROBIAL STRAINS

From Microkit Laboratories, standard Escherichia coli strains (ATCC 25922, E Coli (CFT073)) were purchased. The bacterial colonies were removed from the agar plate and added to 5 mL of Müller-Hinton Broth. This mixture was then incubated at 37°C for 2–5 hours or until the turbidity level reached 0.5 (or roughly 1×10^8 UFC/mL), at which point it was spectrophotometrically quantified. To obtain a lawn of growth, this broth culture was then equally distributed on Mueller Hinton Agar plates..

Determination of MIC

In order to achieve a density of 2×10^4 cells per ml, the cultures were first incubated and then serially diluted. 100 L of cell culture were injected into tubes with two millilitres of MHB broth. Then, 100 L of various test drug concentrations were added to each tube. For 48 hours, anaerobic jars were used to incubate all of the experimental tubes. Following the incubation period, the optical density at 600 nm was determined. MIC was defined as the lowest test medication concentration that resulted in a 20% suppression of test microorganism growth. Each experiment was run in a set of three copies. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration that prevented colour change.

Statistical analysis:

Results were given as mean SD. One-way analysis of variance (ANOVA) and the post hoc least-significant difference test were used to establish the statistical significance. P values under 0.05 were deemed significant.

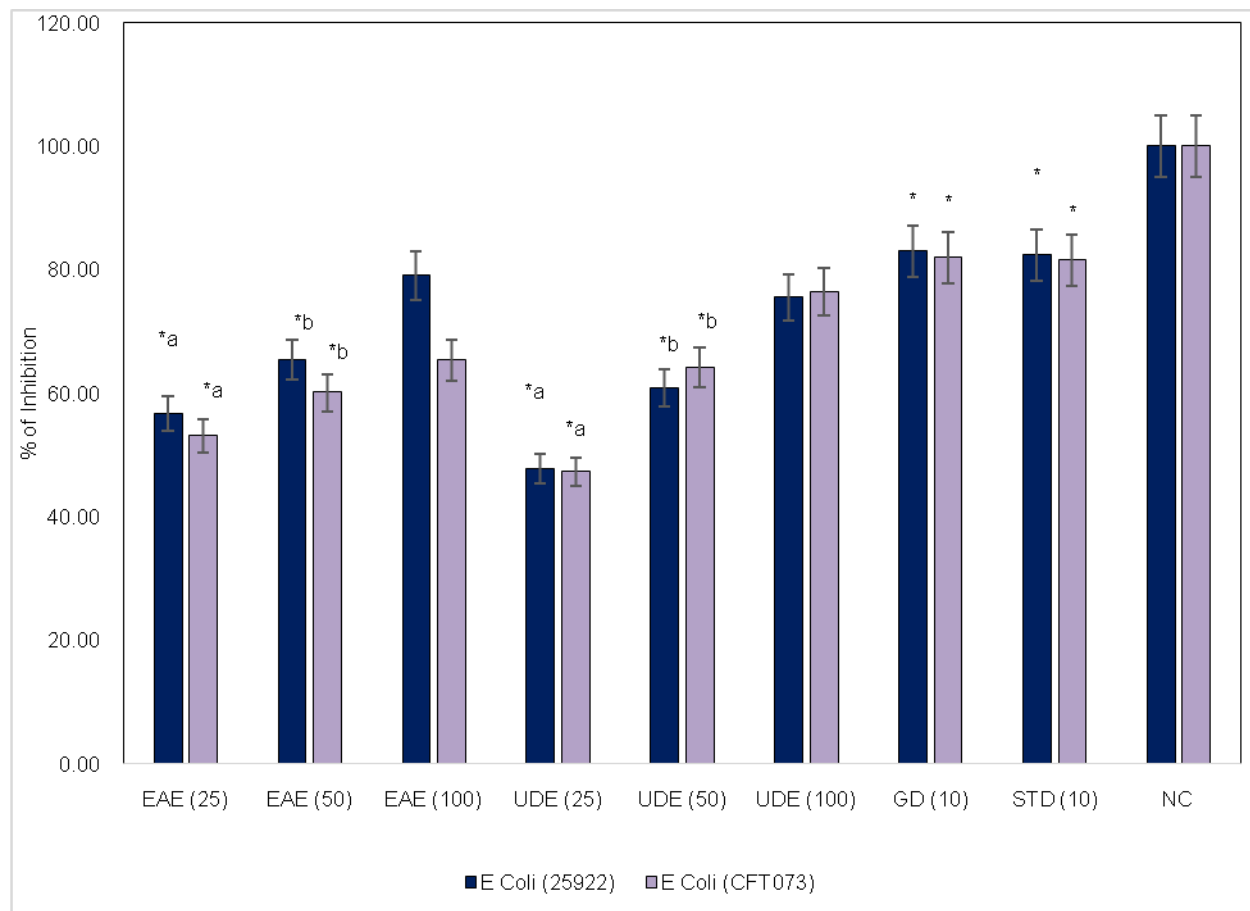
Determination of MIC

	Conc (mg/ml)	E. coli (ATCC 25922)	E Coli (CFT073)
EAE	25	$0.219 \pm 0.15^*$	$0.241 \pm 0.15^*$
	50	$0.175 \pm 0.14^*$	$0.205 \pm 0.18^*$
	100	$0.106 \pm 0.11^*$	$0.178 \pm 0.10^*$

UDE	25	0.264 ± 0.25*	0.271 ± 0.39*
	50	0.198 ± 0.28*	0.184 ± 0.21*
	100	0.124 ± 0.29*	0.121 ± 0.17*
Generic drug	10	0.086 ± 0.15*	0.093 ± 0.15*
Amoxicillin	10	0.089 ± 0.02*	0.095 ± 0.03*
Negative control	-	0.506 ± 0.06	0.514 ± 0.05

All the values mentioned above are Mean ±SD. * p<0.001 statistically significant as compared with negative control.

MIC, mg/ml	E. coli (ATCC 25922)	E Coli (CFT073\
EAE	8.81	9.41
UDE	10.45	10.59
Generic drug	2.46	2.44
Amoxicillin	2.42	2.45



This graph represents the percentage of microbial inhibitory growth. Results are expressed as Mean \pm SD. ^aP<0.001 statistically significant as compared with negative control. ^aP<0.001; ^bP<0.01 statistically significant as compared with standard. GD- Generic drug ; STD- standard; NC-Negative control; EAE - Equisetum arvense extract; UDE - Urticadioica extract

The results of this study indicates that efficacy of generic drug is equivalent to standard drug. The results for the MIC of generic drug, antibiotic and 2 herbal extract were presented in this study.



Equisetum arvense

Urticadioica



Equisetum arvenseeethanolic
extract powder

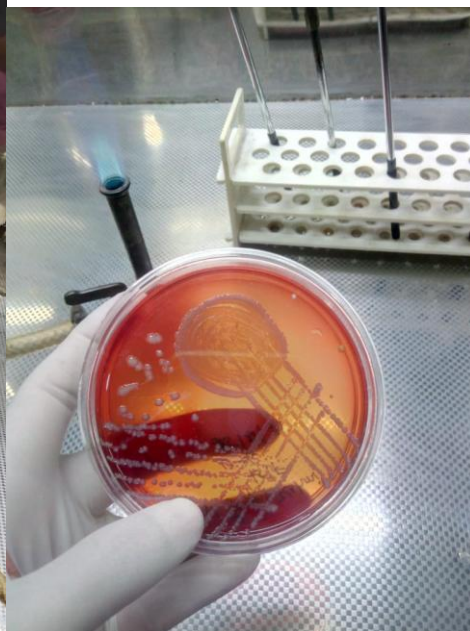
UrticadioicaUrticadioica
extract powder



Incubation of culture plates



Microbial strains streakings



E.Coli growth

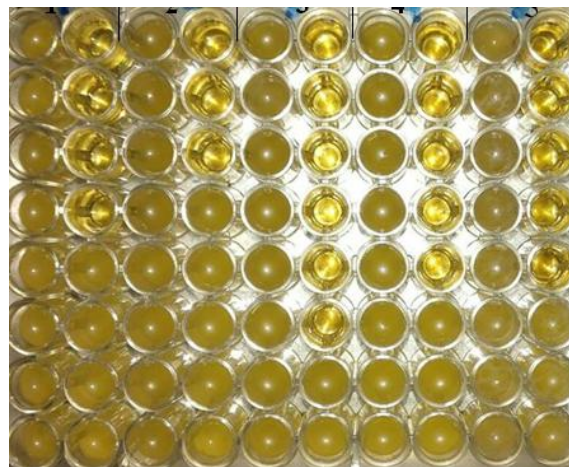
Incubating the tubes for 12h and then for 24hrs at 37°C



Measuring the turbidity using visible spectrophotometer



Addition of herbal drugs to the microplate



MIC of the drugs



MIC reading using microplate reader

Results and discussion

The results of this study indicates that efficacy of generic drug is equivalent to standard drug. The results for the MIC of generic drug, antibiotic and 2 herbal extract were presented in this study. Ping Wang *et al.*, (2021) reviewed that Antioxidant activity differs dramatically between fresh walnut kernels (FW) and dry walnut kernels (DW). It is still unknown what metabolomic mechanism underlies these variations. Here, we assessed the antioxidant properties of four

different types of walnuts' FW and DW and used GC-MS and LC-MS/MS to identify their metabolites. Using a multivariate analysis, it was determined how various metabolites affected the antioxidant activity of walnut kernels. Comparing DW to FW, antioxidant activities were substantially higher in DW. All samples contained 144 different metabolites in total. The antioxidant activity is highly influenced by phenolic metabolites and polyunsaturated fatty acids, according to a Pearson correlation analysis. DW's increased antioxidant activity may be mostly related to its higher level of, according to a subsequent network analysis of 21 target differential metabolites. Aiysha Thompson (2013) reported that Numerous essential oils had antibacterial activity in the three assays, indicating that they might make excellent candidates for investigation in clinical trials. In a published clinical experiment, the effectiveness of a combination of coriander, lemon balm, and mint extracts against IBS was supported by the observed antibacterial activity of ethanolic extracts of spearmint, coriander, and lemon balm leaves. Dashen (2022) indicated that 45 samples of herbal remedies were examined, 15 of which were each of the "MaganinShawara," "Maganintsusanciki," and "MaganinBasir"—all of which were said to treat piles, typhoid fever, and intestinal parasites, respectively. Standard techniques were used to isolate, identify, and determine the antibiotic susceptibility pattern of the bacterial contaminants of the herbal items. The purpose of the study was to evaluate certain liquid herbal medicines available in Jos, Nigeria for their bacteriological purity and to identify the isolates' susceptibility to various antibiotics. The analysis of 45 liquid herbal samples revealed contamination with 21 (46.67%) *Escherichia coli*, 8 (17.78%) *Staphylococcus aureus*, 6 (13.33%) *Salmonella sp.*, 7(15.56%), and 3(6.67%) *Bacillus sp.* and *Proteus vulgaris*, respectively. All of the isolates tested positive for high levels of resistance to the antibiotics used in this investigation, according to Abah and Jassim's 2017 testing for antibiotic reactivity. The results of this study suggest that herbal remedies have poor bacteriological quality and may be a source of multi-drug resistant bacteria. We looked at 45 herbal remedy samples, 15 each of "MaganinShawara," "Maganintsusanciki," and "MaganinBasir," which are allegedly effective against pile, typhoid fever, and intestinal parasites. The bacterial contaminants of the herbal products were isolated, identified, and their pattern of antibiotic susceptibility was determined using standard procedures. The study's goal was to evaluate the bacteriological cleanliness and antibiotic susceptibility of several liquid herbal treatments sold in Jos, Nigeria. For many years, both in human and animal medicine, antibiotics were the answer to all problems. Bacteria that

are resistant to the effects of antibiotics have evolved as a result of the massive amount of antibiotics that are used to promote the growth and maintain the health of farm animals.

Conclusion

Many illnesses, including bacterial infections, have been treated for centuries with herbal remedies. Numerous plants have been found to have antibacterial properties, making them a potential antibiotic substitute. The individual plant, the technique of preparation and the quantity of active ingredients can all have a significant impact on how effective a herbal medicine is. While their effectiveness is typically well-established, generic drug preparations are typically subjected to more stringent testing and regulation. However, the development of antibiotic-resistant bacteria brought on by the overuse of antibiotics has become a major global public health concern. Overall, additional research is required to assess the effectiveness of herbal treatments and over-the-counter medications in combating pathogenic E. coli's antibiotic resistance. The best method for treating bacterial infections and halting the emergence of strains resistant to antibiotics may combine the two techniques. It is crucial to remember that any medication should only be used as prescribed by a doctor.

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