

Original Research Article

In vitro evaluation of an antibacterial effect of rose leaves and petals extract along with antibiotics on *Escherichia coli*

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ABSTRACT

Background: Today Herbal medicine are commonly used because of their easy availability and affordability. It is more closely corresponds to the patient's belief, concerns about the adverse effects of chemical (synthetic) medicines, persuade a desire for more personalized health care, and allows significant public access to health information. The major use of herbal medicines is for health promotion and treatment for chronic, as opposed to life-threatening, conditions. Usage of traditional remedies increases when conventional medicine is less effective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases. The hibiscus *Rosa sinensis* plant extract have multiple organic component like flavonoids, tannins, alkaloids, triterpenoids which are known to have antibacterial activity against *E. coli*, *P aeruginosa*, Salmonella species. Phytotherapy is considered to be less toxic and minimal or no side effects in comparison to modern allopathic medicines. Therefore, in today's scenario there has been reappearance of interest developed in herbal medicine. Therefore, the study was planned to look for antibacterial activity of extract of petals and leaves of hibiscus *Rosa sinensis* against.

Methods: *Escherichia coli* isolated from different clinical samples along with resistant antibiotics. All resistant isolates were tested along with hibiscus *Rosa sinensis* extract both leaves and petals ex-tract with resistant antibiotics.

Results: We found that out of total 51, 35 (68.63%) were resistant *E. coli* isolates to different antibiotics and 85.72% were from urine samples. Enhancement effect in zone size with many resistant antibiotics was seen individually with both extracts. Antibiotics like ampicillin, cefotaxime, cotrimaxazole, cefuroxime, piperacillin, gentamycin showed more enhancement with rose petal extract than leaves. While antibiotic like levofloxacin (5%) showed enhancement only with leaves extract and not with the petals extract.

Conclusions: It was found that Rose petal extract showed more antibacterial activity along with the antibiotics as compare to Rose leaves extract.

Keywords: Leaves extract, Petal extract, Rose

INTRODUCTION

Members of the genus *Rosa* (Family Rosaceae) are considered as one of the world's most popular ornamental plants because of their beauty and fragrance.^{1,2} In addition to fragrance, there has been an interest in the medicinal applications of roses. In ancient medicine, *R. damascena* was used for the treatment of abdominal and chest pain, menstrual bleeding, digestive problems,

depression, grief, nervous stress, tension, skin problems and headaches.³ Flavonoids of petals were found to possess strong resistance to UV radiation (254 nm) and they potentially could be used in antisolar creams.⁴ Flavonoids have also antioxidant activity and were found to be protective to DNA from oxidative damage.^{5,6}

One of the major problems that concerns public health, is bacterial resistance against antibiotics.

However, environment of hospitals serves as a reservoir for various microorganisms; which may be resistant to multiple antibiotics and the selective pressure of antimicrobial used in hospitals, therefore makes an environment a repository for these resistant strains.⁷⁻⁹ However, selective pressure exerted by antimicrobials drug use also has been the major driving force behind the emergence and spread of drug resistant traits among pathogenic and commensal bacteria. Surveillance data showed that resistance in *E. coli* is consistently highest for antimicrobial agents.¹⁰ The emergence of *E. coli* isolates with multiple-antibiotic resistance phenotypes, involving coresistance to four or more unrelated families of antibiotics has been reported and is considered a serious health concern.¹¹

Antimicrobial drug resistance (ADR) hampers the control of infectious diseases and has potential to threaten health security, damage trade and economies but it is difficult to think of “the world without antibiotics”. Most of the pathogenic bacteria have developed resistance to modern antibiotics also. We know that, nature has been a source of medicinal agents for thousands of years. As the plants have coevolved with pathogens, they have also developed the chemical protection pathways against the organisms. Therefore, varieties of plants compounds are associated with antibacterial activities. Therefore, in today’s scenario there has been reappearance of interest developed in herbal medicine. So, the study was planned to evaluate the antibacterial effect of petals and leaves extract of rose along with resistant antibiotic against *Escherichia coli* isolated from different clinical samples.

METHODS

This is a hospital based prospective study from April to September 2019. We have included 51 *Escherichia coli* isolated from different samples during the study period. *E. coli* were isolated and identified as per routine standard protocol. All *E. coli* isolates were first tested for routine antibiotic by Kirby Bauer method as per CLSI guideline.¹² Then, isolates which was found to be resistant to one or more antibiotics was further tested by using Rose petals and leaves ex-tract for their antibacterial activity.

Rose both leaves and petals (plant material)¹³

The fresh flowers and leaves of rose were collected. The flowers and the leaves washed thoroughly with tap water and then with sterile distilled water, dried in hot air oven and powdered. This powder of plant material was stored in the dry place for further using as a raw material for the extraction of antibacterial compounds.

Method of extraction by using soxhlet apparatus¹³

A total of 10gm of air dried powder of flower petals and leaves was weighed with the help of electronic weighing machine and was placed in 100 ml organic solvents,

ethanol in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 hrs. And then it was filtered with the help of muslin cloth and was centrifuged at 1000 rpm for 5 min. The supernatant was collected and the solvent was then evaporated by solvent distillation apparatus to make the final volume of one fourth of the original volume, giving a concentration of 40mg/ml. It was stored in airtight bottles for further studies.

Testing of antibacterial effect of leaves and petals of rose

All *E. coli* isolates which was found to be resistant to one or more antibiotics was included in study. Lawn culture of *E. coli* isolates was done on Muller Hinton agar, resistant antibiotics alone and same with extract of leaves and petals each was placed on same Mueller Hinton Agar plate. About 15ul containing 40 mg/ml of extract was inoculated on the plain sterile disc pre-pared homemade and then allowed to dry. The plates were incubated for 24 hrs at 37°C.

RESULTS

During the study period, total 51 *Escherichia coli* isolates was recovered from different samples like urine, sputum, wound swab and blood which were received in the laboratory for routine testing. Identification of organism was done as per routine standard protocol and antibiotic susceptibility testing of all *E. coli* isolates was done as per CLSI guideline.¹² In routine, *E. coli* was tested for antibiotics like ampicillin, gentamycin, nitrofurantoin, norfloxacin, ceftazidime, amikacin, cefuroxime, cefotaxime, meropenam, piperacillin, levofloxacin, ESBL panel and other antibiotics on special request. Out of total 51 *E. coli* isolates, 35 (68.63%) *E. coli* was found to resistant to the tested antibiotics and 16 (31.37%) was found to be susceptible to all the tested antibiotics. All 35 resistant *E. coli* isolates was further tested for antibacterial effect of extract of rose both leaves and petals with the antibiotics and also the antibiotic without extract.

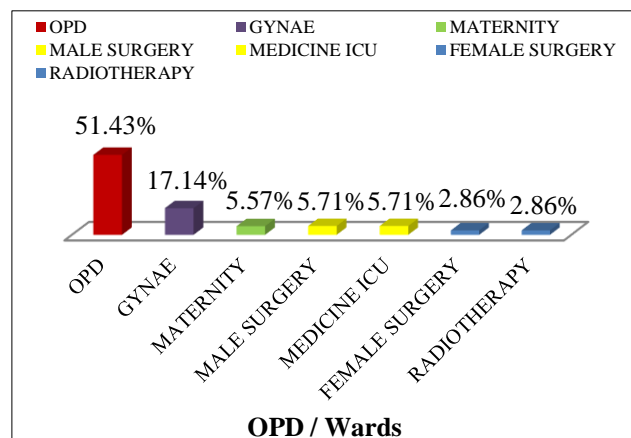


Figure 1: Distribution of resistant *E. coli* as per outdoor and indoor admission.

Out of 35 (68.37%) resistant *E. coli* isolates, 30 (85.71%) were from urine, 2 (5.71%) each from sputum and wound swab and 1 (2.86%) from blood samples. Out of total 35 resistant *E. coli*, 18 (51.43%) from OPD, and 17 (48.57%) were from IPD patients (Figure 1).

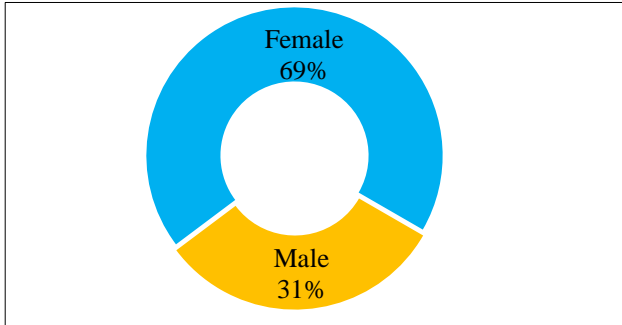


Figure 2: Sex wise distribution of *E. coli* resistant isolates.

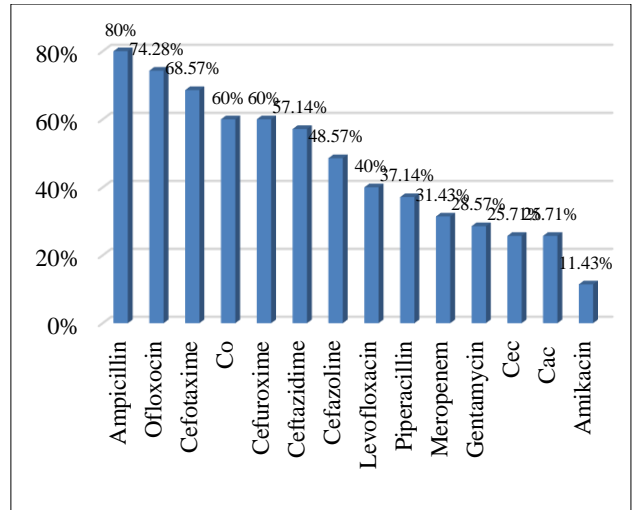


Figure 3: Distribution of resistant *E. coli* as per antibiotic resistance.

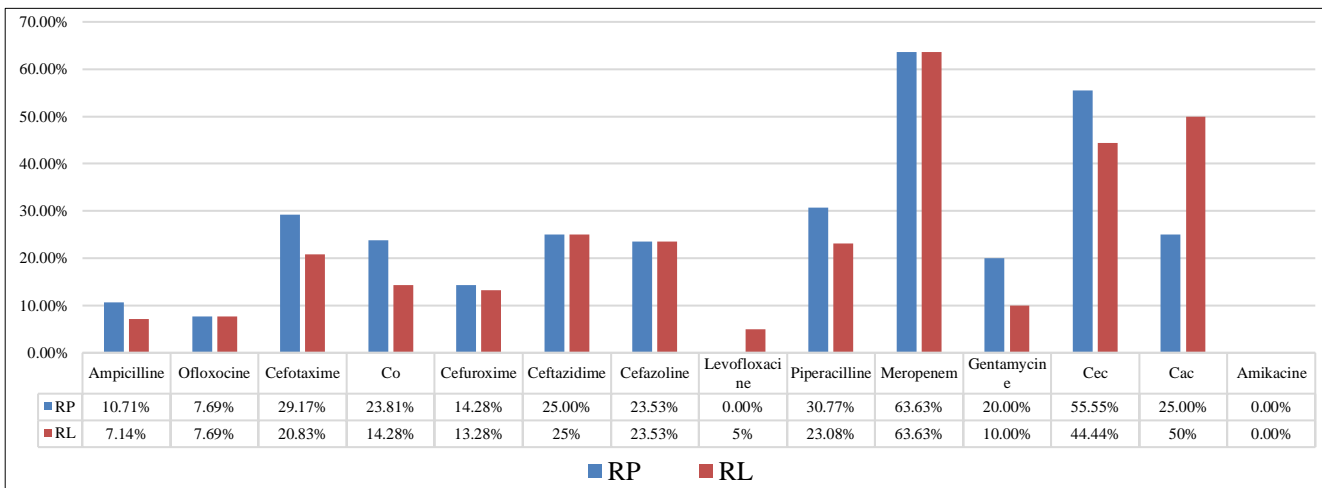


Figure 4: Effect of Rose petal and leaves extract on *E. coli* along with antibiotics.

In our study, 24 (68.57%) were female patients and 11 (31.43%) male (Figure 2).

Out of total 35 resistant *E. coli* isolates, we found that these isolates were resistant to Ampicillin 28 (80%), ofloxacin 26 (74.28%), cefotaxime 24 (68.57%); cotrimaxazole 21 (60%); cefuroxime 21 (60%), ceftazidime 20 (57.14%), cefazolin 17 (48.57%), levofloxacin 14 (40%), piperacillin, 13 (37.14%), meropenam 11(31.43%), gentamycin 10 (28.57%) and amikacin 4 (11.43%) showed in (Figure 3).

After testing these resistant antibiotics individually with Rosa leaves and petals extract, we found that few antibiotics enhances the antibacterial action more with

either leaves or petals and some able to show enhancement equally with both extract.

Antibiotics like ampicillin (10.7%), cefotaxime (29.1%), cotrimaxazole (23.8%), cefuroxime (14.2%), piperacillin (30.7%), gentamycin (20%) showed more enhancement with rose petal extract than leaves. While antibiotic like levofloxacin (5%) showed enhancement only with leaves extract and not with the petals extract. But antibiotics like ofloxacin (7.69%), ceftazidime (25%), cefazoline (23.5%) and meropenem (63.6%) showed enhancement with both extract equally. We also found that rose both petal and leaves extract did not show any effect with amikacin as there is no enhancement in zone size (Figure 4).

DISCUSSION

Plant materials have been widely used for the treatment of infectious diseases and have gained popularity as they decrease the side effect seen with the use of systemic antimicrobials. These antibacterial properties are noticed in the active compounds that are extracted from the plants and the extraction of same depends on the solvent that is used for the extraction process. The most commonly used solvent are methanol and ethanol considering above benefits, in the pre-sent study ethanol was used as a solvent for extraction.⁹

The past two decades have witnessed major increase in emergence and spread of multi-drug resistant bacteria and increasing resistance to newer compounds, such as fluoroquinolones and certain cephalosporins.

In our study, total 35 (68.63%) *E. coli* isolates were found to be resistant to maximum antibiotics routinely used, showing an increasing resistance trends for ciprofloxacin, trimethoprim/ sulfamethoxazole and amoxicillin clavulanic acid for the *E. coli* isolates. Out of these resistant isolates, 85.71% were from urine samples.

We found that out of 35 resistant isolates, maximum resistance was showed to Ampicillin 80%, followed by ofloxacin 74.28 % (Figure 3). In our study, amikacin resistance was less i.e. 11.43% as compared to other and gentamycin resistance was 28.57% which was contradictory to the findings reported by Daniel et al that gentamycin resistance was rare in human *E. coli* isolates.^{10,4}

This antibacterial effect of the extract could be due to presence of active compounds like flavonoids, tannins, alkaloids, and triterpenoids in the extract. These active compounds may act alone or in combination to inhibit the bacterial growth. The remedies based on these plants have a minimal side effect.^{13,14,8,10} Plant extract contain phenolic compounds like tannins that are very good antimicrobial agent. Antibacterial activity of *R. damascena* against *E. coli*, *B. subtilis* has been reported by Vasanthakumar et al.¹⁵

We found that rose petal extract showed more antibacterial activity along with the antibiotics as compare to rose leaves extract. The activity is attributed to their ability to complex extra cellular and soluble proteins and with bacterial cell wall. There are several reports published on antibacterial activity of different herbal extract.

The resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plants extract are required.

There were also some limitation in this study like sample size was small as the duration of study was short, authors

have not tested the effect of plant extract in patients directly.

CONCLUSION

Our study concluded that plant extracts have antimicrobial potential. These flower petals contains flavonoids, cyanidine, quercetin, calcium oxalate, thiamine, ribofalavin, niacin, ascorbic acid and oxalic acid. Plant derived medicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents. Medicinal plants as alternative agents are effective to treat infectious diseases and mitigate the side effects associated with synthetic antimicrobials. From the above study, it is clear that rose petals and leaves extracts have antibacterial activity against *Escherichia coli* along with antibiotics. The application of herbal extracts can decrease the use of antimicrobial agents and have excellent potential in reducing the antimicrobial resistance of *E. coli*.

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