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PREPARATION AND ANTIMICROBIAL STUDIES OF UDAYABHASKAR RASA : A HERBOMINERAL DRUG

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ABSTRACT

Five different preparations of *Udayabhaskar Rasa* were evaluated for their antimicrobial properties against different pathogenic micro organisms on agar plate medium and the zone of inhibition was calculated. Well as disc diffusion methods were tried during the studies of antimicrobial properties of drug. It was observed that Well method of drug sensitivity was more efficient than disc diffusion method. Preparations U_2 and U_5 were observed with highest sensitivity than U_1, U_3 and U_4 respectively. *Pseudomonas aeruginosa* was found highly sensitive to all different preparations of Udayabhaskar Rasa except sample U_4.

Key words :*Udayabhaskar Rasa*, Antimicrobial study*, Rasa Karpura* , *Pseudomonas aeruginosa,* Disc diffusion method., Well method.

INTRODUCTION

Ayurveda is one of the most ancient medical sciences of the world. This discipline was evolved through intuitive, experimental and perceptual methodology. The main objective of Ayurveda is to promote health thereby preventing the ailments and to relieve the humanity from all categories of miseries i.e. physical, mental, intellectual and spiritual. The approaches are essentially holistic. The Ayurvedic system of medicine is prevalent in India since the Vedic period and as early as the dawn of human civilization. Ayurveda gets flourished in the course of long history.The branch which deals with Rasa (Mercury), metals, minerals, herbals and other herbo-mineral preparation is known as "Rasa Shastra" which has become an integral part of Ayurveda. Since the time of Vedas, Rasaushadhis play an important role in the management of various ailments. The innate qualities like quick action, less dues, tastelessness, prolonged shelf life, better palatability of Rasaushadhis have helped them to conquer the compliance of the patients. As per other sciences; this science too, needs further probes for its total scientific progress according to the demand of present era.

Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of Udayabhaskar Rasa preparations in vitro (i.e. culture and

sensitivity Tests).Total 14 ingredients are used in this formulation but Rasakarpura, Jaypala and Bhavanas of Bijpuraka Rasa are most important ingredients present in this formula.

AIMS AND OBJECTIVES

- Pharmaceutical standardization of Udayabhaskar Rasa.
- Physico chemical standardization of Udayabhaskara Rasa.
- To evaluate the anti-microbial activity of Udayabhaskara Rasa against common pathogenic bacteria.
- A comparative anti microbial study to evaluate the role of Rasakarpura. Rasapushpa and Jaypala seed.

For the above aims and objectives, five different samples of Udayabhaskara Rasa were prepared, as follows with their codes: -

- **U_1-** As per the specification of Rasa Kalpa Lata (Rasa Kalpa Lata formulation No.158, Chapt. 7, Pg. No. 22 Verse no. 45-46)
- **U_2-** As per the specification of Rasa Yoga Sagar.(Rasa Yoga Sagar, Part- I , formulation No. 369 , Verse No. 1520-1522, Pg. No. 162)
- **U_3-** This was prepared from Rasapushpa in place of Rasakarpura.
- **U_4-** Devoid of Rasakarpura and Rasapushpa.
- **U_5-** Devoid of Jaipala seed (*Croton tiglium Linn)*

PLAN OF STUDY

A. Pharmaceutical Study

In this study, five different samples were prepared including standard formulation of Udayabhaskara Rasa as per the specification of Rasa KalpaLata in the departmental laboratory of Rasashastra and Bhaishajya Kalpana, NIA, Jaipur The main implication of this study was to standardize the processes involved in it.

B. Physico-Chemical Study

In present research work, for physico-chemical standardization, standard sample of Udayabhaskara Rasa as per Rasa KalpaLata specifications prepared (U_1), Sample U_2 as per the specification of Rasa Yoga Sagar and Sample U_3 (prepared from Rasapushpa in place of Rasakarpura) were also taken for the standardization of process involved in it. Materials and methods were followed according to "Pharmacopoeial Standards for Ayurvedic Formulations" – C.C.R.A.S.

C. Antimicrobial Study

The aim of this study was to assess the comparative antimicrobial activity of different samples. For this study, Aqueous and Methanol extracts of the samples were prepared. In vitro studies were undertaken to assess the antimicrobial activity. For both types of extracts, disc diffusion method and for different samples, which were in fine powder form, Well method was employed.

Following common pathogenic strains of bacteria were procured from "Institute of Microbial Technology" (IMTECH), Chandigarh.

Table- Showing the Species of Bacteria and their MTCC NO. -

S. NO.	NAME OF SPECIES	**MTCC NO.**

I.	Staphylococcus aureus	3160
II.	Streptococcus pyogenes	1928
III.	Escherichia coli	901
IV.	Pseudomonas aeruginosa	424
V.	Salmonella typhi	733

These stock cultures were maintained on solid media, Blood Agar for Streptococcus pyogenes and Nutrient Agar for other bacterias, of Himedia Lab. Pvt. Ltd., Mumbai. This anti-microbial study was carried out at 'Birla Institute of Scientific Research', Jaipur. The antimicrobial effects were assessed on the basis of the scale developed by *Arora D.S. et al* (1997).

2.Relevance of the study:

In Ayurvedic literature the word "krimi" has very much potential. There are abundant materials available regarding krimi in Vedic literatures especially in Atharvaveda. Maharishis classified them on the basis of visibility, colour, size, shape and their place in human body. Size dependent classification has two divisions, one is **Sukshama** (Microorganisms) and another is **Sthula** (Macroorganisms). The details of krimi have also been described very efficiently in Samhita-Granthas.

The word 'krimi' was used in a broad sense in Ayurvedic literature i.e. it includes all the pathogenic and non-pathogenic organisms covering a wide range of infection and infestation caused by a host of agent ranging from viruses to worms. As per Charaka Samhita,(Ch.Su., 11/45) 'Bhutabhishanga' is also responsible factor to cause diseases[1]. One of the three major divisions of disease i.e. 'AgantujaVyadhis' is also caused due to krimi[2]. Similarly, Acharya Sushruta has illustrated the means of spread of 'AaupsargikaRogas' (Infectious disease) [3].

According to the Dalhana Commentary on Sushruta

"Upasargaja Iti Upasrijyanta Ityupasargah,

Piditajanasamipotpannah Jvaradayah"

(Dalhana Commentary on Su.Su. 24/7)

That means in the etiology of many diseases, microbial relation plays a role that was realized by the modern medicine only a century ago. But the specific idea of the nature of disease producing germs did not develop till modern microbiology came into existence. Now, it has become possible to investigate the action of drugs on pathogenic microorganisms. Therefore, to make our treatment scientifically more validated, we assess the antimicrobial activity of such preparations in vitro (i.e. culture and sensitivity tests).

Since long, a number of Ayurvedic classical formulations were being used in cases of infections, and they were being found to be effective clinically. But it is a matter of great concern that such a famous formulation "Udayabhaskar Rasa" is not studied in detail. The first reference of the formulation was quoted in Rasa Kalpa Lata by Magniram[5]. Total fourteen formulations were mentioned in Rasa Yoga Sagar[6] as Udayabhaskara Rasa, but Udayabhaskara Rasa (8^{th})[7] is found similar to the Udayabhaskara Rasa (1^{st}) of Rasa KalpaLata. Two gunja matra

with suitable anupana has mentioned in the treatment of Jalodara, Gulma and Aaamvata rogas and also in some other diseases[8].

Similar formulation can be found into Bharat Bhaishajya Ratnakara[9] as Udayabhaskara Rasa with some modifications. Many of these preparations have not even evulated for their antibacterial properties.

3. UdayabhaskarRasa:

The use of herbomineral drug in the therapeutics was found since Samhita period. Health is the supreme foundation of virtue, wealth, enjoyment and salvation. In Ayurveda, it is described in one of the quadrupies of the treatment next to the physician. When the multiple groups of symptoms present, single drug formulations might cover only a fraction of the treatment. Hence, the compound preparations or 'Yogas' (combinations) came into practice.

The Khalveeya Rasayanas are the combination of herbal, mineral and animal products and effective against a number of diseases in a single formula. These are administered in smaller doses to get faster relief and combating many ailments by proper Anupana and Sahapana. It takes less space for manufacturing and storing. The most important aspect is that it preserves the properties of freshly added churnas, swarasa etc. with the help of Moorchhita Parada i.e. Kajjali, Rasasindura, Rasakarpura, Rasapushpa and Hingula etc. Because of which Khalveeya Rasaushadhis occupies greater portion in therapeutics as compared to other kalpanas such as Vati, Gutika,Taila, Ghrita, Avaleha etc.

"Udayabhaskar Rasa" (UBR) is also a compound drug which comes under "Khalveeya Rasayana Kalpana." Most of the Khalveeya Rasayanas come under "Sagandha and Niragni Moorchhana preparations. Udayabhaskar Rasa is one of such preparation. Some of Khalveeya Rasayanas viz. Kaphaketu Rasa, Bhuvaneshwara Rasa etc. are termed as Rasayoga but are not having any type of Moorchhita Parada preparations.Table 1 shows different ingredients of Udayabhaskar Rasa as described in R.K.L[10].

Table 1: Ingredients of Udayabhaskar Rasa (UBR).

S. No.	Ingredients	English/Botanical Name	Part used	Quantity
1.	Shu. Parada	Mercury	---	1 Part
2.	Shu. Gandhaka	Sulphur	---	1 Part
3.	Shunthi	*Zingiber officinal Roxb.*	Rhizome	1 Part
4.	Maricha	*Piper nigrum Linn.*	Fruit	1 Part
5.	Pippali	*Piper longum Linn.*	Fruit	1 Part
6.	Saindhava Lavana	Rock Salt	---	1 Part
7.	Sauvarchala Lavana	Black Salt	---	1 Part
8.	Vida Lavana	Ammonium Chloride	---	1 Part
9.	Sita	Crystalline Sugar	---	1 Part
10.	Dhanyaka	Coriandrum sativum Linn.	Fruit	1 Part
11.	Brihadela	Amomum subulatum Roxb.	Seed	1 Part
12.	Rasakarpura	Mercuric Chloride	---	1 Part

13.	Shuddha Jaipala	*Croton tiglium* Linn.	Seed	12 Part (Equal to total of above all ingredients)
14.	**Bijapuraka Swarasa**	***Citrus medica* Linn.**	**Fruit**	**Q.S. for 7 Bhavana**

Preparation of different samples of Udayabhaskar Rasa:

We have prepared five different combinations of Udayabhaskar Rasa by varying its constituents as shown in Table 2 and evaluated the antimicrobial properties against different pathogenic bacterial strains.

Table 2: Ingredients of five different samples of UBR.

S.No.	Material	U_1	U_2	U_3	U_4	U_5
1.	Shudha Parada	+	+	+	+	**+**
2.	Shudha Gandhaka	+	+	+	+	**+**
3.	Shunthi (Rz.)	+	+	+	+	**+**
4.	Maricha (Fr.)	+	+	+	+	**+**
5.	Pippali (Fr.)	+	+	+	+	**+**
6.	Saindhava Lavana	+	+	+	+	**+**
7.	Sauvarchala Lavana	+	+	+	+	**+**
8.	Vida Lavana	+	+	+	+	**+**
9.	Sita	+	+	+	+	**+**
10.	Dhanyaka	+	+	+	+	**+**
11.	Brihadela	+	+	+	+	**+**
12.	Rasakarpura	+	+	-	-	**+**
13.	Rasapushpa	-	-	+	-	**-**
14.	Shuddha Jaipala (Sd.)	+	+	+	+	**-**
15.	**Bijapuraka Swarasa (Fr.) for 7 Bhavanas**	**+**	**+**	**+**	**+**	**+**

Each drug was taken in 10 gm of quantity except Jaipala seed which quantity was the total sum of other ingredients.

3.1.1 Preparation of sample U_1 (R.K.L.)[10]:

Shuddha Parada was taken into khalva yantra and then Rasakarpura was added in equal quantity and triturated well. Afterwards Trilavana was mixed followed by Suddha Gandhaka and finally other ingredients were added one by one and mixed well. Bhavana was performed with fresh juice of Bijapuraka in 50 ml. quantity and triturated until whole swarasa had dried up and powdered lastly.

3.1.2 Preparation of sample U_2 (R.Y.S.)[11]:

Sample U_2 had same ingredients as sample U_1 but procedure for its preparation was somewhat different. In this preparation, firstly Shuddha Parada was triturated with equal quantity of sulphur, then Trilavana followed by Rasakarpura. The ingredients were mixed and grinded. After that one by

one other ingredient were added and properly triturated. Seven Bhavanas of Bijapuraka swarasa were given in the quantity of 50 ml. for each Bhavana. After complete drying of the drug, it was collected and stored till further use.

3.1.3 Preparation of sample U_3:

The procedure of preparation of sample UBR_3 was same as sample U_1 but, Rasapushpa was used in the place of Rasakarpura.

3.1.4 Preparation of sample U_4:

The sample U_4 is devoid of both the ingredients i.e. Rasakarpura and Rasapushpa. Other pharmaceutical procedure was same as the formulation of U_2.

3.1.5 Preparation of sample U_5:

It is devoid of Suddha Jaipala seed. Other ingredients and pharmaceutical process for preparation of this sample was same as sample U_1.

After preparation of all five different samples of UBR, the total weight of drugs was recorded

Table 3:Weight of different samples of Udayabhaskar Rasa.

S.No.	Sample Code	Weight of the total Ingredients before Bhavana(In gm)	Weight of the final product (drug) after 7 Bhavanas (In gm)	Total Weight increased (In gm)	Weight gain in %
1.	U_1	240.0	279.0	39.0	**16.25 %**
2.	U_2	240.0	280.0	40.0	**16.67 %**
3.	U_3	240.0	260.0	20.0	**8.33 %**
4.	U_4	220.0	261.0	41.0	**18.63 %**
5.	**U_5**	**120.0**	**157.0**	**37.0**	**30.83 %**

Therapeutic Applications:

In the treatment of Aamvata, Jalodara, Gulma etc. at a single dose of two gunja (250 mg).

4 Antimicrobial Studies:

Five different pathogenic bacteria were procured from Microbial Type of Culture Collection, Institute of Microbial Technology, Chandigarh (Table 4) for evaluating the antibacterial properties of different Udayabhaskar Rasa. The cultures were maintained on solid agar plate media and stored at 4^0C till further use. Well and disc diffusion methods were followed for the testing the antimicrobial properties of different samples. 100 µl of well grown bacterial cell were spreaded on the agar plate and a hole of 6 mm diameter was made aseptically. 25 mg of drug was placed in the well (well method) and incubated at 37^0 C for two days and zone of inhibition was recorded. In case of disc diffusion assay, different concentration of herbal drug was loaded on sterile disc of whatman paper no 1 (diameter 6 mm) and placed on the agar plate to observe the zone of inhibition at optimal conditions.

Table 4: Pathogenic bacterial strains.

S. No.	Name of Bactera	MTCC No
1	*Staphylococcus aureus*	3160
2	*Streptococcus pyogenes*	1928

3	*Escherichia coli*	901
4	*Pseudomonas aeruginosa*	424
5	*Salmonella typhi*	733

Results and Discussion:

The antibacterial activity of different formulations of Udayabhaskar Rasa were evaluated against all pathogenic bacterial strains and zone of inhibition was calculated based upon the scales mentioned in Table-5[12].

Table 5: Correlation between zone of inhibition and sensitivity of drug

S. No.	Zone of Inhibition (mm)	Sensitivity of drug
1.	N.I. (below 6)	**Insensitive**
2.	6 < 9	**Less sensitive**
3.	9 < 12	**Moderate sensitive**
4.	**> 12**	**Highly sensitive**

The results of in vitro studies (well method) are shown in Table 6 and in the Figures 1 though 5.The study showed that different formulations of Udayabhaskar rasa were found highly sensitive (>12 mm inhibition) against all the pathogenic bacterial strains. It was also observed that preparation U_4 has less sensitivity against all the bacterial strains when compared to other formulations. U_2 has highest sensitivity followed by U_5.Similar trend was observed in case of disc diffusion method (Figures 6 through 10). Though, in case of disc diffusion method the sensitivity against different pathogens varied with the concentrations of Udayabhaskar Rasa.

S. pyogenes was highly sensitive to all the concentrations of sample U_3 and U_4. It was also found that the strain is highly sensitive to 75 and 100 mg/ml concentrations of sample U_2 and 100 mg/ml concentration of sample U_1. The antibacterial activity of U_5 sample is found less as compared to other formulations.

S. aureus was found less sensitive to all formulations except 75 and 100 mg/ml concentrations of sample U_2 and U_5 respectively.

The ***E. coli*** was found moderately sensitive to all the formulations at highest concentration of 100 mg/ml.

Pseudomonas aeruginosa was highly sensitive to all the concentrations of the sample U_5. Formulation U_1, U_2 and U_3respectively are moderate sensitive at higher concentrations while, least sensitivity was noted with U_4 formulation of U.B.R.

The formulation U_5 was found moderate sensitive against *S. typhi* at all the concentrations while other formulations showed weak sensitivity even at highest concentrations of drug.

Table 6: Sensitivity of different formulations against pathogens.

S. No.	Samples (25 mg each)	*S. pyogenes* MTCC – 1928	*S. aureus* MTCC – 3160	*E. coli* MTCC – 901	*P. aeruginosa* MTCC – 424	*S. typhi* MTCC – 733

1.	U_1	20,21,19,20 Mean -20	35,36,35,36 Mean -35.5	22,23,22,23 Mean -22.5	33,34,34,33 Mean -33.5	30,31,30,31 Mean -30.5
2.	U_2	22,23,21,22 Mean -22	35,36,36,35 Mean -35.5	31,32,31,32 Mean -31.5	37,38,37,38 Mean -37.5	35,36,35,36 Mean -35.5
3.	U_3	19,20,18,19 Mean -19	28,29,28,29 Mean -28.5	26,27,27,26 Mean -26.5	27,28,27,28 Mean-27.5	24,25,24,25 Mean -24.5
4.	U_4	13,14,13,14 Mean -13.5	25,26,26,25 Mean -25.5	21,22,21,22 Mean -21.5	27,27,27,27 Mean -27	23,24,23,24 Mean-23.5
5.	U_5	21,22,20,21 Mean -21	34,35,34,33 Mean -34	25,26,26,25 Mean -25.5	34,35,34,35 Mean -34.5	29,30,28,29 Mean -29
6.	Control Sterile (Charcoal powder)	Nil	Nil	Nil	Nil	Nil

CONCLUSIONS

The antibacterial properties of different formulations of Udayabhaskar rasa (UBR) proved the importance of compound drugs in the treatment of a number of diseases caused by a number of "krimi's". In the treatment of infectious diseases due importance should also be given to restoration of Tridosaha-equilibrium i.e. immunity and bala. Comparing the well and disc diffusion method for antimicrobial assay, the former gave maximum inhibition against all pathogens. In case of disc diffusion method, the formulation U_5 was least sensitive against *S. pyogenes* at all concentrations. *S. aureus*was highly resistant against all formulations of Udayabhaskar rasa.

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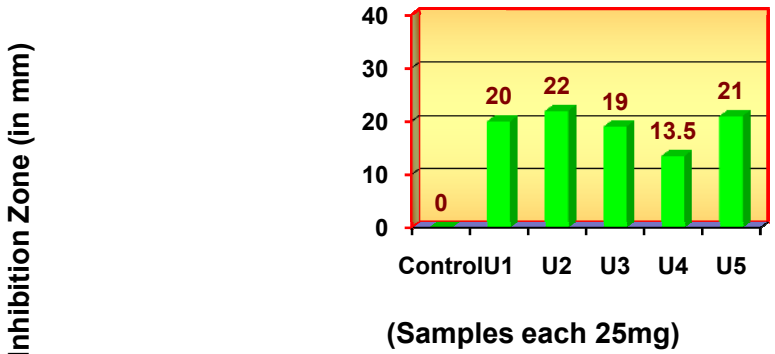

Fig. 1: Effect of drugs U1 to U5 on Streptococcus pyogenes

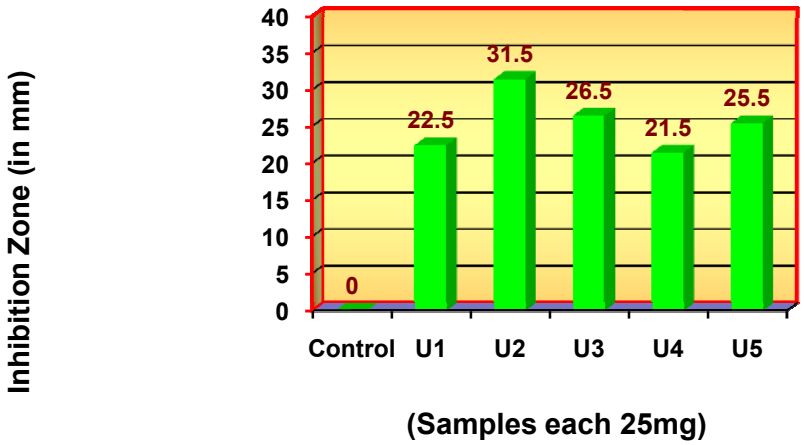

Fig. 3: Effect of drugs U1 to U5 on Escherichia coli

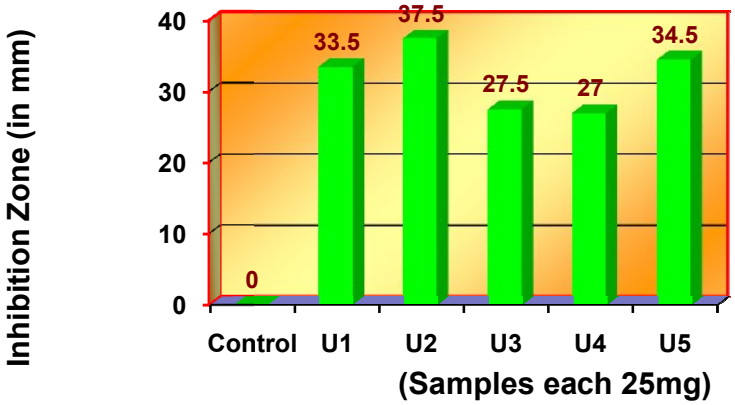

Fig. 4: Effect of drugs U1 to U5 on Pseudomonas aeruginosa

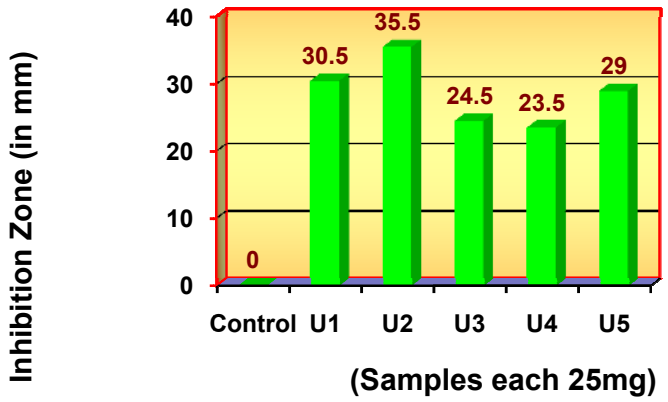

Fig. 5: Effect of drugs U1 to U5 on Salmonella typhi

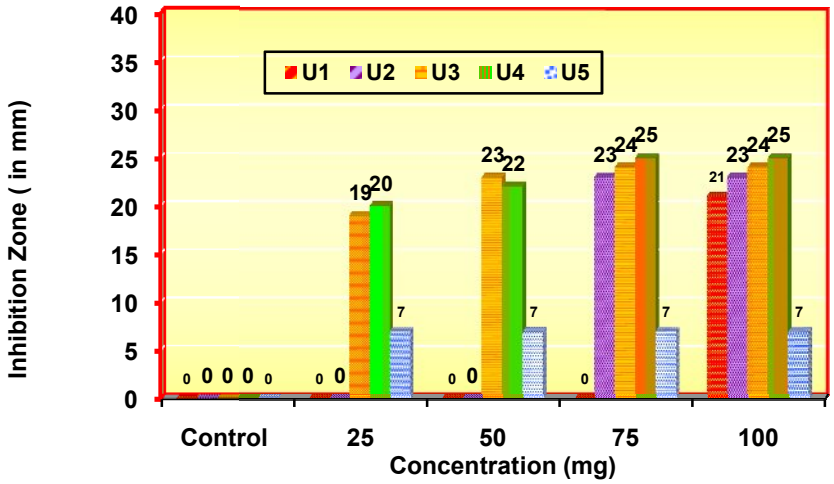

Fig. 6: Effect of different concentrations of drugs on Streptococcus pyogenes (Disc diffusion method)

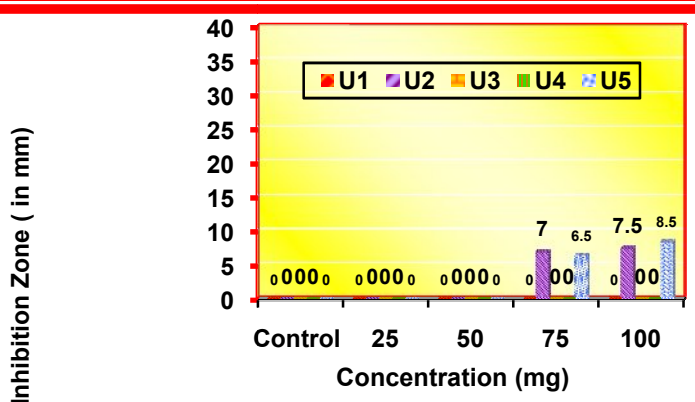

Fig. 7: Effect of diffefern concentrations of drugs on *Staphylococcus aureus* (Disc diffusion method)

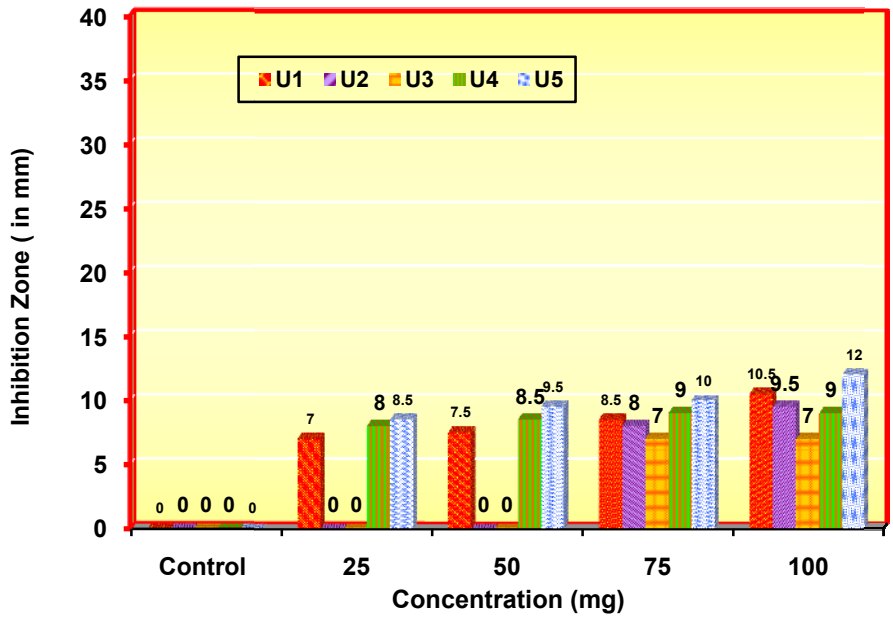

Fig. 8: Effect of different concentrations of drugs of *Escherichia coli* (Disc diffusion method)

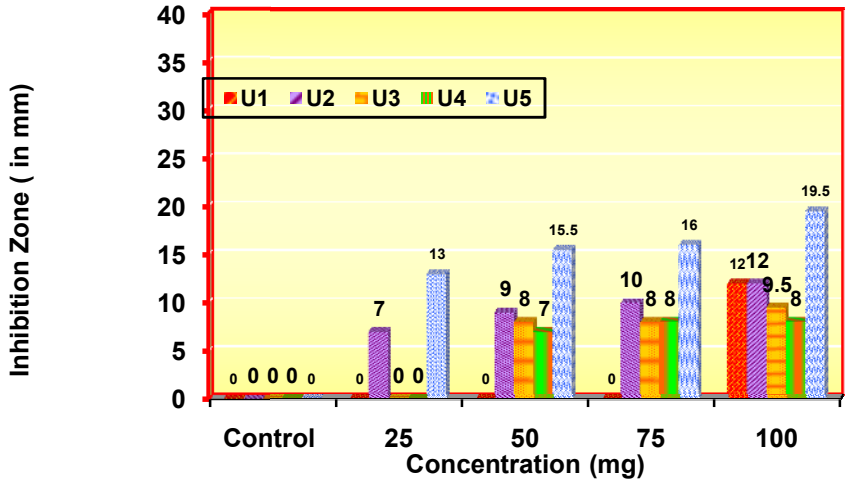

Fig. 9: Effect of different concentrations of drugs on Pseudomonas aeruginosa (Disc diffusion method)

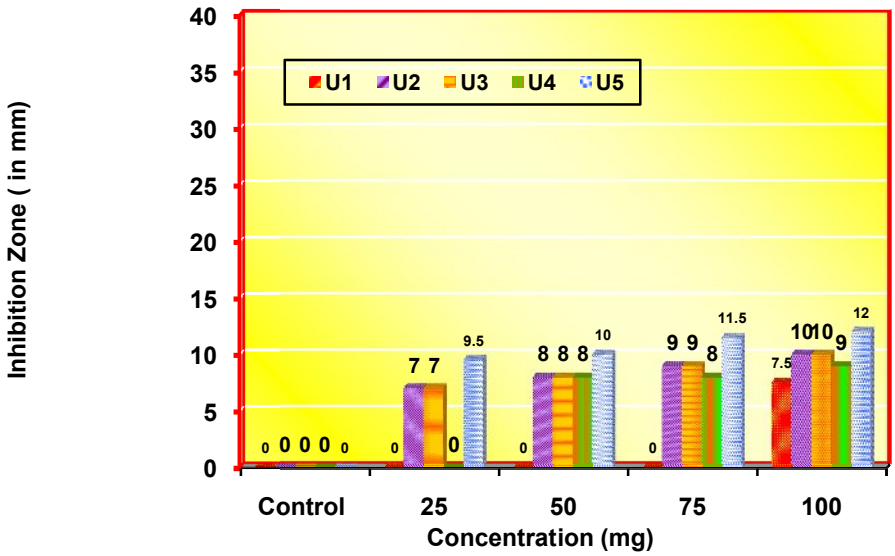

Fig. 10: Effect of different concentrations of drugs on Salmonella typhi (Disc diffusion method)

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