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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF HERBS

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ABSTRACT:

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants. Wild plants have been reported to have antimicrobial and antioxidant properties for centuries, and indigenous plants have been used in herbal medicine for curing various diseases. The development of bacterial resistance to currently available antibiotics has necessitated the search for new antibacterial agents. In lieu of the above justification, present study aimed at evaluating the *In vitro* antibacterial studies on the extracts of three herbs namely Punica Granatum, Ricinus communis and Zingiber officinalis carried out on five medically important bacterial strains (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Proteus vulgaris). Based on the present investigation results, extracts has great potential against different microorganisms tested and has inhibitory effect. It can be concluded that these plants can be used as therapeutic natural agents that may serve as lead for the development of new pharmaceuticals addressing the major therapeutic needs.

KEY WORDS: Bacterial pathogens, Punica granatum, Zingiber officinalis, Ricinus communis, antibacterial activity, cup plate method.

INTRODUCTION:

The usage of herbs to treat a variety of different ailments is universal, and exists in every human culture on Earth[1]. As modern medicine developed large numbers of the pills we take today have their origins in those humble herbs gathered from the waysides and stream beds. Even today, the "miracle drugs" being developed can regularly be traced back to some little known plant growing in some remote part of the world[2]. Herbs have been with us throughout history and they will be with us for as long as we continue[3]. The WHO has indicated that as many as 80% of all people living in the world make use of herbal medicine as their main source of healthcare. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain[4]. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural[5]. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. There are a number of herbal systems that dominate the world today, and these systems are Chinese herbs, Ayurvedic medicine, Roman and Greek herbs, and Shamanic herbs.

MATERIALS AND METHODS:

The leaves of *Ricinus communis* were collected during the month of December from Vignan Hills of Deshmukhi. The authentification was done by Asst.Prof. K.Chaitanya sravanthi, Department of Pharmacognosy, Vignan Institute of Pharmaceutical Sciences. Required amount of rhizomes of *Zingiber officinale* and fruits of *punica granatum* were collected from market.

***Ricinus Communis*:** The leaves of *Ricinus Communis* were shade dried at room temperature and undergone size reduction using pulverizer. It is sieved several times to ensure uniformity of the powdered plant material. The powdered leaves (135g) were undergone ethanolic extraction using soxhlet apparatus. After extraction the solvent is removed. Trace amounts of the solvent may be present in the semi solid extract obtained. This solvent remnant was removed by subjecting it to desiccation. A desiccator with activated silica was used for this purpose. The percentage yield of castor leaves was 14.8%.

***Zingiber Officinalis*:** Required amount (135 g) of ginger was collected and washed with water. Fresh ginger was cut into small pieces and grinded to obtain fine paste. The liquid extract from this paste is obtained by decanting with muslin cloth. The liquid extract is refrigerated. The percentage yield of Ginger was 37.5%.

***Punica Granatum*:**

Seeds extract: Required amounts (135 g) of punica seeds was taken and grinded. The juice was filtered through muslin cloth. The fresh juice so obtained is used for the experiment. The Percentage yield of crude extract is 65%.

Seeds with rind: 135g of *Punica granatum* fruit (rind and seed) was taken and grinded. The obtained liquid was filtered through muslin cloth. The fresh crude extract was used for experiment. The Percentage yield of crude extract is 20%.

TEST ORGANISMS:

The test organisms includes the following Gram positive bacteria: *Staphylococcus aureus*(MTCC740), *Bacillus subtilis* (MTCC441) and Gram negative bacteria: *Pseudomonas aeruginosa* (MTCC424), *Escherichia coli* (MTCC41), *Proteus vulgaris* (MTCC426). All the strains were obtained from Institute of Microbial Technology, Chandigarh, India.

EXPERIMENTAL MODEL:

Preparation of pure cultures:

A small amount of culture is placed on the tip of an inoculation loop/needle is taken into flask containing nutrient broth. This is carried out in laminar airflow. The flask is plugged with cotton. These flasks were incubated to allow the growth of organisms for 18 to 24 hrs which is used as pure cultures for activity.

Spread Plate Method:

20ml of nutrient agar media was transferred into each petri plate. The petri plates were left undisturbed for 1-2hrs. 100 µl of each pure culture was transferred into petri plates using micro pipette. The pure cultures were evenly spread with the help of sterile bent glass rod. They were kept for incubation for 24hrs.

PROCEDURE FOR ANTIBACTERIAL ACTIVITY TESTING:

Drug substances that either suppress or influence the growth of microorganisms are generally analyzed by microbial method. The procedure employed for this testing is Cup plate method or Agar well diffusion method.

Cup plate method:

The antibacterial activity of the extracts was determined by using the agar well diffusion technique. Mueller- Hinton agar plates (Himedia, Mumbai) were seeded with 0.1 ml of overnight culture, allowed to incubate for 24hrs. Cups were made in Petri plates using sterile cork borer (0.85 cm) and 50 µl of each extract was added into each well. Then bacterial plates were incubated at 37° C 24 hrs[6]. Each test compound has got six bores for which zone of inhibition diameter and mean values were determined. Antibacterial activity was determined by measurement of zone of inhibition around each well in plate using zone reader[7]. Measured inhibition zones were recorded as mean diameter in mm[8]. Gentamycin antibiotic was used as control.

RESULTS AND DISCUSSION:

The results of zone of inhibition which is the parameter observed for antibacterial activity of *Ricinus communis, Zingiber officinalis, Punica granatum* is as shown in table 1:

Table 1: Zone of inhibition of extracts and standard, Gentamycin against test organisms

Plant extracts	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus vulgaris*	*Staphylococcus aureus*
Ricinus Communis	13.1± 1.06	16.5± 1.7	15.1± 2.51	16.3± 1.24	19.1± 2.3
Zingiber Officinalis	15±0.87	19.8± 1.34	13.6± 1.24	10± 1.15	21± 1.91
Punica Granatum					
Rind+Seed	20.3± 1.24	15.6± 0.94	24.3± 2.05	17.3± 0.47	19.3± 2.04
Seed	9±0.78	18± 1.63	15± 0.81	18.6± 1.2	22.3± 1.23
Combined activity (R.C+P.G +Z.O)	18.5± 1.5	20.6± 1.1	17.8± 1.86	19.3± 2.1	16.8± 2.26
Standard (Gentamycin)	14.1± 1.4	10±0.67	12.8± 1.06	10±0.86	11.6± 2.2

R.C- *Ricinus Communis* P.G- *Punica Granatum* Z.O- *Zingiber Officinalis*

Ethanolic extract of *Ricinus communis* (10mg/µl) has good inhibitory action on S.aureaus (19.1± 2.3). This is assessed from the data, which is far higher (2 folds) when compared to that of standard (11.6± 2.2). We also found that *Ricinus communis* has comparatively less inhibitory effect on B.subtilis (13.1±1.060) even though both S.aureaus and B.subtilis are gram positive bacteria.

Ricinus communis has shown good activity on P.aurigenosa (16.5± 1.7), E.coli (15.1± 2.51) and P.vulgaris (16.3± 1.24) which are gram negative bacteria. The results so obtained were comparatively more than the standard. This helps us to draw conclusion that *Ricinus communis* has potent inhibitory action towards P.aurigenosa, E.coli, P.vulgaris.

Zingiber officinalis has good inhibitory action towards gram positive bacteria B.subtilis (15±0.87) and S.aureaus (21± 1.91). B.subtilis was more inhibited by Zingiber officinalis compared to standard (14.1± 1.4). Gram negative bacteria P.aurigenosa, P.vulgaris, E.coli were inhibited by Zingiber and we found that it has more potent activity towards P.aurigenosa when compared to other two gram negative strains and also showed twice the action of standard.

Punica granatum rind has broad spectrum activity from the experiment. It has shown twice the action of standard in case of Gram positive bacteria B.subtilis (20.3± 1.24) S.aureaus (19.3± 2.04). The rind has more potent antibacterial activity towards gram negative bacteria P.aurigenosa (15.6± 0.94), E.coli (24.3± 2.05) and P.vulgaris (17.3± 0.47), which are nearly 1.5 to 2 folds more than the standard.

Punica granatum seed has broad spectrum activity from the experiment. It is active against Gram positive bacteria B.subtilis (9±0.78) and S.aureaus (22.3± 1.23). Seed extract had greater inhibitory effect on Gram negative bacteria P.aureginosa (18± 1.63), E.coli (15± 0.81) and P.vulgaris (18.6± 1.2) than standard.

As the individual plants *Punica granatum, Zingiber officinalis, Ricinus communis* has showed good antibacterial activity, so we made an attempt to assess their potency when they are taken in combination. We found that the combined activity of the 3 plants enhanced the potency of anti microbial action. The combined extract i.e; (1:1:1) ratio of 3 plants showed broad spectrum antibacterial activity which is supported by the statistical analysis. This discussion helped us to draw a conclusion from our study that combined extract as well as individual extracts of the plants have good or sometimes high potent activity towards bacteria when compared to Gentamycin.

CONCLUSION:

With the current spread of antibiotic resistance almost at geometric scale and obvious challenges confronted with medical practitioners in the treatment of infectious diseases, proper attention should be given to such plants to reap the potential antimicrobial benefits inherent in them, However actual antimicrobial ingredients need to be extracted and identified, also its tolerable levels in the human body as well as any toxic effects on human and animal tissues be investigated accordingly.

Punica granatum has got high potent activity towards B.subtilis when compared to *Ricinus communis, Zingiber officinalis* and standard drug Gentamycin. Combined extract has shown high activity towards P.aeruginosa. *Punica granatum* rind was highly active against E.coli when compared to other extracts and standard. P.vulgaris was highly inhibited by combined extract. S.aureaus was highly inhibited by crude seed extract of *punica granatum*.

This helped us to draw a conclusion from our study that combined extracts of *Punica granatum, Zingiber officinalis, Ricinus communis* (1:1:1) as well as individual extracts of plants under research have potent activity towards pathogenic bacteria and they have broad spectrum of Antimicrobial activity. This study helps us to develop antibacterial herbal formulations with no or less side effects.

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